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EXPLOITING DIVERSE DATA SOURCES TO INVESTIGATE EMERGING SAFETY ISSUES OF DRUGS IN POST-MARKETING PHASE: A RESEARCH FRAMEWORK FOR CLINICAL PHARMACOLOGIST

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Science does not exist, unless it has been written down'
'Further the judgement is upon the reader'

J.P. Vandenbroucke
(Ned Tijdschr Geneeskd 2002; 146:1699-1703)

TABLE OF CONTENTS

ABSTRACT

New therapeutic products are typically approved based on their demonstrated efficacy and safety in a series of clinical trials. Randomized, controlled, phase 3 studies are considered to be the most rigorous means for studying the efficacy/safety profile of drugs. Various aspects of a medicinal product's safety may not be known from clinical trials and until the product is used in routine clinical settings. Research efforts by clinical pharmacologist towards predicting safety, efficacy and effectiveness of drugs require a multidimensional approach. Such a multidimensional approach should encompass related discipline of clinical pharmacology, such as pharmacoepidemiology, pharmacovigilance and clinical pharmacy that can provide the tools for rigorous assessment of the good and harm that specific medications provide.

Real-world data (RWD) and evidence provide the potential to address the effectiveness and safety of drugs. With the emerging RWD sources, clinical pharmacologist should able to effectively and comprehensively exploit RWD sources depending on the research problem. Towards this goal, this dissertation has proposed and investigated a research framework for clinical pharmacology, if this would produce reliable evidence to answer given research question by exploiting and combining evidence from different data sources, namely pharmacovigilance and observational studies. To answer the initial research questions, several studies were conducted. The work described in this thesis is based on five original studies.

In study I-II, an association between psychotropic drugs (anti-psychotics/antidepressants) use and hyponatremia was studied. The pathophysiology of hyponatremia induced by psychotropic drug remains unclear. To gain knowledge into this rare and severe pathology, we performed a study combining a real-world pharmacovigilance safety data and the pharmacodynamics properties of given drug class. These studies highlight the potential roles of receptor targets in psychotropic induced by hyponatremia. Overall, these studies contribute to a better understanding of hyponatremia induced psychotropic and to identify the potential target of interest that needs to be further explored.

In study III, the potential association between the use of dipeptidyl peptidase-4 (DPP-4) inhibitors and bullous pemphigoid was analysed. Since 2012, an increased risk of bullous pemphigoid among DPP-4 inhibitor users has been reported in case series, pharmacovigilance reports, and case-control studies. To date, the mechanism by which DPP-4 inhibitors induce BP is not well understood. This is the first study aimed at evaluating the potential role of pharmacological properties of different gliptins in the occurrence of BP as a result of exposure to DPP-4 inhibitors using the global SRS. Several molecular targets were analysed and we found a clinical relevance of gliptins selectivity for DDP-4 in the development of BP as a result of exposure to these drugs.

In study IV-V, we studied the association between anthropometric changes in inflammatory bowel disease (IBD) patients following anti-tumour necrosis factor (TNF)- α therapy using three different methodologies. In study IV, we performed a systematic review and meta-analysis of the studies on anti-TNF-α in adults and paediatric IBD patients reporting changes in anthropometric parameters. Subsequently, A longitudinal case series analysis was performed to assess anthropometric and glucometabolic changes in paediatric IBD patients following anti-TNF treatment. Finally, to map a broader safety profile of anti-TNF-α therapy in a real-world setting, we carried out a comprehensive analysis of pharmacovigilance database in parallel to quantify the association between TNF-α therapy and the rate of body-related changes.

In conclusion, the research presented in this dissertation has produced several novel insights and provided new answers towards the challenging drug safety questions. This dissertation developed research framework including technical and statistical methods for examining ADEs and/or beneficial effect of drugs that might occur among new or established users of pharmaceuticals. This work provides a template to clinical pharmacologist in establishing a continuous learning system for other priority conditions and drug classes, other populations, and other databases which could potentially unveil drug safety profiles and novel adverse events (AEs).

RIASSUNTO

I farmaci di neo-immissione in commercio vengono approvati sulla base delle prove di efficacia e sicurezza dimostrate nel corso dei trial clinici pre-registrativi. Gli studi clinici randomizzati e controllati di fase 3 sono infatti considerati il mezzo più rigoroso per studiare il profilo di efficacia/sicurezza dei farmaci. Vari aspetti legati alla sicurezza di un medicinale possono però non essere individuati durante la conduzione di tali studi clinici e vengono resi noto solo quando il prodotto viene utilizzato nella pratica clinica quotidiana.

Le attività di ricerca del farmacologo clinico nel prevedere la sicurezza e l'efficacia dei farmaci richiedono un approccio multidimensionale. Un tale approccio multidimensionale dovrebbe includere tutte le discipline correlata alla farmacologia clinica, come la farmacoepidemiologia, la farmacovigilanza e la farmacia clinica, che possono fornire gli strumenti per una valutazione rigorosa dei benefici e dei possibili effetti collaterali dei farmaci. Le evidenze scaturite dai dati del mondo reale (RWD) forniscono il potenziale per affrontare gli aspetti legati all'efficacia e sicurezza dei trattamenti. Grazie al numero crescente di fonti di RWD, il farmacologo clinico potrebbe sfruttare appieno ed efficacemente la possibilità di utilizzare queste fonti, in accordo allo specifico quesito clinico.

A tal fine, l'elaborato di tesi è incentrato sulla proposta e validazione di un modello di approccio multidisciplinare per la farmacologia clinica, in modo tale da verificare se tali strategie possono produrre prove affidabili per rispondere a determinate quesiti clinici sfruttando e combinando prove da diverse fonti di dati, vale a dire i sistemi di segnalazione spontanea, la letteratura e la conduzione di studi osservazionali. Sono stati condotti diversi studi per affrontare determinati quesiti clinici. Il lavoro descritto in questa tesi si basa su cinque studi originali.

Negli studi I-II, è stata studiata l'associazione tra l'uso di farmaci psicotropi (antipsicotici/antidepressivi) e l'insorgenza di iponatriemia. La fisiopatologia dell'iponatriemia indotta da farmaci psicotropi rimane poco chiara. Per acquisire conoscenze su questa patologia rara e grave, abbiamo eseguito uno studio che combina i dati di monitoraggio post-marketing con le proprietà farmacodinamiche dei farmaci in oggetto. Questi studi evidenziano i potenziali ruoli dei bersagli recettoriali dei vari agenti psicotropi nella comparsa di

iponatriemia. Nel complesso, questi studi contribuiscono a una migliore comprensione ti tale associazione e all'identificazione di potenziali bersagli di interesse che devono essere ulteriormente esplorati.

Nello studio III, è stata analizzata la potenziale associazione tra l'uso di inibitori della dipeptidil peptidasi-4 (DPP-4) e la comparsa di pemfigoide bolloso. A partire dal 2012, è stato segnalato un aumento del rischio di pemfigoide bolloso tra gli utilizzatori di farmaci inibitori della DPP-4 nell'ambito di studi di farmacovigilanza e caso-controllo. Ad oggi, il meccanismo con cui gli inibitori della DPP-4 inducono la BP non è stato caratterizzato. Questo è il primo studio volto a valutare il ruolo potenziale delle proprietà farmacologiche delle diverse gliptine nella comparsa di pemfigoide bolloso a seguito dell'esposizione a tali farmaci, utilizzando i dati provenienti dal sistema di segnalazione spontanea internazionale. Nell'analizzare diversi bersagli molecolari, abbiamo riscontrato una rilevanza clinica della selettività delle gliptine per la isoforma DDP-4 nello sviluppo del pemfigoide bolloso come risultato dell'esposizione a questi farmaci.

Negli studi IV-V, abbiamo studiato l'associazione tra i cambiamenti antropometrici nei pazienti con malattia infiammatoria intestinale (IBD) e la terapia con farmaci inibitori del fattore di necrosi tumorale (TNF)-α utilizzando tre diverse metodologie.

Nello studio IV, abbiamo eseguito una revisione sistematica e una meta-analisi della letteratura sull'utilizzo dei farmaci anti-TNF-α negli adulti e nei pazienti pediatrici con IBD che riportavano cambiamenti nei parametri antropometrici. Successivamente, è stata eseguita un'analisi longitudinale di case series per valutare i cambiamenti antropometrici e glicometabolici nei pazienti pediatrici con IBD dopo il trattamento con anti-TNF-α nella pratica clinica quotidiana. Infine, per mappare un profilo di sicurezza più ampio della terapia anti-TNF-α in un contesto reale, in parallelo, abbiamo effettuato un'analisi del database di farmacovigilanza per quantificare l'associazione tra la terapia anti-TNF-α e il tasso di cambiamenti antropometrici.

In conclusione, la ricerca presentata in questo elaborato di tesi ha prodotto diversi nuovi spunti di discussione e ha fornito nuove risposte ai numerosi quesiti clinici irrisolti sulla sicurezza dei farmaci, sviluppando contestualmente un approccio multidisciplinare che include metodi tecnici e statistici per esaminare gli ADE e/o gli effetti benefici dei farmaci che potrebbero verificarsi

tra nuovi e vecchi utilizzatori. Questo lavoro fornisce al farmacologo clinico una strategia di intervento pluridisciplinare per la valutazione dei rischi e benefici dei farmaci da adattare potenzialmente ad altri ambiti di ricerca prioritari, così come ad altre popolazioni e fonti dati in grado di contribuire alla caratterizzazione dei profili di sicurezza dei farmaci.

CHAPTER 1: INTRODUCTION

CHAPTER 1: GENRAL INTRODUCTION

Every year prescribers and patients have more medications at their disposal, each with its own efficacy, side effects, and cost. When a new drug is introduced, its benefit/risk relationship is often understood in only a preliminary way, as is its cost-effectiveness. This provides a limited perspective on how it ideally should be used. This chapter provides an overview of the problem and significance of safety and effectiveness with the use of drugs in post-marketing phase and when one needs to consider drug as safe, i.e., the risk of adverse reactions as 'tolerable'. The chapter will continue with a discussion of the need for real-world data and how it gives the opportunity to bring safest, most effective treatments. The chapter will conclude with the aim and objectives of the present thesis.

1.1. PROBLEM AND SIGNIFICANCE OF SAFETY, EFFICACY, AND EFFECTIVENESS OF DRUGS IN THE POST-MARKETING PHASE

1.1.1. Adverse drug Events

An adverse drug events (ADEs) includes all undesirable events temporally associated with the use of a medicinal product, but not necessarily causally related to the product [1]. This includes medication errors (ME), adverse drug reactions (ADRs) and overdoses. ADEs can happen anywhere: in hospitals, long-term care settings, and outpatient settings. There is a diversity of terms used to describe the harmful effects of medicinal products. For example, the terms adverse effect, ADRs, side effect, and others are used interchangeably in clinical practice.

1.1.1.1 Adverse Drug Reactions

It is perhaps a fundamental truth in medicine that there is no medication that is without risk [2], although the most rigorous efforts in drug approval and regulation, unanticipated ADRs may occur. The burden of ADRs worldwide is high, accounting for considerable morbidity, mortality, and extra costs [3]. The United States Institute of Medicine reported in January of 2000 that an estimated 7,000 deaths per year occur due to ADRs [4]. Another study

estimates that 6.7% of hospitalized elderly patients had a clinically ADRs with a mortality rate of 0.11% [5]. Also, it was estimated that the costs due to preventable ADRs in an inpatient setting had a wider range than outpatient setting: a minimum of €2,851 to a maximum of €9,015 (inpatient setting) versus a minimum of €174 to a maximum of €8,515 (outpatient setting) and the impact of preventable ADRs in terms of hospital length of stay was higher in the outpatient setting (9.2 ± 0.2 days) than in the inpatient setting (6.1 ± 2.3 days) [3]. A comprehensive meta-analysis indicated that the mean prevalence of fatal ADRs was 0.20% (95% CI: 0.13–0.27%) among patients with ADRs that lead to hospitalisation [6]. Therefore, knowledge, minimization, and prevention of undesirable and harmful effects of medicinal products are important objectives of successful pharmacotherapy.

1.1.1.2. Medication Errors

Medication errors (ME) are a leading source of unintended patient harm worldwide [7]. Although estimating the overall prevalence of ME is difficult due to the varying definitions and classification systems employed [8, 9], their prevention is recognized internationally as a healthcare priority. Errors in the prescribing and management of drug therapy are common and have been identified as a major cause of ADEs. A systematic review reported the prevalence of prescribing errors was ranged widely from 2% to 94%; inappropriate prescribing was the most common type of error reported while the incidence of preventable ADEs was estimated as 15/1000 person-years [10].

1.1.1.3. Drug-drug interactions

Often preventable, a drug-drug interactions (DDIs) occurs when two or more drugs interact with each other, resulting in altered drug effectiveness or toxicity [11]. DDIs have the potential to cause serious harm to patients [12]. The prevalence of potential DDIs has been estimated at between 15 and 45% of general hospitalised patients, while the percentage is higher in intensive care patients (67%) [12, 13]. Some studies linking DDIs with increased length of hospital stay and increased healthcare costs [14, 15]. There is a general consensus that polypharmacy is a contributing factor to the occurrence of

DDIs, this is particularly important in patient with extreme age groups because of greater end-organ sensitivity to drugs due to reduced renal drug clearance and reduced drug clearance by metabolism that may lead to higher concentrations of medicines and greater potential for adverse effects from drug interactions.

1.1.2. Drug treatment in special population

1.1.2.1. Elderly

It is generally recognized that elderly patients have multiple co-morbid conditions and, as a result, interventions that target single diseases may have limited efficacy in this population. For example, a hypertensive older patient may have diminished renal function, insulin resistance and low levels of physical activity. Treating increased blood pressure has clear benefits but may have a limited (to no) effect on renal function, risk of diabetes, functional capacity or reduced physical activity. Thus, conducting a clinical trial in older individuals is intrinsically more difficult than in younger adults and may lead to disease-specific improvements that have little relevance for the life and well-being of the individual.

The elderly population has a high rate of medication usage; approximately 87% to 90% use at least one medication [16] while 65% use three or more medications [17]. A recent cross-sectional analysis in 17 European countries reported that 32.1% of the older adults take 5 or more medications per day [18]. The use of multiple medications increases the risk for DDIs, non-compliance, ME, and ADRs. These patients are also more susceptible to ADEs—including ADRs—resulting from the physiologic changes of ageing. By one estimate, ADRs are about seven times more common in those older than 70 than in younger persons [19]. One out of every 30 acute hospital admissions in patients ages ≥65 is related to an ADR [20]. From a clinical perspective, ADRs in elderly are often difficult to diagnose and are sometimes mistaken as a new disease and treated with additional medications, a phenomenon termed as termed the "prescribing cascade”. Discontinuation of previously prescribed therapy in the elderly is rarely considered. As a result,

the older population receives a considerable quantity of doses per day of different pharmacological principles.

1.1.2.2. Paediatrics

Because of limited drug development for the paediatric age group, sufficient safety, efficacy, and dosing information for children are lacking in labels of numerous medicinal products. At the time of approval, the majority of medications have not been evaluated appropriately for use in children with a dedicated paediatric study, resulting in the lack of sufficient metabolic, safety, efficacy, and dosing information for children for most medications approved in adults. A review of drug product labelling listed in the electronic Physicians' Desk Reference showed that only 46% of products had some information on paediatric use in labelling [21]. The lack of paediatric-specific information in product labels can lead to "off-label use" of medications in the paediatric population. In fact, the use of off-label medications in children is a common practice for paediatric providers, with off-label prescription rates from 3.2 % to 95% [22]. This becomes a significant issue in specific populations of children, including preterm infants and new-borns, and in children with chronic or rare diseases [23, 24]. Moreover, unlike adults, children respond differently to drugs and are more susceptible to ADEs. This is due to the immaturity of the immune system and the developmental pharmacology [25]. A systematic review of observational studies covering the period from 2000 to 2019 reported preventable ADE rates of 2.3 per 100 patients and 21–29 per 1000 patient-days in paediatric intensive care units [26].

1.1.3. Effectiveness of medicines, particular dose selection, sequence of therapies, subpopulations for drug use, and co-prescribing

After the approval of a drug, it is important to know relative advantages and disadvantages for patients who would have been treated with the old drug, one needs to be more certain of its effectiveness in terms of cost as well as risk. The presence of significant ADR, or the absence of beneficial effects, is

less likely to be tolerated for a drug that does not represent a major therapeutic advancement.

Trials in special populations or rare diseases are more challenging than clinical trials in more frequent diseases. The classical randomized controlled trial (RCT) cannot always be conducted, because of small numbers of eligible patients and the heterogeneity of the patient groups. Patients may be in various stages of a disease and disease courses may be far from uniform. Likewise, in paediatrics, where therapy is often based on expert opinion, extrapolated from studies performed in adults and from descriptive studies in youngsters, rather than the best evidence supported by well performed and well-presented RCTs in children. Moreover, ethical considerations of medical experts on performing placebo-controlled trials in children and in patients with rare disease limit the available evidence based on the placebo-controlled trials.

1.1.4. Drug Safety and Tolerability

The legal standard for any drug that must be met before a drug is approved for marketing is that it needs to be proven to be "safe and effective under conditions of intended use." However, it is important to differentiate safety from risk. Practically nothing is without some risks and undoubtedly no drug is inherently safe. Use of a "safe" drug, however, still carries some risk. It would be better to consider safety in terms of 'degrees of safety'. Specifically, a drug "is safe if its risks are judged to be acceptable" [27, 28]. Measuring risk is an objective but probabilistic pursuit. A judgment about safety is a personal and/or social value judgment about the acceptability of that risk. The tolerability of the medical product represents the degree to which overt adverse effects can be tolerated by the subject. Whether one's perspective is that of a manufacturer, regulator, academician, or clinician, one needs to consider the risk of ADR that one is willing to accept as tolerable [29]. Several factors can affect one's willingness to tolerate the risk of adverse effects of drugs. Some of these factors are related to the adverse outcome being

studied. Others are related to the exposure and the setting in which the adverse outcome occurs. Therefore, safety assessments may need to vary for different age groups, the presence of particular set of medical conditions or certain individuals with genetic variants [30, 31].

1.2. THE NEED OF REAL-WORD EVIDENCE REGARDING SAFETY, TOLERABILITY, EFFICACY, AND EFFECTIVENESS OF DRUGS.

Once a drug is approved for marketing, it enters a complex healthcare system in which, its use by patients and its outcomes often goes largely unassessed. The common view has been that after the approval of a drug, it is used at the discretion of the clinician, with little formal follow-up of the appropriateness or consequences of such decisions. The fact that many regulatory agencies purposely do not base their approval decisions on a medication's clinical or economic value compared to similar products; often superiority over placebo is sufficient for a drug to be approved have further aggravated the problem. This section provides an overview of the shortcomings of premarketing trials and how the Real-world data (RWD) and its methods can contribute to addressing this problem in the field of clinical pharmacology.

1.2.1. Shortcomings of premarketing trials

New therapeutic products are typically approved based on their demonstrated efficacy and safety in a series of clinical trials. The foundation of this evidence from carefully designed, adequately powered RCTs, which seek to evaluate the efficacy and safety of medications in a specifically enrolled and well-controlled target population. However, various aspects of a medicinal product's safety may not be known from clinical trials and until the product is used in routine clinical settings.

- *Small sample sizes in clinical trials.* Generally, the number of patients exposed to any new medicinal product in clinical trials is considerably smaller than the number of patients exposed to the product after commercialization [32]. Therefore, rare ADRs may not be encountered in clinical trials due to the low number of patients exposed.
- *Importance of naturalistic settings*. Clinical trial data are generally

obtained in controlled settings where patients are carefully chosen and followed under strict conditions to assess particular efficacy endpoints [33]. Real-life patients may also have more complex comorbidities and concomitant medication regimens. Some ADRs (e.g., cancer) can only be detected after prolonged exposure to a medicinal product, which may not be feasible in clinical trials of limited duration.

- *Insufficient safety data in special populations*. Clinical trials are generally conducted in certain countries and limited patient populations and may not comprehensively obtain medicinal product safety information in special populations (ethnicity, pregnancy, paediatrics, geriatrics).
- *Broader aspects of product safety.* A medicinal product's abuse potential and effects of an overdose may become known only after product commercialization. Similarly, MEs may not be recognized until the routine clinical use of the product. The United States Food and Drug Administration (FDA) advisories about confusing Brintellix (Takeda's brand of vortioxetine) with Brilinta (AstraZeneca's brand of ticagrelor) [34] and ME involving Avycaz (Allergan's brand of ceftazidime–avibactam) [35] are two such recent examples. With the passage of time, information about interactions between a new medicinal product and older products may become evident. The burgeoning medical device industry also presents possibilities of novel AEs involving product–device interactions (e.g., heparin-induced thrombocytopenia leading to left ventricular assist device thrombosis) [36]. With an increasing public tendency toward the use of herbal products and dietary supplements, there is an ever-greater need for identifying their potentially harmful effects and interactions [37, 38].
- *Improved characterization of adverse effects*. The availability of advanced diagnostic tools may facilitate better characterization of adverse effects and predisposed population groups. For example, the presence of the human leukocyte antigen B*1502 variant allele in Han Chinese (and other Asians) has been associated with carbamazepine-induced, serious, cutaneous ADRs [39]. In December 2007, the FDA recommended

genotyping patients of Chinese ancestry for this allele before starting treatment with carbamazepine [40]. The timing of this safety communication is remarkable when one considers the fact that carbamazepine was first approved by FDA in 1968.

Increasingly, more attention is being paid to assessing the outcomes of medication use on a population level, considering what its useful and harmful outcomes are when it is taken by hundreds, thousands, or even millions of patients rather than by single individuals in a clinical trial or in routine practice.

1.2.2. Opportunity of Real-World Data

In clinical practice, data on medication use are routinely captured in different formats, including information on efficacy and tolerability in populations that extend beyond those enrolled in clinical trials. RWD refers to data associated with patient health, collected from sources other than RCTs; these data may be used for decision-making purposes [41, 42]. RWD can be analysed to produce real-world evidence (RWE), that is, evidence from RWD on the usage and/or benefits and risks of a medication or a medical product. Not only do RWD support clinical interpretation of how products act in more diverse patient populations, but they may also point to additional therapeutic benefits or uses beyond those originally studied in RCTs.

RWE can be used for developing medical products and informing healthcare practise and policymaking. Examples of its uses include support for identification of unmet medical needs [43], design of registered clinical trials [44], post-approval drug safety assessment and pharmacovigilance [45], payment and coverage decisions [42], healthcare quality improvement [46], new indications of medical products [47], assessment of healthcare technologies [48], and clinical practice guideline development [49]. In addition, the abundance and diversity of data allows exploration of clinical research questions other than healthcare interventions, such as disease burdens, prognoses, and clinical predictions.

Previously unavailable, novel sources of RWE and experimental data in digital form have also become available for pharmacovigilance purposes [50]. The confluence of these events has spurred the development of automated, quantitative big data methods to analyse ADE reports to supplement and complement traditional qualitative pharmacovigilance methods [51]. Beside the classic role of pharmacovigilance, many other issues are also of relevance to pharmacovigilance-related activities and include MEs, lack of efficacy reports, off-label use, acute and chronic poisoning, assessment of drug-related mortality, abuse and misuse of health products, identifying substandard, adulterated and contaminated, wrong medicinal and adverse interactions of medicines with chemicals and other drugs. Data mining of drug safety report databases, medical literature, and other digital resources could play an important role in augmenting the information about the aforementioned aspects of medications [52].

1.3. RATIONALE

Research is a vital part of the training and everyday work of a clinical pharmacologist. The endeavour of a pharmacologist working in the clinical environment is to develop methods and strategies that improve the quality of drug use in individual patients and patient populations. Since the first WHO report on clinical pharmacology in 1970 [53], research roles of clinical pharmacologist were mainly in a translational sense; research in drug evaluation, drug utilization, pharmacovigilance and pharmacoepidemiology areas are now the priority. All these research areas have great potential for supporting healthcare personnel in rational drug use. Research in clinical pharmacology therefore also includes studies that elicit new data about drugs in use such as new ADR, indications, comparative effectiveness, safety aspects and treatment of neglected patient populations (children, elderly). It also includes research on pharmacogenetics and drug interactions. RWD and RWE have the potential for filling gaps in knowledge that can be harnessed to further refine clinical practise guidelines and treatment decisions. Evidence derived from such data could be utilized to better characterize a product’s

clinical outcomes in more diverse, real-world patient populations.

Research efforts by clinical pharmacologist towards predicting safety, efficacy and effectiveness of drugs require a multidimensional approach. Such a multidimensional approach should encompass related discipline of clinical pharmacology, such as pharmacoepidemiology, pharmacovigilance and clinical pharmacy that can provide the tools for rigorous assessment of the good and harm that specific medications provide. With the emerging RWD sources, clinical pharmacologist should able to effectively and comprehensively exploit RWD sources depending on the research problem. Equally importantly, such multidimensional approach can bring together the expertise of several groups whose skills may be complementary in addressing the difficult methodologic issues inherent in studies of drug use and outcomes. Moreover, it should also include selected special opportunities from the critical appraisal of clinical evidence to address major issues of importance. These are of particular interest as the field continues to mature and turn its attention to questions beyond just those of ADRs.

Driven by these rationales, this project elaborated on the research framework to train the clinical pharmacologists who are equipped to effectively exploit diverse data resources to study the research question at hand. The proposed research framework is intended to effectively utilize the principles, methods, and strategies of investigating ADRs, as well as other questions of drug effects. Methods developed and findings of each research objective studied during the work on this thesis should facilitate clinical pharmacology research units to generate evidence from exploiting diverse data sources that improve the quality of drug use.

1.4. AIM, OBJECTIVES AND OUTLINE OF THIS THESIS

1.4.1. General aim

The current PhD thesis aims at assessing the safety, efficacy and effectiveness profile of drugs by exploiting heterogeneous data sources.

1.4.2. Objectives

The specific objectives outlined for this project were the following:

1. To apply a novel approach to gain a better understanding of the underlying mechanisms of serious ADRs associated with specific pharmacological classes.
2. To develop novel methods for reducing confounding by concomitant medications and demographic factors based on spontaneous reporting systems (SRSs).
3. To evaluate the errors in medication, use and associated potential harm.
4. To identify unanswered questions that are important to improve drug treatment outcomes that can usefully be addressed by systematic reviews/meta-analyses.
5. To conduct a medical chart review in order to assess treatment outcomes.
6. To identify clinically relevant information regarding the safety of drugs by using pharmacovigilance databases.

To fulfil points **1)** and **2)**, the studies entitled "Association of Hyponatraemia and Antidepressant Drugs: A Pharmacovigilance–Pharmacodynamic Assessment Through an Analysis of the US Food and Drug Administration Adverse Event Reporting System (FAERS) Database" [54], "Hyponatraemia following antipsychotic treatment: In-silico pharmacodynamics analysis of spontaneous reports from the US Food and Drug Administration Adverse Event Reporting System Database and an Updated Systematic Review", and "Bullous pemphigoid induced by dipeptidyl peptidase-4 (DPP-4) inhibitors: a pharmacovigilance-pharmacodynamic/pharmacokinetic assessment through an analysis of the VigiBase®" [55] were conducted; to fulfil point **3)**, the study entitled "Prevention of medication errors at hospital admission: a single-centre experience in elderly admitted to internal medicine" [56] and "A characterization and disproportionality analysis of medication error related adverse events reported to the FAERS database" [57] was conducted; to fulfil point **4)**, the study entitled "Changes in Anthropometric Parameters after

Anti-TNF-alpha Therapy in Inflammatory Bowel Disease: A Systematic Review and Meta-Analyses" [58]; to fulfil point **5)**, the study entitled "Anthropometric changes following Anti-TNF-alpha treatment in children with Inflammatory Bowel Disease: a retrospective case series analysis with new insights from the Real-World pharmacovigilance study using the FDA Adverse Event Reporting System (FAERS) database" was conducted. Several other studies were done to fulfil point **6)** as that were based on different SRS databases.

1.4.3. Outline of this thesis

The work described in this thesis is based on various data sources both Global, European and US-based, mostly SRSs but also in-patient electronic health records, as presented in **Table 1.1**. Though several studies were conducted under proposed framework, however, we focus here on a subset of that content which mostly related to safety issues of drugs. The interested reader is referred to the studies listed in doctoral activity report.

Table 1.1: Summary of research objectives and corresponding data sources used in this thesis

Chapter	Specific research objective	Data-source	Source Type	Setting
Chapter 3: Study I	To investigate the putative relationship between different antidepressant pharmacological targets and the risks of hyponatraemia induced by antidepressant drugs using the 'pharmacovigilance–pharmacodynamic' method. Leveraging information on concomitant medications and demographic factors for reducing confounding by concomitant medications and demographic factors based on SRSs.	FAERS	Spontaneous reporting database	USA
Chapter 4: Study II	Hyponatraemia following antipsychotic treatment: In-silico pharmacodynamics analysis of spontaneous reports from the US Food and Drug Administration Adverse Event Reporting System Database and an Updated Systematic Review	FAERS/ RWD from observational studies	Spontaneous reporting database/Literature	USA/others
Chapter 5: Study III	To examine the potential role of pharmacological (pharmacodynamic/pharmacokinetic parameters) characteristics of different gliptins in the occurrence of BP risk as a result of exposure to DPP-4 inhibitors.	VigiBase®	Spontaneous reporting database	Worldwide
Chapter 6: Study IV	To investigate the anthropometric and glucometabolic outcomes following anti-TNF-α drugs in paediatrics with Inflammatory Bowel Disease.	In patients Electronic medical record/FAERS	RWD/Spontaneous reporting database	Tertiary-care institution (data from KSA)/World wide
Chapter 7 Study V	To evaluate and appraise the evidence on anthropometric changes following anti-TNF-α treatment: Systematic review and Meta-analysis.	Literature	Observational studies	

CHAPTER 2: BACKGROUND

CHAPTER 2 BACKGROUND

In this chapter, we provide an overview of the key data sources from the real-world. Methods that are state of the art centering on these data. We also review relevant work concerning synthesizing evidence from multiple data sources and describe related techniques used for conducting studies discussed in this thesis.

2.1. REAL-WORLD DATA

Definition of RWD has not achieved a wide consensus [59]. As an umbrella term, RWE comes from a spectrum of studies that apply various epidemiological methods to data collected from real-world settings [60].

2.1.1. Potential sources of Real-World Data

RWD can be derived from a wide range of sources, such as routine healthcare (e.g., electronic medical records), traditional epidemiological studies (e.g., classical cohort studies), surveillance (e.g., spontaneous adverse drug events monitoring), administrative databases (e.g., death registers, medical claims), or personal devices (e.g., regular blood pressure measured with mobile devices). Study designs are generally classified into three categories: (i) pragmatic clinical trials, which may or may not be randomised; (ii) observational studies involving the prospective collection of data; and (iii) observational studies using retrospective administrative databases. RWD originate from a variety of sources that are summarized in **Table 2.1** and only relevant data sources that are used in the work described in this thesis has been discussed here.

2.1.1.1. Electronic Medical Record Databases

The primary function of electronic medical records (EMRs) systems is to capture clinical information generated from patient-provider interactions.

Traditionally, EMRs were developed and used by localized health systems. More recently, it is common for providers and health systems to license EMRs systems [61]. This has allowed EMRs vendors to collect de-identified clinical data from users and consolidate the data into large research databases. Researchers now recognize the wealth of the real-world clinical information in EMRs data sets and are using them to study clinical outcomes in a variety of diseases. The distinguishing value of EMRs as a data source is that they are clinically rich and include patient-level information on features such as height, weight, chief complaint/diagnosis, vital signs, prescription orders, medication history, and laboratory test results [62]. These well-defined data elements are generally captured as structured data or information that is codified in a discrete field. This allows records to be queried and sorted on these data fields. Additional clinical detail, such as patient history, disease status, and treatment rationale is captured in unstructured text fields, which are considerably more difficult to query.

In addition, EMR systems capture data at the point of care and EMR research data sets support the examination of treatment patterns and outcomes across time. Thus, EMR data can be used to assess longitudinal patient outcomes, which is particularly useful when studying chronic diseases. Additionally, EMRs capture data on all patients seen by providers or clinics regardless of the method of payment, which may increase the generalizability of study findings. EMR database is cost-effective as the data already exist in charts/computer systems (economy of data collection). EMR Data reflect real-world patterns of care, not affected by the study protocol and data mining approaches can uncover key relationships, not on the clinical radar.

Table 2.1: Summary of real-world data sources

RWD Source	Data Description	Strengths	Limitations
Interventional			
Pragmatic clinical trials	measure effectiveness-the benefit the treatment produces in routine clinical practice.	Enhanced external validity Assess effectiveness Ability to enrol socially disadvantaged populations Relevant patient-centred outcomes Directed towards decision-making	Lack of experience and training Selection and ascertainment of outcomes Achieving large separation between comparison groups Informed consent and regulatory oversight
Observational			
Disease registries	Repository or predefined clinical, demographic, and disease characteristics based on a particular disease or on patients' use of a specific product or device.	uniformly collected and defined patient characteristics and clinical outcomes Often contain relatively long follow-up on patients May have elements tailored to research objectives at study initiation Enhance understanding of the natural history of diseases, comparative effectiveness, and quality of life Uncover adverse events in understudied patient populations	Missing data is common Represent biased sample of enrolled patients costly to maintain Lack of standardized therapy among enrolled patients Lack of uniform assessment for response and progression
Patient surveys	Designed to collect descriptions of health status and well-being, health care utilization, treatment patterns, and health care expenditures from patients, providers, or individuals in the general population	Provide unique contributions about the generalizability of treatments and their impacts, and about use of and expenditures for health services Methodologically rigorous, relying on complex sample survey designs	Lacking relevant data on specific products Data subject to issues of subjectivity and recall bias
Administrative claims databases	Retrospective, longitudinal, and cross-sectional analyses of clinical and economic outcomes at the patient level. Claims data are collected	Large size of databases allows for identification of outcomes of patients with rare events Analyses can be performed at low cost and over a short time	Nonrandomized design Potential for reduced data quality (missing data, data entry/coding

	primarily for reimbursement, but databases may also contain some clinical diagnosis/procedure information and details on related resource use and costs	frame	errors) Limited comprehensive clinical data across health care settings Lack of distinction between costs and charges
Patient's medical record (electronic health record [EHR] databases),	Used for medical chart reviews to produce specific information on the real-world use of specific tests or medications for particular conditions	Important sources for RWD from a wide range of clinical settings throughout the world Expansion of electronic data capture is lowering the cost of the medical chart reviews May contain detailed, longitudinal information, including patient-level, disease-specific symptoms	High-end statistical analysis tools required to transform the information for research purposes
Encounter Databases	Encounter databases contain electronic records of healthcare encounters for large, defined populations. They capture information on patient characteristics, prescription fills, and medical services, as part of the routine administration or reimbursement of healthcare. When combined, these data can be used to infer a longitudinal picture of a person's medical and treatment history.	largest available population-based healthcare databases. rapid and cost-efficient assembly of extremely large cohorts of patients and provide data on drug exposures, health outcomes, and potential confounding factors. support the full range of epidemiologic study designs facilitate systematic or ad hoc linkage to nonencounter data resources, including disease registries, laboratory results, or patient and provider surveys	uncertain validity of diagnostic information lack clinical detail such as markers of disease severity and lifestyle factors (tobacco and alcohol use, physical activity). in certain situations, medication dispensing information may not available for specific drugs or drug classes.
Spontaneous adverse drug events databases	Repositories for spontaneous reports of ADEs made to regulatory agencies by health care professionals (HCPs), consumers, medical product companies, and other sources.	allows signal detection, the further exploration of drug safety hypotheses, and appropriate regulatory decision making and action when necessary a well-designed and effectively utilized postmarketing pharmacovigilance reporting system is that, in certain cases, the relationship of a drug to	heavily depends on the quality of reports Underreporting Nonuniform Temporal Trends in Reporting

		an AE/ADR can be established with sufficient confidence allow for direct engagement of healthcare professionals in the drug safety monitoring system The system can cover all medicines used in the population, and it can receive reports of AEs/ADRs occurring in any member of the population	
Medical Literature			
	Information published in clinical studies, observational studies, case reports, and other articles	qualitative, as well as quantitative, analyses of published studies is possible to generate evidence or assess comparative effectiveness Identify drug–ADE signals Predict drug–ADE associations based on chemical structure	Inherent biases in the component studies and the great diversity in study designs and populations Publication Bias Combinability of Studies is often difficult because Different studies will look at different outcomes, with different treatments in different patients.
Social media			
	range of computer-based technologies that allow the creation and sharing of information, ideas, photographs, and other messages via electronic communication	a potential source of patient-and consumer-generated information about adverse events Wide coverage	underreporting, duplicate reports, lack of relevant details, and stimulated reporting. lack of structure of social media posts, informal nature of writing, the use of "street names" for established pharmaceuticals.
Information summarized from ref [42, 63-65]			

There are also several limitations of EMR data sets in regards to supporting outcomes research. First, EMR data sets may provide an incomplete and fractured medical history for any given patient. This is due to the fact that EMR systems and, therefore, EMR data sets may be limited to a specific provider or health system and other providers may use different EMRs [66]. Furthermore, EMR systems may be developed to support care in a specific setting, such as ambulatory care clinics or hospitals. Although providers may document other significant encounters in a patient note if reported by either the patient or other health care providers (HCPs), clinical detail will likely be lacking. Another limitation of EMR databases is that they contain information on physician orders, but may not have data on whether or not the order was carried out. For instance, an EMR captures prescription orders but generally does not have data on medication fills. Additionally, patients may be "lost to follow-up" in an EMR system for any number of reasons, including death, moved to a new location, or switched to a new Hcp. These may also lead to an incomplete medical history and make it difficult to study longer-term outcomes, such as mortality [67]. Finally, as mentioned above, important clinical detail may be captured as unstructured data and is not easily identified for research. Capturing information from unstructured data is complex and requires efforts such as manual chart reviews.

2.1.1.2. Spontaneous reporting system

SRS databases are repositories for spontaneous reports of ADEs made to regulatory agencies by HCPs, consumers, medical product companies, and other sources [68]. These reports are comprised of RWD about suspected safety issues regarding drugs [69]. SRS reports generally include information concerning the patient, drug, event, and concomitant drugs that may have caused or contributed to the ADE. Therefore, the analysis of individual or a case series of SRS reports may be an important source of information to identify potential safety concerns. Most of the case reports collected by the SRS centres are either required to be submitted by pharmaceutical companies or are voluntarily reported by healthcare professionals and consumers. The

FAERS and Vaccine Adverse Event Reporting System (VAERS) databases, the European Medicines Agency's (EMA) EudraVigilance, and WHO's VigiBase® are among the largest SRS databases worldwide (see **Table 2.2**).

Spontaneous reports that the FDA receives regarding drug ADEs are entered into the FAERS. The FAERS receives approximately 1.5 million ADEs, product complaints, and user error reports each year from HCPs, consumers, companies, and other sources, concerning drugs, vaccines, and medical devices for human use [50].

The number of safety reports made to the FDA annually is continuously expanding due to increases in the type and number of products the agency regulates, awareness of the importance of these reports, ease of submitting reports (i.e., digitally), and a larger population [70].

VigiBase® is the largest, with more than 18 million reports from 156 countries over the five continents [71]. Relevant National Pharmacovigilance Centers are responsible for collecting, processing, and evaluating the spontaneous ADR reports prior to their submission and registration in VigiBase®.

EudraVigilance database, which is developed and maintained by the EMA to collect reports of suspected ADRs. It contains adverse events reported in relation to any drug authorised or being studied in the European economic area. EudraVigilance contains spontaneous reports from healthcare professionals, patients and marketing authorisation holders and clinical trial safety data dating back to 1995. The number of new reports in this database increases each year, with more than 2 million ADRs report collected in 2018 [72].

Table 2.2: Major Spontaneous Reporting System Databases					
Name	**Region**	**Catchment Period**	**Total^ Number of Reports**	**Report Sources**	**Report Content**
FDA Adverse Event Reporting System (FAERS)	Mostly United States	1969–present	> 104,000,000	HCPs, pharmaceutical companies, patients, consumers	Mandatory post-marketing ADE reports submitted by pharmaceutical companies Voluntary ADE reports from HCPs and the public (via MedWatch)
FDA Vaccine Adverse Event Reporting System (VAERS)	United States	1990–present	> 740,000	HCPs, pharmaceutical companies, patients, consumers	Reports of ADEs concerning vaccines
European Medicines Agency Eudravigilance	European Union, Worldwide #	2001–present	> 2,400,000	Marketing authorization holders	Reports of suspected ADEs concerning medical products
World Health Organization	Worldwide *	1968–present	> 19,000,000	National pharmacovigilance centres	Reports of suspected ADEs concerning medical products Reports of ADEs from studies or other social monitoring situations

^ as of 20th November 2019
* Although more than 150 member countries participate, the majority of reports come from the U.S. and European Union.
# Under the condition that the drug associated with the ADR has marketing authorisation by EMA
Information summarized from references [35, 69-73]

Although each database is dedicated to a different geographical area, there may be some duplication or overlap in the reports that they contain [69]. This is particularly true for serious or severe ADEs, which are often reported to national or regional authorities, such as FAERs or EudraVigilance, respectively, as well as to the global repository, VigiBase®.

The SRSs have both advantages and disadvantages. Their advantages include a large catchment population, low cost, and coverage of virtually all licensed drugs [74]. The most important limitations are under-, over- and duplicate reporting, missing and incomplete data, lack of denominator data and unknown causality [75]. Underreporting is one of the most notorious limitations and is very hard to overcome since nothing can be done in the absence of data. Although it is difficult to provide an accurate estimate of the level of underreporting, a review [20] has shown that it may be as large as 90%, even for serious events. Public campaigns on ADR reporting can increase the reporting rate, however this may also lead to skewed reporting and false-positive signals [76]. Sampling biases whereas all the reports are related to corresponding ADRs so that information on the number of patients who take a drug of interest but do not develop an ADR is unknown and duplicate reporting whereas multiple reports referring to the same adverse events are collected from different sources such as consumers, drug manufacturers and investigators. Ultimately, some researchers argued that spontaneous reports are flawed and we should look for better alternatives [77]. One of these alternatives is the electronic healthcare data.

2.1.1.3. Medical Literature

The medical literature is another major source of data that is expected to improve the understating of treatment-related outcomes and detection of drug–ADE associations [50, 70]. Important medical questions are typically studied more than once, often by different research teams in different locations. In many instances, the results of these multiple small studies of an issue are diverse and conflicting, which makes the clinical decision-making difficult. The need to arrive at decisions affecting clinical practise fostered the

momentum toward "evidence-based medicine". Information published in clinical studies, observational studies, case reports, and other articles can be analysed to identify pertinent information on safety, efficacy and effectiveness of drugs.

Systematic reviews (SR) and meta-analyses (MA) are the central approaches in evidence synthesis. In fact, in the hierarchy of evidence, where clinical evidence is ranked according to the strength of the freedom from various biases that beset medical research, meta-analyses are in the top. SR refers to a collection of all empirical evidence that fits pre-specified eligibility criteria to answer a specific research question. MA is the use of statistical methods to summarize and combine the results of independent studies. Since much of the general framework for conducting SR and MA are similar, and the term "SR" may be used synonymously with "MA", here and henceforth in section 2.3 I use the term SR/MA.

A SR/MA can explore the sources of heterogeneity, and identify subgroups associated with the factor of interest, potentially providing new insights for future studies. SR/MA is a more efficient and effective standard method for summarizing the results of many studies than is subjective judgment; therefore, it has become an important research strategy and is progressively expanding. MA may be a valuable tool when there is the need to explore inconsistencies across studies previously conducted, to evaluate subgroups of patients in whom 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, MA allows discussing the magnitude of the effect between the intervention being evaluated and the selected comparator [78].

Both regulatory agencies and product manufacturers routinely consult and track the medical literature to identify undetected drug–ADE associations [50]. Because the content of medical literature is peer-reviewed, it is considered to be a highly reliable source of ADE information. 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 [79]. In several occasions, the study of rare AEs relies on pooled analysis. Data mining published medical literature can also provide evidence for mechanisms of action for possible DDIs [52].

2.1.2. Choosing among the available Data-source to study the research question at hand

Once one has decided to study a research problem by using RWD, one needs to decide which of the resources described earlier in this chapter should be used. The choice may too often be based upon availability of resources, and researcher's familiarity with given data resources. Most importantly, the researcher needs to examine the characteristics of the research question at hand to determine which resources can best be used to answer it. No single resource is gold standard for the RWD, and researcher has to decide the best optimal resource for addressing a question, that indeed also depend on the nature of the research question at hand. Otherwise, the use a number of approaches that complement each other can be useful. Certainly, this is probably the preferable approach for addressing important questions.

RWD can be used to generate hypotheses about drug effects, to strengthen hypotheses, and/or to test a priori hypotheses about drug effects. Hypothesis-generating studies are designed to raise new questions about possible unexpected drug effects, whether adverse or beneficial. Hypothesis-strengthening studies are designed to provide support for existing hypotheses (with no definitive evidence), whereas hypothesis-testing studies are designed to evaluate in detail hypotheses raised elsewhere. SRSs has been the most productive source for raising new hypotheses about drug effects, however, they are not very useful for providing additional support for those hypotheses. Conversely, pragmatic trials with randomization can certainly test and strengthen hypotheses raised elsewhere but are generally too costly and logistically too complex. **Table 2.3** summarized RWD sources assessment weights for three types of studies based on the characteristics of research questions. Of note, the assessment and weights provided in this **Table 2.3** are arbitrary and not represented as an established consensus. Indeed, there are many data resources/approaches that are not described in the present

thesis. The comparative characteristics of RWD sources have been discussed in detail elsewhere [80] and the interested reader should refer to relevant reference.

Table 2.3: Comparison of selected real-word data sources depending on characteristics of research questions

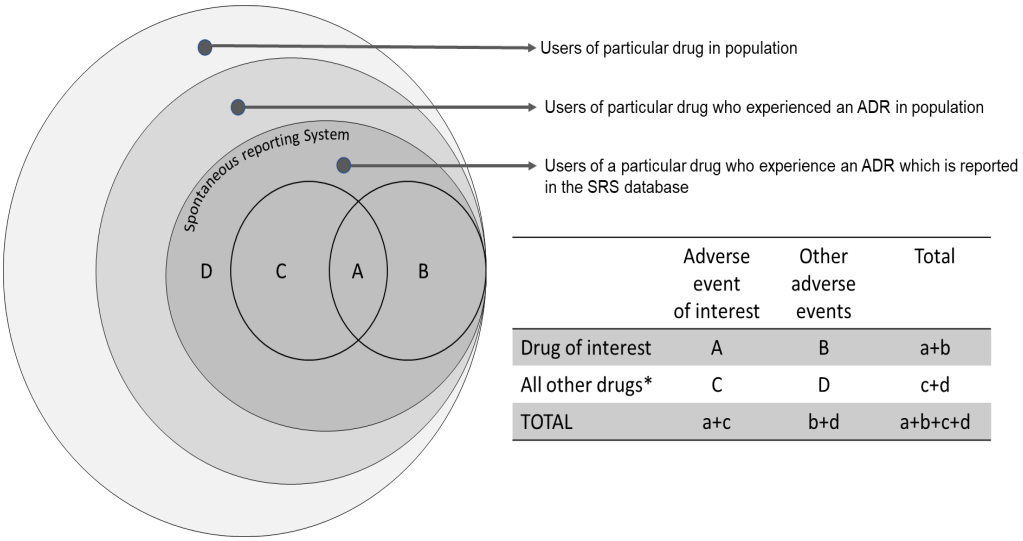
RWD Source/Approach	Hypothesis Generating	Hypothesis strengthening	Hypothesis testing	Study of benefits (versus risk)
Spontaneous reporting	++++	+	—	—
Electronic medical records	++	++++	+++	++
Disease registries	++	++++	+++	++
Patient surveys	++	++++	+++	++
Administrative claims databases	++	++++	+++	+++
Patient's medical record	+	++++	+++	++
Encounter Databases		++++	+++	++
Medical Literature	+	++	+	++
Pragmatic Trial	+	++	++++	++++

2.2. METHODS APPLIED IN SPONTANEOUS REPORTING SYSTEM DATABASES

2.2.1. Disproportionality (Case/non-case) analysis

The objective of studying the disproportionality of spontaneous reports is to generate pharmacovigilance signals concerning unknown or underestimated ADRs, as early as possible after marketing by using data mining algorithms. The fundamental feature of data mining techniques used to analyse SRS databases is that each is based on finding "disproportionalities" in data; that is, the finding that a given AE/ADR is reported for a particular drug more often than would be expected based on the number of reports of that AE/ADR for all other drugs in the database. This ratio is based on an 'artificial denominator' since in SRSs there are no untreated patients, only patients treated with other drugs which experienced a certain ADR. These patients form the denominator [74], see **Figure 2.1**.

Figure 2.1: Relationships among patients exposed to drugs, experiencing ADRs and actual reporting with 2x2 contingency table for a drug-adverse event combination, in spontaneous reporting databases



	Adverse event of interest	Other adverse events	Total
Drug of interest	A	B	a+b
All other drugs*	C	D	c+d
TOTAL	a+c	b+d	a+b+c+d

Adapted and modified from Poluzzi Elisabetta ER. Data Mining Techniques in Pharmacovigilance: Analysis of the Publicly Accessible FDA Adverse Event Reporting System (AERS), PhD thesis. 2012.

The most common disproportionality methods are: proportional reporting ratio [81] and reporting odds ratio (ROR) [82]. Using the ROR allowed adjustments using multivariate logistic regression analysis and offered the advantage of controlling covariates [83]. Their estimates are easy to calculate; however, the results tend to become unstable when the number of events is small, resulting in potentially high estimates with wide confidence intervals. This instability led to the development of more advanced detection techniques based on Bayesian statistics. The Bayesian techniques try to adjust for uncertainty in the data by shrinking the estimates depending on the amount of data available. The commonly used Bayesian methods are the Multi-item Gamma Poisson Shrinker (MGPS) [84] and the Bayesian Confidence Propagation Neural Network (BCPNN) [85].

Another serious concern is due to potential interactions between several drugs taken simultaneously in relation to the occurrence of an ADR. The techniques discussed so far assess the risk of 2-way drug-event combinations, i.e., one drug and one ADR. DuMouchel and Pregibon introduced the 3-dimensional MGPS to deal with multi-item sets of a size n > 2 (e.g., n= 3; drug-drug-event interactions) [86]. An overview of the most commonly used methods, their computation, advantages and limitations are presented in **Table 2.4**.
All the data mining algorithm discussed above, however, measure lower order associations without considering the effect of confounding factors. A confounder is an extraneous variable, either observed or unobserved, that mediates an association between two other variables. Such confounder could be patient demographics, an indication of a drug, concomitant medications, and weber effect. For example, alcoholism is a confounder that could lead to a suspicious relationship between the medication Naltrexone and pancreatitis because Naltrexone treats alcoholism, which often leads to pancreatitis. If not properly accounted for, confounding may lead to the discovery of suspicious associations and therefore erroneous study conclusions. This is referred to as indication bias or co-prescription bias.

2.2.2. Logistic regression to leverage information of possible confounders in SRS

Unlike RCTs, SRS and observational healthcare databases where data have already been collected, characteristics of patients in exposure or unexposed group could not be balanced through randomization, confounding should be addressed in the analysis stage.

Table 2.4: Summary of major DMAs used for signal detection						
Name	**Point estimate**	**Confidence interval**	**Published threshold criteria**	**Institutions which use it**	**Advantage**	**Limitations**
Frequentist methods						
Reporting Odds Ratio (ROR) [82]	$\frac{ad}{bc}$	$95\%CI = e^{ln(ROR)\pm 1.96\sqrt{(\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d})}}$	95%CI> 1, N≥2	LAREB (Netherlands)	Easy to apply and Interpret More sensitive as compared to Bayesian Method Allows adjustment for covariates in logistic regression analysis	Higher rate of false positives Unreliable at low numbers
Proportional Reporting Ratio (PRR) [81]	$\frac{a/(a+b)}{c/(c+d)}$	$95\%CI = e^{ln(ROR)\pm 1.96\sqrt{(\frac{1}{a}+\frac{1}{a+b}+\frac{1}{c}+\frac{1}{c+d})}}$	PRR≥2 χ^2≥4 N≥3	EMA (Eudravigilance), Italian Regulatory Agency (AIFA)		
Bayesian methods						
Bayesian Confidence Propagation Neural Network (BCPN) [85]	$log_2 \frac{a(a+b+c+d)}{(a+c)(a+d)}$	–	$IC_{0.25}$ SD>0	UMC (WHO VigiBase®)	Higher specificity as compared to frequentist method* Can be used for pattern recognition in a higher dimension	Relatively non-transparent for users not familiar with Bayesian statistics Lower sensitivity
Multi-item Gamma Poisson Shrinker (MGPS) [84]	$\frac{a(a+bc+d)}{(a+c)(a+d)}$*	–	EBGM05 > 2 N>0	FDA MHRA		
3-dimensional (3D) EBGM [86]	–*	–	interaction signal score > 1		Allows to identify multi-item sets i.e. drug-drug-event interactions).	
** Complex formulae, not entirely reproduced above., N denotes number of cases or reports*						

Solutions for confounding generally include subgrouping, stratification or adjustment. Stratification is a standard procedure to alleviate confounding effects but it is not effective in situations where a large number of potential confounders need to be examined. A more appropriate approach to handling confounding is by the use of multiple logistic regression. However, it has also been suggested that subgroup analyses (in which a signal is identified if it is present in any of the subgroup strata) tend to perform better than stratified/adjusted analyses [87].

Several studies, mostly on VigiBase®, used logistic regression to adjust for potential confounders. Although most of studies except by Mannesse et al. [88], were designed as a nested case-control, i.e., these investigators adjusted potential confounders in base cohort consisted of all cases of ADRs associated with the use of pharmacological class of interest. For example, Willemen et al. investigate the association of gliptins and the reporting of infections with all potential confounders among antidiabetic drug user [89]. Similarly, Suzuki et al studied the Interaction between clopidogrel, aspirin, and proton pump inhibitors in FAERS, in which they refined the signal by using logistic regression to adjust for possible demographic confounders as well as two way and three-way interactions between pertinent drugs [83]. Mannesse et. al. studied the association of antipsychotic associated hyponatremia in VigiBase® while adjusting for age, sex and concomitant medications associated with hyponatraemia in the whole database [88]. Most researcher tends to adjust confounders in SRSs in a selected cohort, rather than the complete database as it seems practically easier.

2.2.3. In Silico approaches to identify the biological basis for signals

Data-mining methods can help provide an understanding of the biological basis for signals by incorporating reference databases regarding drug chemistry and physiology. Curated knowledge databases can link ADEs with the chemical properties of drugs and their effects on physiological pathways and organ systems, helping to identify the mechanisms by which ADEs develop [50]. Such systematic approaches can be used to investigate the biochemistry and pharmacogenetics of drugs and how drug–receptor

interactions lead to ADEs and DDIs [50]. This may provide a more advanced approach and potentially deeper understanding than traditional drug safety surveillance systems that are based on a drug's basic targets and chemical structure [90]. In addition, such systematic approaches to drug safety surveillance can be predictive, offering the potential to identify potential ADEs before they are observed. Using the SRSs data source, an original and attractive way to illustrate this continuous approach is to combine pharmacodynamics and pharmacovigilance and/or pharmacoepidemiologic data.

One of the first applications of the combination of pharmacodynamics data and disproportionality analysis in the WHO international pharmacovigilance database was performed by De Bruin et al.in 2005 [91]. Coupling disproportionality analyses in pharmacovigilance databases or computerized health databases, with pharmacological characteristics of drugs (receptor affinity, for example) allows investigating in humans, the mechanism of ADRs. Examples of such analyses include investigating the risk of movement disorders, diabetes-related to psychoactive drugs [92], or the risk of adverse cardiac outcomes with different drugs (classical drugs or protein kinase inhibitors) [91]. The increasing number of research works investigating this topic underlines the importance of this relatively new approach, which gives significant inputs for better knowledge of drug safety. Studies using this approach are summarized in **Table 2.5**.

Table 2.5: Summary of studies used pharmacodynamic data with drug safety signals

First author, ref no.	Pharmacological Class	Pharmacodynamic target/ parameter	Adverse Event	Data source
De Bruin et al. [93]	Any drug associated with the studied event of interest	Anti-HERG activity expressed as ETCP unbound/IC50	Drug-induced arrhythmias and sudden death	VigiBase®
Patras et al [94]	Protein kinase inhibitors	Binding affinity towards 21 selected protein kinases.	Cardiac failure	VigiBase®
Nguyen et al [92]	Antipsychotic	Dopamine (D_2), serotonin $5HT_2A$, and muscarinic M_1 receptor occupancy expressed as percentage	Movement disorders (extrapyramidal symptoms)	VigiBase®
Siafis et al [95]	Antidepressants	occupancy on serotonin, alpha adrenoreceptors, dopamine, muscarinic, histamine receptors and SERT, NET, DAT transporters, expressed as percentage	Diabetes Mellitus	VigiBase®
Cornet et al [96]	Protein kinase inhibitors	Binding affinity towards selected 35 protein kinases	Pulmonary arterial hypertension	VigiBase®
Nguyen et al [97]	Antidepressant	binding affinities for nine targets (serotonin, norepinephrine, dopamine transporters, 5-HT2C serotonin, D2 dopamine, α1, α2 adrenergic, M3 muscarinic and H1 histamine receptors).	Diabetes Mellitus	VigiBase®
Montastruc et al [98]	Antipsychotic	Serotonin $5\text{-}HT_1A$, $5\text{-}HT_2A$, $5\text{-}HT_2C$, histamine H_1, muscarinic M_3, adrenergic α_1, α_2 or dopaminergic D_2 D_3 occupancies	Diabetes Mellitus	VigiBase®

2.3. SYSTEMATIC REVIEWS/META-ANALYSES

Systematic reviews/Meta-analyses in medicine have been developed mainly for combination of data from randomized trials, however, in the present work we also include evidence from observational studies as some question of interest cannot be answered by RCTs, and review authors may be justified in including non-randomized studies. In fact, Cochrane Collaboration, global independent network of researchers, has been realized that observational studies may make an important contribution about the harms and benefits of health interventions [99]. The current version of the Cochrane Handbook [100] has chapters on "Including nonrandomized studies" and "AEs".

There are a number of reasons why a clinical pharmacologist might be interested in conducting a SR/MA such as investigation of AEs, study new indication of existing therapies, explore differentiating effect of among subgroups of patients, detect early harm signals, indirect comparison of several treatments and simultaneous evaluation of several treatment options/ interventions for the same indication. In **Table 2.6**, I summarized the general principles of SR/MA and a framework for the methods typically employed in a SR/MA. The explanation of the methods typically employed in a SR/MA and methodological problems encountered in the conduct of SR/MA have been presented in review articles in major clinical journals and I made no attempt to include them all [101-103].

The Council for International Organizations of Medical Sciences (CIOMS) X report suggests a meta-analysis protocol should include but is not limited to, content implied by a series of topic headings [104]. That report elaborates on the content in the context of planning the SR/MA. The reader is referred to the CIOMS publication for more detail. I focus here on a subset and tried to provide succinct descriptions of that content.

Table 2.6: Steps involved in conducting a systematic review/meta-analysis	
STEP	***PRACTICAL CONSIDERATIONS***
Define the Purpose Define precisely the primary and secondary objectives	A well-formulated question should have a clearly defined PICOTS [patient, intervention, comparison, outcome, timing and study design].
Perform literature search Retrieve all relevant published studies by computerized searches of the literature	Ideal search strategy should be highly sensitive search strategy by excluding publication types that are almost certain not to provide primary data, such as commentaries, editorials, SR/MA, reviews, or practice guidelines may decrease the number of nonrelevant citations. Review of the reference sections of retrieved publications found to be relevant, and manual searches of relevant journals, are also recommended
Establish Inclusion/Exclusion Criteria A set of rules for including and excluding studies should be defined during the planning stage of the SR/MA and should be based on the specific hypotheses being tested in the analysis.	Defining the question being addressed by the SR/MA is key to in establishing inclusion/exclusion criteria. If broad inclusion criteria are established, then a broad, and perhaps more generalizable, hypothesis may be tested. Exclusion criteria should be based on a priori considerations of design of the original studies and completeness of the reports and, specifically, should not be based on the results of the studies. Prespecification of the protocol and adherence to it (or at least a clear justification for departures) provides some limited protection against bias related to this step. There are registries of protocols for observational studies and one that is specifically for SR is "PROSPERO" (International Prospective Register Of systematic reviews) (www.crd.york.ac.uk/prospero/). Studies may often generate more than one published paper that might report on outcomes not addressed in earlier papers, this may lead to duplication. Therefore, contacting the authors of primary studies may be of some help in determining if there is duplication in order to maintain statistical validity of the review as validity of the statistical methods depends on the assumption that the different studies represent different groups of individuals.
Collect the Data Important information regarding study design and outcome needs to be extracted	Typically, data abstraction forms are developed, pilot-tested on a few articles and revised as needed. Careful specification in the protocol for the SR/MA of the design features and patient characteristics that will be of clinical or academic interest may help avoid over-or under collecting information. Each included study should be assessed for the risk of bias that likely results and hence that of the SR/MA. The

	Cochrane Collaboration recommends the use of the "risk of bias". Studies should be read independently by two readers and subsequently assess for risk of bias. Judge whether sufficient quantitative data is available to combine overall results and whether there is heterogeneity problem in combining results? This is crucial step to decide if it is possible to conduct MA or only a SR.
Perform Statistical Analyses Summary statistics from each study are combined to yield an overall result by using an appropriate statistical measure	Three summary measures of effect size that can be used in MA when the outcome of interest is binary (e.g., proportion of subjects with pain relief): relative risk, odds ratio, and risk difference. A summary measure used does not affect the statistical significance of the results, the choice of effect measure could affect the transferability of results of the MA into clinical practice. Which summary measure to select depends on the ease of interpretation, the mathematical properties, and the consistency of the results when the particular effect measure is used.

Information summarized from ref [101-104]

CHAPTER 3: STUDY 1

CHAPTER 3: STUDY I

Association of Hyponatraemia and antidepressant drugs: A Pharmacovigilance-Pharmacodynamic Assessment through an analysis of the US Food and Drug Administration Adverse Event Reporting System (FAERS) Database

ABSTRACT

Background: Hyponatraemia induced by antidepressant drugs is a rare but potentially life-threatening adverse reaction. Whether it is associated with all or only some antidepressant drugs is still unclear. This needs to be clarified to guide antidepressant therapies, especially in patients with electrolytic imbalances.

Objectives: The primary objective was to quantify the strength of association between the use of different antidepressant drugs and hyponatraemia by using information reported to the US Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS). The secondary objective was to investigate the putative relationship between different antidepressant pharmacological targets and the risks of hyponatraemia induced by antidepressant drugs using the 'pharmacovigilance -pharmacodynamic' (PV-PD) method.

Methods: We used the FAERS database to conduct a case/non-case analysis on spontaneous reports, focusing on events of hyponatraemia/syndrome of inappropriate antidiuretic hormone secretion (SIADH) reported in connection with the use of antidepressants. Risk was expressed as a measure of disproportionality using the reporting odds ratio (ROR) while adjusting for sex, age, and concomitant medications associated with hyponatraemia/SIADH. We assessed to what extent the receptor binding properties of antidepressants could associate to the RORs of hyponatraemia/SIADH of antidepressants, building a linear regression model that included as independent variables the binding affinities (pKi) to the serotonin transporter (SERT), dopamine transporter, norepinephrine transporter, and serotonin 5-HT_{2C}, 5-HT_{2A}, 5-HT_{1A}, α_1 and α_2 adrenergic receptors.

Results: 2,233 reports were identified. The adjusted ROR for the association between antidepressant drug use and hyponatraemia was 1.91 [95% confidence interval: 1.83, 2.00]. The association was strongest for mirtazapine, followed by selective serotonin reuptake inhibitors (SSRIs), and lowest with serotonin modulating antidepressant drugs. A significant linear correlation was found between the adjusted RORs for hyponatraemia and pKi for the adrenergic receptors α_1 and α_2.

Conclusions: Hyponatraemia is reported at a disproportionately higher level with classes of antidepressant drugs [noradrenergic and specific serotonergic antidepressant (mirtazapine) and serotonin modulators (vortioxetine)] that are in general considered to have a better profile of tolerability in terms of hyponatraemia. In the light of the results herein presented, the risk of hyponatraemia with mirtazapine appears to be greater than what was reported in the literature, however, confounding by indication cannot be ruled out. Our PV-PD analysis indicates also that inhibition of SERT may not be involved in the hyponatraemia linked to the use of antidepressant drugs.

3.1. INTRODUCTION

Hyponatraemia/syndrome of inappropriate antidiuretic hormone secretion (SIADH) induced by antidepressant drugs is a rare but under-diagnosed and potentially life-threatening adverse reaction. Antidepressant-induced hyponatraemia may be asymptomatic or may result in polydipsia, weakness, lethargy, weight gain, headache and anorexia as the most common early symptoms; [105] it can develop very slowly, to the point that symptoms are never noticed as ADRs, especially in the case of long-term psychiatric rehabilitation therapies. Rarely, it may have serious consequences, with seizures, delirium or even death [106]. The plausible relationship between antidepressant drug use and hyponatraemia was first described in 1974, with amitriptyline, and has been increasingly debated following the introduction of selective serotonin reuptake inhibitors (SSRIs) [107].

Over the past two decades, numerous newer antidepressant drugs have been added to the market. The so-called second-generation antidepressant drugs include SSRIs, serotonin-norepinephrine reuptake inhibitors (SNRIs), noradrenergic and specific serotonergic antidepressant drugs, and drugs with mixed serotonergic effects. They have different pharmacological mechanisms, fewer side effects, and are better tolerated than tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) [108].

The relationship between hyponatraemia and antidepressant drugs has been examined in multiple population-based studies over recent years [109-113]. Most studies have suggested that SSRIs [odds ratio (OR) 1.5–21.6] have a higher risk than TCAs (OR 1.1–4.9) [107]. A head-to-head comparison of these two pharmacological classes of antidepressant drugs in a prospective cohort study confirmed this, by reporting a significantly ($p = 0.002$) lower hazard ratio for TCAs than for SSRIs (hazard ratio 1.44) [109]. Among SSRIs, escitalopram and citalopram were consistently noted to be associated with higher incidences than other SSRIs [110, 113]. Recently, hyponatraemia has also been reported for vortioxetine, a new multimodal antidepressant that has been considered to be a safe treatment for depression [114]. Despite this information, a firm conclusion regarding the frequency of hyponatraemia with

different antidepressant classes or agents has not been drawn to date [109, 111, 113]. This is attributed to clinical heterogeneity and differences in study designs and sodium cut-off values; it is also unclear if all antidepressant drugs pose an equal risk of hyponatraemia.

Better awareness and knowledge of the association of hyponatraemia with specific classes of antidepressant drugs could promote safer treatments for at-risk patients, such as those with a history of hyponatraemia/SIADH or those presenting with the first signs of hyponatraemia associated with any given antidepressant drugs. However, in light of the inconsistencies within the literature, most antidepressant drugs are associated with hyponatraemia [115], and it has not yet been established whether hyponatraemia is truly a "class-specific adverse effect".

To provide information that is more conclusive, we conducted a case/non-case analysis; we used the US FAERS database as it allows for the quantification of the association between drugs and reporting of adverse events, in our case, different antidepressant drugs and the occurrence of hyponatraemia. Further, to clarify whether the tendency for hyponatraemia induced by antidepressant drugs is driven by their receptor-binding characteristics, we applied a combined 'pharmacovigilance–pharmacodynamic' (PV–PD) analysis [116]. This method has been previously used to study the pharmacological mechanisms of ADRs to antipsychotics [116] and antidepressant drugs [117]; it can be used to establish a correlation between the binding affinities of drugs for their pharmacological targets and their corresponding reporting risks for ADRs observed in a large pharmacovigilance database.

3.2. METHODS

3.2.1. Case/Non-Case Analysis (Pharmacovigilance Study)

3.2.1.1. Data source, Data acquisition and Data processing

Adverse event reports from the FAERS database were used for this study. We performed FAERS data extraction, data acquisition and processing as previously described [57]. In brief, all the zipped JSON (JavaScript Object Notation) endpoints for drug events from the first quarter (Q1) of 2004

(representing the beginning of freely available FAERS data through to the fourth quarter (Q2) of 2018 (last accessed 30 June, 2018) were downloaded from the openFDA download webpage [118]. A local server was set up as described on the openFDA Github developer platform [119], containing all downloaded JSON endpoints for the openFDA application program interface. Adverse events were queried using fields specific to the "drug/event.json" endpoint following openFDA instructions. Multiple drug name variants were standardised to make the drug name consistent with the international non-proprietary nomenclature defined by the World Health Organization (WHO) Anatomical Therapeutic Chemical classification. Before analysis, data were further scrutinised manually based on similarities in patients, ADRs and medicinal product data; duplicate records were detected and deleted accordingly.

3.2.1.2. Selection of cases and non-cases

Cases were defined as all Individual Case Safety Reports (ICSRs) where at least one MedDRA® lower-level term from the Standardized MedDRA® query (SMQ) for "hyponatraemia/SIADH (narrow)" (released in March 2014 with MedDRA® version 15.0) [120] has been coded in the adverse reaction section (outcome of interest). We included all preferred terms specifically related to hyponatraemia, SIADH, and increased or decreased serum sodium or antidiuretic hormone concentrations.

Non-cases (controls) were all other ADRs reported in the database during the same period of time (i.e., all ADR reports without the outcome of interest). Cases and non-cases with no information on age or sex were excluded. This was done as the final step in the preparation of the dataset.

3.2.1.3. Exposure Definition

Exposure group was defined as all the reports in which the reporter designated antidepressant drug(s) (classified according to Anatomical Therapeutic Chemical classification second-level code beginning N06A) as suspect (primary or secondary) in the development of hyponatraemia and related events. Antidepressant drugs mentioned more than five times were classified

by using the pharmacological classification of antidepressant drugs [121, 122].

3.2.1.4. Co-variates

Concomitant medication associated with hyponatraemia, sex [106, 123-125] and age [126, 127] were considered as potential covariates (confounders). Drug classes/drugs associated with hyponatraemia were identified as potential confounders through extensive searches of the literature [128-130]. Concomitant medications belonging to these classes (**Table 3.1**) were not suspected by the reporter as a plausible cause of the hyponatraemia.

3.2.2. Pharmacodynamic Data

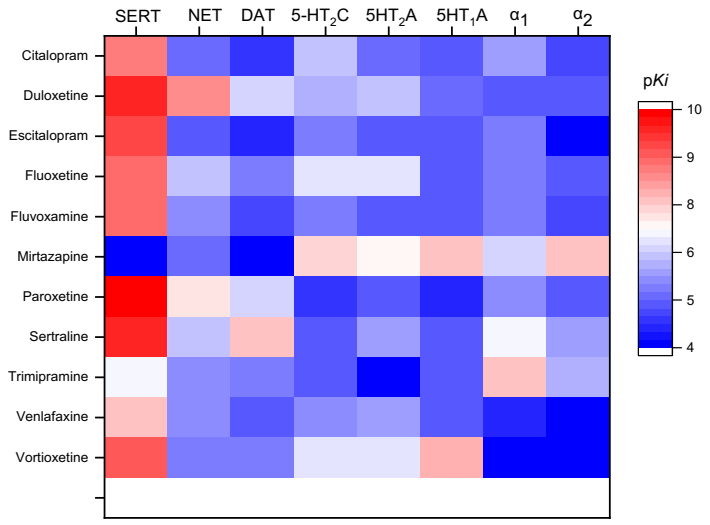
For each antidepressant drug of interest, values of binding affinities (Ki) at three transporters (serotonin transporter [SERT], dopamine transporter, norepinephrine transporter), and five receptors (5-HT_{2C}, $5HT_{2A}$, $5HT_{1A}$, α_1- and α_2-adrenergic receptors) of interest potentially involved in hyponatraemia induced by psychotropic drugs were searched in the European Bioinformatics Institute-ChEMBL [131] International Union of Basic and Clinical Pharmacology [132], Psychoactive Drug Screening Program [133] and Drug-Bank databases [134]. The Ki for a given ligand is determined in a competitive radioligand binding study under equilibrium conditions [134].

Table 3.1: Drugs associated with hyponatremia [references from text]

Category	Associated pharmacological classes/drugs
CNS	Benzodiazepines, antipsychotics, valproate, carbamazepine, oxcarbazepine, lamotrigine, phenytoin, NSAIDs, opioids, MDMA (ecstasy), levamisole
Cardiovascular	Diuretics, beta-blockers, ACE inhibitors, nitrates, calcium channel antagonists, amiodarone, Clofibrate.
Endocrine	Chlorpropamide, sulphonylureas, biguanides, thiazolidinediones, vasopressin, desmopressin, bromocriptine, oxytocin, terlipressin
Cytotoxic drugs	Vinca alkaloids, platinum compounds, ifosfamide, melphalan, cyclophosphamide, methotrexate, pentostatin
Miscellaneous	Interferon, interlukin-2, nicotine, proton pump inhibitors, monoclonal antibodies, antimicrobials (trimethoprim-sulfamethoxazole, ciprofloxacin, cefoperazone/sulbactam, rifabutin)

The pKi values (logarithm with the base 10 of Ki) of each antidepressant drug of interest at different targets are summarised in **Figure 3.1**. Values of pKi are expressed as the geometric mean of values determined in competition binding assays, where appropriate. Where pKi values obtained from assays on human receptors were available, we considered only such data. Where pKi values regarding human receptors were not available, we considered data from mouse receptors as replacements, thus working with a mixed set of human/ mouse pKi values.

Figure 3.1: Heat map representation of receptor binding affinities for selected antidepressant drugs at different selected monoamine transporters and receptors.



Binding affinities (in pKi values; logarithm with the base 10) range from 4 (inactive, blue) to >9 (highly active, red). Binding affinity data were retrieved from the European Bioinformatics Institute-ChEMBL, International Union of Basic and Clinical Pharmacology, Psychoactive Drug Screening Program and DrugBank databases.

3.2.3. Data Analyses

For cases and non-cases, descriptive analysis was performed for antidepressant drug users, in terms of age, female sex and use of concomitant medication associated with hyponatraemia. The Student's t-test was used to assess whether age was distributed differently between cases and non-cases, whereas the Pearson's Chi-square test was used to assess whether categorical variables (sex and the presence of concomitant medications associated with hyponatraemia) were differently distributed between cases and non-cases.

To identify drug/adverse event pairs that were reported more frequently than expected, we used the reporting odds ratio (ROR) as a measure of disproportional reporting. This method allows the comparison of drug exposure (antidepressant) among cases and non-cases by calculation of the

ROR and their corresponding 95% confidence intervals (CIs). This is a validated method of safety signal detection [135] and, in the present case, it provided an estimate of the extent to which hyponatraemia was reported in association with an antidepressant drug designated as 'suspected medication' as compared with reports of hyponatraemia in association with other drugs. For each antidepressant drug, the ROR was calculated. The ROR of a drug–ADR combination was defined as the ratio between proportions of reports containing the drug of interest in the "case" (reports containing the ADR of interest; hyponatraemia) and in the "non-case" (reports containing other ADRs) group. All analyses were performed using counts of unique cases. A signal of disproportionate reporting (SDR) was defined when the lower limit of the 95% two-sided CI for the ROR exceeded the threshold value of 1 [136]. To assess the potential influence of covariates (due to concomitant medications associated with hyponatraemia, sex, and age), we further analysed the unique drug–event combination identified in the previous step using a logistic model. The ROR was adjusted for concomitant medications associated with hyponatraemia, sex (reference category: female) and age (reference category: ≥ 60 years). These reference categories were selected because increased age and female sex are considered as risk factors for the development of hyponatraemia. The crude RORs and adjusted RORs were estimated for each antidepressant drug class and for individual antidepressant drug.

Multiple linear regression models were used to investigate the association between the adjusted ROR estimates for hyponatraemia/SIADH and the receptor-binding characteristics for each individual antidepressant drug. The adjusted ROR for hyponatraemia/SIADH was treated as the dependent variable and pKi as the independent variable. A significant correlation suggests, while not proving with absolute certainty, that the binding affinity of antidepressant drugs for the SERT explains to some extent the different level of hyponatraemia/SIADH reporting (quantified as adjusted ROR). Prior to each analysis, the overall fit and accuracy of the model were ensured by residual analysis and Cook's D; outliers were identified and managed

accordingly. Linear regression analysis was conducted only on statistically significant adjusted RORs. Statistical analyses were conducted using the software package STATA® (StataCorp, College Station, TX, USA).

3.3. RESULTS

3.3.1. Case–non-case analysis

After deduplication and standardisation, we retrieved from the FAERS database between *Q1*, 2004 and *Q2*, 2018 2,784,530 ICSRs with information on age and sex. Of 2,784,530 ICSRs, 13,307 (0.50%) were related to hyponatraemia (cases). These cases were matched with 2,771,233 non-cases. Univariate analyses of demographic and drug-class exposure characteristics of cases and non- cases are presented in **Table 3.2**. Of 13,307 cases, 2233 (16.80%) mentioned an antidepressant drug as a suspected medication compared with 220,773 (8.00%) among the non- cases ($p < 10^{-3}$). All antidepressant drugs, with the exception of levomilnacipran and amoxapine, were reported as suspect in the occurrence of hyponatraemia/SIADH.

Of note, in 2019 cases, antidepressant drugs were designated as concomitant, an aspect we decided not to analyse further. As compared with non-cases, hyponatraemia cases had a higher prevalence of female individuals (57.30% vs. 55.60%; $p < 10^{-3}$). The mean age of cases was significantly higher than that of non-cases (61.47 vs. 49.1 years; $p < 10^{-4}$). Concomitant medication associated with hyponatraemia was used in 38.3% of the cases and in 51.0% of the non-cases ($p < 10^{-4}$).

Table 3.2: Characteristics of cases and non-cases

Characteristic	**Cases (N=13,307)** [n (%)]	**Non-cases (N = 2,771,223)** [n (%)]
Antidepressant user	2233 (16.8)	220773 (8) *
Sex, females	7633 (57.3)	1541780 (55.6) *
Concomitant use of medication associated with hyponatremia	4536 (38.3)	1413323 (51) *
	y (±SD)	**y (±SD)**
Mean age	61.47 (±20.2)	49.1 (±23.5) #

*P <0.001 [chi-square test]

# P < 0.0001 [t-test]

Crude and adjusted RORs for the association between different antidepressant drugs (grouped by pharmacological class) and hyponatraemia are presented in table 3.3. SSRIs were used in 1,367 cases and SNRIs were used in 549 cases, with sertraline (n = 356 cases), and citalopram (n = 339 cases) among SSRIs, and duloxetine (n = 292 cases) and venlafaxine (n=227 cases) among SNRIs being the antidepressant drugs recorded most frequently in association with hyponatraemia (**Table 3.3**). For the individual antidepressant drugs, the highest adjusted RORs were found for moclobemide [n=6; aROR: 8.4 (95%CI: 3.92-18.10)], clomipramine [n=34; 5.1 (3.62-7.26)] and trimipramine [n=5; 4.01 (2.33, 9.10)].

Regarding antidepressant drugs classes, the highest adjusted RORs were observed for MAOIs 8.4 [95% CI 3.92, 18.1] and for the selective serotonin and α2-adrenergic antagonist (mirtazapine) 3.8 [95% C I 3.51, 4.39], respectively (**Figure 3.2**). Noteworthy, there were only 6 cases for the MAOI class (and that this class only included one drug - moclobemide), versus 230

for mirtazapine. Finally, the adjusted ROR for the pooled antidepressant drugs included in our analysis was 1.9 [95% CI 1.83, 2.00]. Because we only included antidepressant drugs that had been reported five or more times to be a suspected cause of hyponatraemia, the number of cases grouped by different pharmacological classes were less than the total number of cases using antidepressant drugs.

Table 3.3: Crude and adjusted reporting odds ratios (RORs) for the association between different antidepressants, grouped by pharmacological class and hyponatraemia.

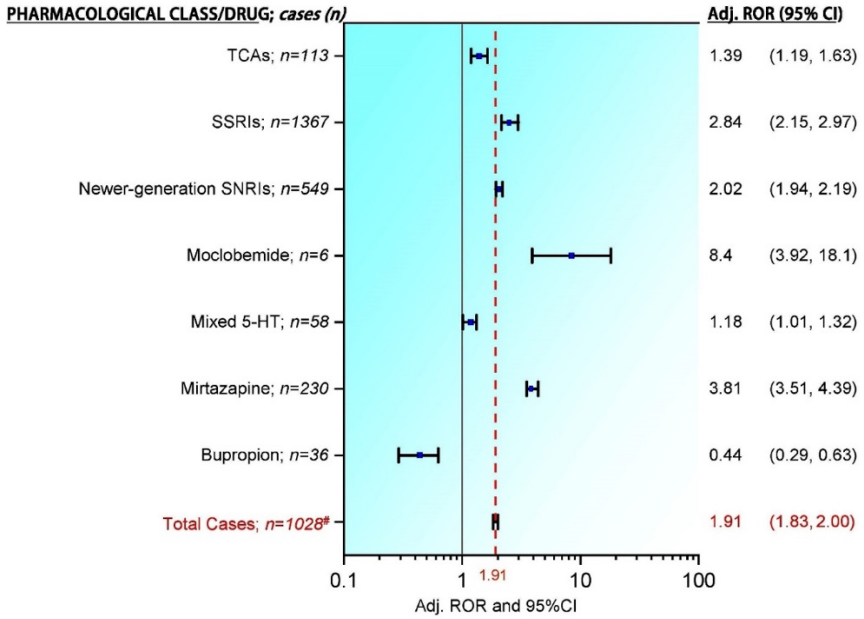
Drug	**Cases (n)**	**Crude ROR (95% CI)**	**Adjusted ROR (95% CI)**
Total	2233*		
Selective-Serotonin- Reuptake Inhibitors (SSRIs)			
Total	1367		
Sertraline	356	2.28 (2.05, 2.54)	2.40 (2.15, 2.84)
Citalopram	339	2.90 (2.60, 3.23)	3.09 (2.83, 3.37)
Paroxetine	241	2.50 (2.20, 2.84)	2.72 (2.36, 2.99)
Escitalopram	234	2.35 (2.07, 2.68)	2.51 (2.26, 2.89)
Fluoxetine	180	1.76 (1.52, 2.04)	1.87 (1.61, 2.19)
Fluvoxamine	17	2.10 (1.30, 3.38)	2.56 (1.81, 3.80)
Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs)			
Newer-generation SNRIs			
Total	549		
Duloxetine	292	2.14 (1.91, 2.41)	2.38 (2.01, 2.56)
Venlafaxine	227	1.83 (1.60, 2.09)	1.90 (1.66, 2.16)
Desvenlafaxine	30	0.87 (0.61, 1.24)	0.97 (0.70, 1.30)
Tricyclic antidepressants (TCAs)			
Total	113		
Amitriptyline	59	0.87 (0.68, 1.13)	0.94 (0.75, 1.25)
Clomipramine	34	4.99 (3.55, 7.02)	5.10 (3.62, 7.26)
Doxepin	9	0.84 (0.44, 1.62)	0.90 (0.59, 1.79)
Imipramine	6	0.95 (0.43, 2.13)	0.99 (0.91, 2.28)
Trimipramine	5	3.57 (1.47, 8.64)	4.01 (2.33, 9.10)
Norepinephrine and Dopamine Reuptake Inhibitor (NDRI)			
Bupropion	36	0.29 (0.21, 0.40)	0.44 (0.29, 0.63)
Mixed Serotonergic Effects			

Total	58		
Trazodone	39	0.53 (0.39, 0.73)	0.61 (0.44, 0.81)
Vortioxetine	12	1.55 (0.88, 2.73)	1.72 (1.03, 2.98)
Vilazodone	7	1.30 (0.62, 2.72)	1.62 (0.92, 2.91)
Serotonin and α_2-Adrenergic Antagonist			
Mirtazapine	230	3.69 (3.24, 4.21)	3.81 (3.51, 4.39)
Monoamine Oxidase Inhibitors (MAOIs)			
Moclobemide	6	7.35 (3.25, 16.59)	8.40 (3.92, 18.10)
Others[#]			
Nortriptyline	4	-	-
Maprotiline	4	-	-
Mianserin	3	-	-
Desipramine	2	-	-
Reboxetine	2	-	-
Phenelzine	1	-	-
Nefazodone	1	-	-
Selegiline	1	-	-
Tranylcypromine	1	-	-

CI: confidence interval, ROR: reporting odds ratio
non-significant RORs are presented in lighter text
*A report may contain more than one suspected antidepressant drug. For this reason, the total number of reports in which an individual antidepressant (n = 2378) was mentioned as the suspected drug was higher than the total number of reports in which an antidepressant drug was mentioned as the suspected drug (n = 2233).
Not included in the statistical analysis because the analysis was based on unique drug-event combinations with at least 5 occurrences.
Significant signal was defined as ROR > 1 and α threshold of 0.05
Adjusted reporting odds ratio (adj.ROR) was calculated in adjusted multivariate logistic regression analysis, with adjustment for age, sex, drugs known to cause hyponatremia

Figure 3.2: Forest plot, on a logarithmic scale, showing adjusted reporting odds

ratios (adj. RORs) of different pharmacological classes of antidepressants.



TCAs: tricyclic antidepressants; SSRIs: selective serotonin reuptake inhibitor; SNRIs: serotonin-norepinephrine reuptake inhibitor; 5-HT: 5-hydroxytryptamine. See table 3.3 for drugs in each class of antidepressants.

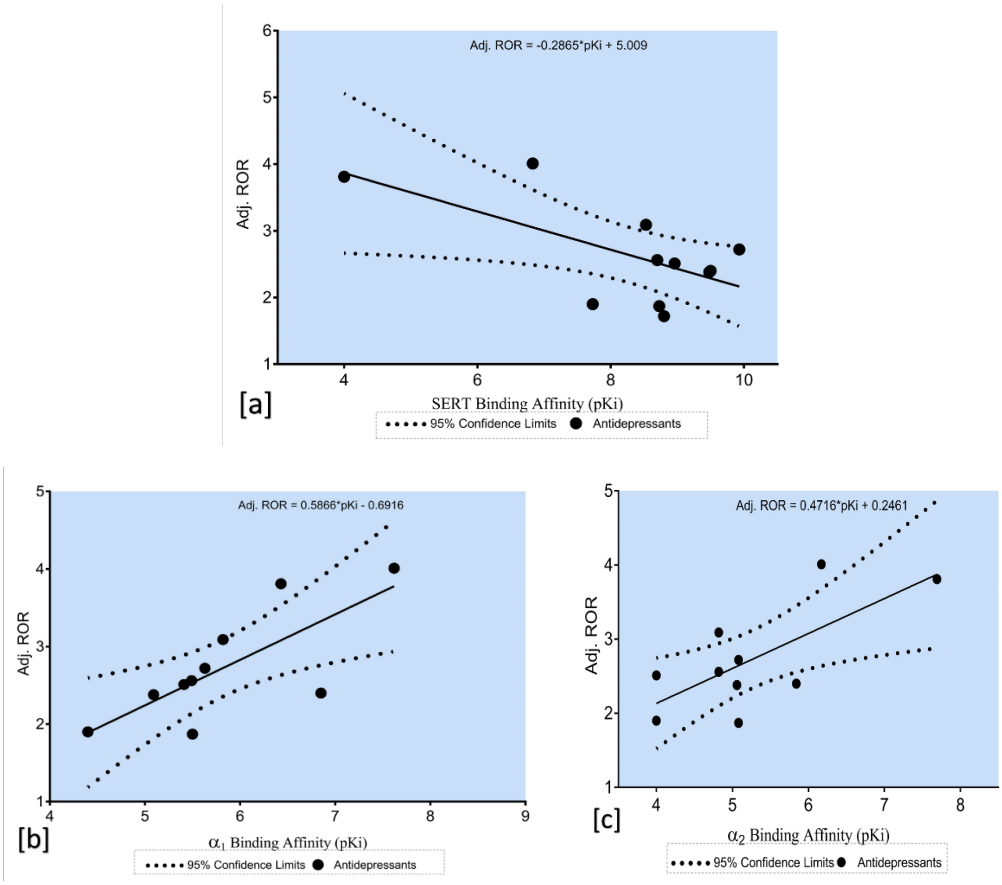
[#]Since we included only antidepressant drugs that had been reported ≥5 times to be a suspected cause of hyponatraemia, therefore the number of cases grouped by different pharmacological classes was less than the total number of cases using antidepressants.

3.3.2. Pharmacovigilance –Pharmacodynamic Assessment

Residual analysis identified clomipramine as an outlier with significant distortion in the model accuracy. Removing this outlier subsequently improved the model accuracy up to ninety percent. For the 10 antidepressant drugs (citalopram, duloxetine, escitalopram, fluoxetine, fluvoxamine, mirtazapine, paroxetine, sertraline, trimipramine, venlafaxine, vortioxetine) considered in the analysis, a significant inverse correlation was found between the adjusted RORs for hyponatraemia/SIADH and pKi for SERT [slope = −0.2865; 95% CI (− 0.5486 to −0.02446); P =0.035; R2 = 0.40] (**Figure 3.3a**), significant linear correlations for α_1 [slope = 0.5866; 95% CI (0.165 to 1.008); P =0.0125; R^2 = 0.56] (**Figure 3.3b**), and α_2 [slope = 0.4716 ; 95% CI (0.09564 to 0.8476); P =0.0201; R2 = 0.51] (**Figure 3.3c**); but not for the remaining tested targets: NET, DAT, 5-HT_2C, 5HT_2A, and 5HT_1A (data not shown).

Figure 3.3: Linear regression plots between hyponatraemia signal ranked

antidepressants and their binding affinities at serotonin transporter (SERT) **[a]**, α_1 adrenergic receptor **[b]**, and α_2 adrenergic receptor **[c]**



3.4. DISCUSSION

In the present analysis of 2,233 reports of antidepressant-induced hyponatraemia, obtained from the FAERS database, we found that all antidepressant drugs, with the exception of levomilnacipran and amoxapine, were reported as suspect in the occurrence of hyponatraemia/SIADH. Most of the antidepressant drugs were significantly associated with disproportionate reporting of hyponatraemia/SIADH. This association was strongest for moclobemide, clomipramine, trimipramine, followed by mirtazapine, SSRIs, SNRIs and lowest for vortioxetine. Of note, trazodone and bupropion were associated with a disproportionately lower reporting of hyponatraemia/SIADH, indicating a lower rate of hyponatraemia events. However, given the potential competition bias in SRSs data, low magnitude of SDRs points to an increased

possibility for confounding as an alternative explanation. Worthy of mention is the very low number of cases for moclobemide, clomipramine and trimipramine, requiring caution in interpretation of these data.

Demographic data (mean age around 61 years, female sex predominance) were consistent with epidemiologic data regarding hyponatraemia [126, 127] as 57.3% of all hyponatraemia cases were reported in women, a proportion in keeping with the more extensive use of antidepressant drugs by women [137-139].

Antidepressant drug users of advanced age, women, and those taking concomitant medications known to cause hyponatraemia are prone to antidepressant drug-induced hyponatraemia. Existing studies using SRS on antidepressant drugs -induced hyponatraemia, had not adjusted for these factors before [105, 140]. To adjust for these influencing factors, we have excluded all reports with missing information on covariates (age and sex) even if, theoretically, this approach could be a potential source of selection bias. However, the control-to-case ratio regarding missing vs complete information was approximately parallel (208 non-cases /case with missing information; 227 non-cases /case with complete information). Moreover, after adjusting the ROR for concomitant medication, in both situations, the adjusted RORs for the total number of antidepressant-induced hyponatraemia cases were comparable. Therefore, selection bias due to the exclusion of reports with missing information on covariates is unlikely. Furthermore, drugs or drug classes that are known to be associated with hyponatraemia (enlisted in table 3.1), used as one of the potential confounding factors, were the most current at the time of data acquisition from FAERS and based on an extensive literature survey. Our list also included drugs that are used in everyday clinical practice (e.g., antibacterials, antihypertensive agents, proton pump inhibitors).

Because several case reports have been published, clinicians are possibly aware of antidepressant-induced hyponatraemia. However, the association between antidepressant drugs and reports of hyponatraemia is largely influenced by the use of concomitant medication associated with

hyponatraemia. We have found that in more than fifty percent of cases, antidepressant drugs were recorded as concomitant. Selective reporting may cause this difference: clinicians reporting hyponatraemia as an ADR might consider the concomitant medication associated with hyponatraemia to be responsible and thus less frequently attribute hyponatraemia to the antidepressant drug used. Inversely, potential confounders in causality assessments such as protopathic bias and confounding by indication also need to be considered, as psychiatric diseases themselves may be associated with hyponatraemia due to SIADH and polydipsia [141]. Because indication is not always recorded in FAERS, it was impossible for us to adjust for confounding by indication.

The mechanism by which antidepressant drugs may lead to hyponatraemia is still unclear. It has been suggested that this mechanism depends mainly on the stimulation of antidiuretic hormone (ADH) secretion and possibly SIADH. However, hyponatraemia was also reported to occur in the presence of normal ADH levels [126]. Indeed, it can have multifactorial causes, including the augmentation of ADH action in the renal medulla, the re-setting of the osmostat that lowers the threshold for ADH secretion, and the interaction with other drugs by inhibition of CYP2D6 [137-139]. From a mechanistic perspective, some animal studies have shown that increased levels of serotonin can increase ADH secretion, through the hyper-activation of 5-HT2 receptors [141, 142]. The mechanistic explanation of this finding is that an excessive increase in serotonin levels can cause a rebound drop in dopamine levels, via 5-HT2 receptors [143, 144] and insufficient dopamine levels may lead to SIADH [145, 146]. Therefore, antidepressants that have strong serotoninergic effects are expected to cause more hyponatraemia/SIADH, while antidepressants that are less serotoninergic, that boost also norepinephrine and dopamine, or that have specific 5-HT2 antagonist properties, should be safer regarding this adverse effect.

Previous studies found that the risk of developing hyponatraemia on TCAs is lower than for SSRIs, with clomipramine being associated with the greatest risk among TCAs [107]. The fact that we found very disproportionate reporting

rates, higher than those of SSRIs, with clomipramine and trimipramine is not aligned with the theory that the serotonin/dopamine imbalances due to antidepressants may determine the extent of their endocrine adverse effects. However, there were scant overall reports available in the FAERS for clomipramine and trimipramine; therefore, there may have been a possibility of risk overestimation.

This same criticism should be considered when evaluating the results for moclobemide. There is little evidence, even in case reports, of an association of hyponatraemia for most of the MAOI antidepressant drugs as they have been largely replaced by newer antidepressant drugs. In FAERS, we retrieved only nine such cases. Of these 9 cases, 6 involved moclobemide—the selective MAO-A inhibitor. Due to insufficient information as a result of the low exposure to MAOIs in the studied dataset, it is inadvisable to compare the evidence of SDRs for moclobemide with other drugs.

Among drugs with a sizeable number of overall reports in the FAERS, mirtazapine was associated with the highest disproportionate reporting of hyponatraemia/SIADH. This result is important in view of the fact that evidence in the literature is both sparse and inconsistent regarding the association of mirtazapine with hyponatraemia. It has been suggested that mirtazapine has a lower risk of hyponatraemia than SSRIs and this drug is considered as an alternative treatment for patients with an increased risk of hyponatraemia [109, 112]. Except for the cohort study performed by Coupland and colleagues [109], most of the evidence regarding the association of mirtazapine with hyponatraemia is derived from sporadic case reports and case series, a finding that weakens the strength of the association. A post-marketing prospective safety study and at least seven case reports found a significant correlation between the use of mirtazapine and the occurrence of hyponatraemia [146]. Recently, a French study using the national SRS found that the evidence of SDR for mirtazapine was comparable to that for sertraline [140]. Consistent with this, our analysis showed that hyponatraemia is reported with mirtazapine at disproportionately higher rate as compared to any other individual SSRIs. Given the high potential for

'confounding by indication' (channelling bias) in SRS data, however, this finding should be interpreted cautiously and needs to be investigated further in large-scale sample observational studies, particularly on patients at risk of hyponatraemia.

Mirtazapine was followed by SSRIs, with a ROR ranking from citalopram, to paroxetine, fluvoxamine, escitalopram, sertraline, and fluoxetine. Previous evidence regarding differences between individual SSRI drugs in their potential for eliciting hyponatraemia or SIADH is inconclusive [125]. In most studies, sample sizes are too small to differentiate between the various risks associated with distinct SSRIs. A review of the literature revealed fluoxetine, citalopram, and escitalopram to be associated with significantly increased risks of hyponatraemia, whereas the risk is lower with paroxetine and sertraline [109, 110, 113, 147]. In contrast, in our study and also in the French one mentioned above [140], the evidence of SDR for hyponatraemia was greater with citalopram, paroxetine, and escitalopram, while lower with sertraline and fluoxetine. Notably, in our dataset the ROR relative to fluoxetine was lower than that of all other SSRIs and SNRIs, possibly supporting the concept that noradrenergic properties and/or dopaminergic properties may contribute to determine risks of hyponatraemia/SIADH.

Although data from the literature concerning the risk of hyponatraemia associated with the use of SNRIs are scant [148, 149], our results regarding the SNRIs: duloxetine and venlafaxine indicated a safer profile as compared to most SSRIs. A few case reports mentioning the occurrence of hyponatraemia with desvenlafaxine, a major active metabolite of venlafaxine, are also reported [150-152]. Non-significant ROR for desvenlafaxine in our analysis could be explained by its safe drug interaction profile.

Given that fluoxetine scored the lowest ROR for hyponatraemia/SIADH among all SSRIs and SNRIs, vortioxetine resulted to have an even lower ROR. Vortioxetine is currently considered to be a safe treatment for depression [153, 154], even for patients above 65 years. In the literature, only one case of hyponatraemia associated with the use of vortioxetine has been reported in a 72-year-old woman, with fatal outcome [114]. Another case report

described the successful use of vortioxetine in a 63-years-old patient who developed hyponatraemia upon initiation of four different antidepressant drug treatments (paroxetine, venlafaxine, mirtazapine, and fluoxetine augmentation) [155]. Twelve cases have been reported to the FAERS in which vortioxetine was deemed to be a causal factor in the development of hyponatraemia. The adjusted ROR for vortioxetine in the present work was statistically significant, however, due to its recent commercialization, additional studies on larger-scale samples are warranted to confirm this finding.

Our findings of low reporting of hyponatraemia with trazodone and bupropion may further support the importance of a serotonin/dopamine imbalance in determining hyponatraemia/SIADH, since both drugs have a scant SERT inhibition capability but a greater noradrenaline and dopamine boosting potential— could possibly be attributed to a disproportionately low reporting.

Concerning the PV–PD approach we proposed, results deserve a critical interpretation, as they indicate that the greater the SERT affinity, the fewer hyponatraemia cases are reported. By contrast, affinity at α1 or α2 receptors showed significant positive linear relationships. Our result showed that α1 or α2 receptors were quantitatively more relevant than the SERT (greater R^2 value) in their association with the RORs of antidepressants. The main issue with this analysis was that, given the low number of molecules examined, we could not build a regression model containing simultaneously all receptor-binding characteristics; rather, we could analyse one receptor at a time. This methodological issue acquires great importance in a psychopharmacological view, as the balance between serotoninergic, noradrenergic and dopaminergic stimulation of each drug should ideally be appraised in its entirety, in order to produce reliable results.

The role of the serotonin-dopamine balance for the adverse potential of an antidepressant is further called into question by the examples of trazodone and bupropion. Trazodone is a selective serotonin reuptake inhibitor and serotonin receptor modulator, known for its capability to preserve physiological dopamine levels [156]; the literature reports no data regarding

its relationship with hyponatraemia/SIADH. Bupropion is a selective noradrenergic and dopaminergic reuptake inhibitor; for which at least six case reports [157-161] describe an association with hyponatraemia. The observation of RORs for hyponatraemia/SIADH below the average for these drugs is consistent with the hypothesis that serotonin-induced SIADH may be mediated by a drop in physiological dopamine levels.

3.4.1. Limitations

The use of a SRS database has some important implicit limitations, reporting being influenced by factors such as the notoriety bias, selection bias and under-reporting [162]. Unlike typical case-control study, cases and non-cases are drawn from different populations, thus, this method cannot be a substitute for the classical case-control but gives relatively reliable results if the database contains several thousand of different drugs−event combinations. RORs are influenced by potential confounders such as selective under-reporting, follow-up period bias and exposure misclassification bias. Neither the prevalence, nor the incidence rate of ADRs can be computed from the analysis of the FAERS dataset since the primary goal of such system is to signal the existence of a possible relationship between a drug or drug class and an ADR, and it does not prove causality. The ICSRs are not necessarily different individuals as one individual may be reported several times in the FAERS. Due to small number of reports for some antidepressant drugs within a drug class we were unable to address apparent inconsistencies within the class.

The PV–PD based on pharmacological receptors theory has its own limitations [163], for instance, receptor affinity does not directly reflect the intrinsic activity of a drug. The PV–PD approach allowed us to determine an inverse relationship between the ROR of hyponatraemia/SIADH and the pKi for SERT, while we found a positive correlation between the ROR of hyponatraemia/SIADH and the pKi for noradrenaline receptors α_1 and α_2. These results, which appear at a first glance counterintuitive, are due to the fact that we could analyse only one receptor at a time. A more proper PD evaluation should comprise all receptors at the same time; the applicability of such an approach, however, is increasingly limited as the pharmacological

complexity of the mechanisms of action increases, like in the case of antidepressants, that are known to act through many receptors simultaneously.

3.5. CONCLUSION

In this work conducted on spontaneous reports of hyponatraemia/SIADH present in the FAERS database, we found that moclobemide, clomipramine, trimipramine, mirtazapine, citalopram, paroxetine, fluvoxamine, escitalopram, sertraline, duloxetine, venlafaxine, fluoxetine and vortioxetine are all associated with a disproportionately high reporting rate, however, for some drugs we retrieved small number of reports. Conversely, trazodone and bupropion are instead associated with a disproportionately low reporting rate; other antidepressants were not associated with significantly disproportionate reporting rates. The most notable results comprise a large ROR of hyponatraemia/SIADH with moclobemide, clomipramine, trimipramine and mirtazapine, while trazodone and bupropion reduced the chance of reporting it, however, an alternative explanation involving chance and confounding by indication could not be ruled out with reasonable confidence. An interesting suggestion from the hierarchy of RORs may be that the serotonin-dopamine hypothesis underlying endocrine dysfunctions (including SIADH) can be supported by FAERS data. Concomitant use of medication known to cause hyponatraemia may have been linked with selective under-reporting of antidepressant-induced hyponatraemia. The application of the PV-PD method remains hypothetical, especially in the presence of drugs that act on multiple receptors, and requires further investigation. The findings presented in this study substantiate previous reports in the literature and reinforce the notion that hyponatraemia can occur also with classes of antidepressant drugs that are deemed to have better tolerability, while the majority of antidepressants may follow a hierarchy of risk that could be explained by their antidopaminergic potential. Further observational studies on large-scale samples are needed to confirm these results.

CHAPTER 4: STUDY 2

CHAPTER 4: STUDY II

Hyponatraemia following antipsychotic treatment: In-silico pharmacodynamics analysis of spontaneous reports from the US Food and Drug Administration Adverse Event Reporting System Database and an Updated Systematic Review

ABSTRACT

Background: Hyponatraemia associated with antipsychotic drugs is a rare but potentially life-threatening adverse drug reaction (ADR); the underlying pharmacological mechanism has not been explained yet.

Methods: We investigated the relationship between pharmacological targets of antipsychotic drugs and the occurrence of hyponatraemia by conducting a nested case-control study using the Food and Drug Administration Adverse Event Reporting System (FAERS) database. Multiple logistic regression was used to determine the associations between antipsychotics receptor occupancy and hyponatraemia. We also performed a systematic review of clinical studies on this association.

Results: Of 139,816 reports involving at least one antipsychotic, 1.1% reported hyponatraemia. Olanzapine was the most frequently suspected drug (27%). A significant positive association was found between dopamine D3, D4 and hyponatraemia, while adrenergic α1, serotonin 5-HT1A, and 5-HT2A receptor occupancies were negatively associated. A multivariable stepwise regression model showed that dopamine D3 (Adj. Odds Ratio=1.21; 95% CI=1.09–1.34; $p<0.05$) predicted the risk for hyponatraemia ($p<0.05$), while serotonin 5-HT2A occupancy (Adj. OR=0.78; 95% CI=0.68–0.90; $p<0.01$) exhibited a protective effect against hyponatraemia. Among the eleven studies included in the systematic review, incidence rates of hyponatraemia diverged between 0.003 % and 86%, whereas the odds of developing hyponatraemia from effect studies ranged between 0.83 to 3.47.

Conclusion: Antipsychotic drugs having a combined modest occupancy for D3 and 5-HT2A receptors and higher levels of D3 receptor occupancy, correspond to different degrees of risk for hyponatraemia. Based on the few, relatively large-scale available studies, atypical antipsychotics have a more attenuated risk profile for hyponatraemia.

4.1. INTRODUCTION

The syndrome of inappropriate antidiuretic hormone (SIADH) occurs when there is persistent stimulation of antidiuretic hormone (ADH) resulting in hyponatraemia. SIADH commonly presents as euvolemic hyponatraemia and it should be suspected in any patient with hyponatraemia, hypoosmolality, and a urine osmolality above 100 mOsmol/kg [164]. Hyponatraemia (serum sodium concentration < 136 mEq/L) is a prevalent and potentially dangerous medical comorbidity in psychiatric patients [165]. It has been reported to be associated with an increase in the risk of mortality of 55% and substantial costs for the health systems [166, 167].

The cause of hyponatraemia/SIADH among psychiatric patients is still unclear, and there are two conflicting possibilities. As any central nervous system (CNS) abnormality, including mental illness and psychosis, can enhance ADH-release from the pituitary gland, one possibility is that hyponatraemia is associated with the exacerbation of the underlying psychiatric conditions such as psychosis-intermittent hyponatraemia-polydipsia syndrome and compulsive water drinking/psychogenic polydipsia [168, 169]. A second possibility is that hyponatraemia/SIADH has an iatrogenic cause; a number of drugs is indeed associated with SIADH by enhancing or affecting the release of ADH. The list of the commonest drugs includes carbamazepine, oxcarbazepine, chlorpropamide, cyclophosphamide, and selective serotonin reuptake inhibitors (SSRI) [169]; however, hyponatraemia/SIADH has been reported also with the use of both typical and atypical antipsychotics [170, 171]. The clear association between antipsychotic drug use in clinical setting and the occurrence of these abnormalities has not been established.

Early observational studies that examined the relationship between hyponatraemia and antipsychotic drugs reported higher incidence with typical antipsychotics than atypical antipsychotics, particularly phenothiazines [172-174]. To date, there has been only one comprehensive systematic review addressing this topic, which was conducted in 2010 [175]. More recently, a systematic review of case reports on hyponatraemia induced only by atypical antipsychotics in

patients with schizophrenia was published [170].

Hyponatraemia/SIADH in atypical antipsychotics may be mediated by the action of serotonin, both by the release of ADH induced by the stimulation of central serotonin 5-HT_2 and 5-HT_{1c} receptors and by the increase in the effects of ADH at the renal medullary level [137, 176]. Prolonged blockade of dopamine D_2 receptors and subsequent stimulation of the release of ADH and increase in its peripheral response [177-179] has also been proposed, limited to typical antipsychotic drugs. Yet, stratified disproportionality analyses of antipsychotics based on chemical structure and receptor affinity profiles of the dopamine D_2 receptor and serotonin 5-HT_{2A} have not shown a variation regarding the risk of hyponatraemia in VigiBase® [171] leaving unanswered the question on how antipsychotic drugs may lead to hyponatraemia.

Given the widespread use of antipsychotics in different neuropsychiatric disorders and in light of the poorly understood pharmacological mechanism of action, it is of clinical relevance to elucidate the plausible pharmacodynamic relationship between hyponatraemia and antipsychotics. Moreover, in the past decade, several studies have been carried out that describe the development of hyponatraemia in association with antipsychotic drug treatment [180-184], however, no review has been conducted to summarise findings.

We thus conducted a case-control study by using the US Food and Drug Administration Adverse Event Reporting System (FAERS) database aimed at quantifying the association between antipsychotics and the occurrence of hyponatraemia/SIADH. To then clarify whether hyponatraemia induction by antipsychotic drugs is driven by their receptor occupancy characteristics (dopaminergic D_1, D_2, D_3, D_4, histamine H_1, muscarinic M_1, M_2, M_3, central adrenergic α_{1a}, α_{2a}, serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}). we carried out a combined 'pharmacovigilance–pharmacodynamic' (PV–PD) analysis. This method has been previously used to study the pharmacological mechanisms of adverse reactions to a variety of drug classes [185-187]. It can be used to establish an association between pharmacological targets of drugs and their corresponding reporting risks for ADRs of interest observed in a large pharmacovigilance database. Finally, we performed an updated systematic review of the current literature to include all additional

data on antipsychotic-associated hyponatraemia.

4.2. EXPERIMENTAL PROCEDURES

4.2.1. In silico pharmacodynamics analysis

4.2.1.1. Setting and study design

Data were obtained from the FAERS, one of the largest spontaneous reporting system databases. The FAERS receives approximately 1.5 million adverse events (AEs), product complaints, and user error reports from healthcare practitioners, consumers, companies, and other sources, concerning drugs, vaccines, and medical devices for human use [50]. The database is updated quarterly and designed in accordance with the international safety reporting guidance issued by the International Conference on Harmonization (ICH 2000). AEs are recorded in the FAERS using the Medical Dictionary for Regulatory Activities (MedDRA®) preferred terms. The number of safety reports sent to the FDA annually is continuously expanding due to increases in the type and number of products the agency regulates, awareness of the importance of these reports, ease of submitting reports (i.e., digitally), and population [188] size.

This study was designed as a nested case-control study. The base cohort consisted of all ADRs involving any antipsychotic drug [Anatomical Therapeutic Chemical (ATC) code N05A, excluding Lithium (ATC N05AN)], as suspected, interacting or concomitant drug, and for which information on binding affinities were available. The study period covered the first quarter of 2004 (representing the beginning of freely available FAERS data) through to the third quarter (Q3) of 2019.

4.2.1.2. Data acquisition and data processing

AEs data recorded in the FAERS were downloaded from the FDA website http://www.fda.gov/Drugs/InformationOnDrugs/ucm135151.htm. The database consists of seven datasets, namely patient demographic and administrative information (file descriptor DEMO), drug and biologic information (DRUG), adverse events (REAC), patient outcomes (OUTC), report sources (RPSR), start and end dates of drug therapy (THER), and indications for use/diagnosis (INDI). These seven datasets were joined by unique identification numbers for each FAERS report and a relational

database was built. Data extraction was restricted to reports without missing values for age and sex. We analysed only reports concerning adults (≥18 years).

Because FAERS may sporadically contain duplicate reports, in case of reports submitted by both the consumer and the sponsor or intentional multiple reporting, data were scrutinised further manually based on similarities in patients, adverse drug reactions (ADRs), and medicinal product data. Duplicate records were detected and deleted accordingly. We further standardised our dataset for possible misspelt or variants of drug names. Drug name text-mapping was accomplished by normalising multiple drug names into a single generic name by automated matching processes through SQL-database schema. Subsequently, an open-source programme, OpenRefine [189] was used to standardise drug name variants in the dataset to make them consistent with the international non-proprietary nomenclature defined by the World Health Organization ATC classification.

4.2.1.3. Definition of cases and controls

Cases were defined as all Individual Case Safety Reports (ICSRs) where at least one MedDRA® lower-level term from the standardised MedDRA® query for "hyponatraemia/SIADH (*narrow*)" (released in March 2014 with MedDRA® Version 15.0) has been coded in the adverse reaction section (outcome of interest) in relation to antipsychotic drug(s). Non-cases (controls) were all other ADRs reported in the database during the same period of time (*i.e.,* all ADRs reports without the outcome of interest).

4.2.1.4. Potential confounding factors

Potential confounding factors retrieved from the case reports included age and sex of the patient, exposure to concomitant medication associated with hyponatraemia, reporting year, year since marketing, characteristics of the reporter (physician; pharmacist; other caregivers; pharmaceutical company, indirectly obtained from a healthcare professional, and patient/consumer). Concomitant use of medication associated with hyponatraemia/SIADH, defined as one of the drugs summarised in **Appendix I,** reporting as a concomitant or interacting drug for an ADR.

4.2.1.5. Pharmacodynamic data sources and methods for calculation of antipsychotics' receptor occupancy

For each antipsychotic drug studied, we quantified degrees of occupancy at dopaminergic D_1, D_2, D_3, and D_4, histamine H_1, muscarinic M_1, M_2, and M_3, central adrenergic α_{1a} and α_{2a}, serotonin 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} (**Appendix II**). Degree of receptor occupancy was calculated according to an equation derived from the pharmacological receptor theory's model [163, 190]. By using this approach, receptor-mediated pharmacological actions of drugs can be estimated quantitatively with reasonable accuracy [191]. The receptor occupancy is expressed by the following equation:

$$\text{Receptor Occupancy } (\%) = \frac{[Cu]}{(Ki + [Cu])} \times 100$$

Where [Cr] represents the concentration of unbound antipsychotic (nmol/L) and constant *Ki* (nM) is the equilibrium dissociation constant of a ligand determined in inhibition studies. [Cr] were estimated according the "therapeutic reference ranges" reported in the "AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry" [192] and data on plasma protein binding reported in DrugBank, Integrated Database of ADMET and Adverse effects of Predictive Modeling (IDAAPM) [193] databases and individual monographs or regulatory documents.

For a given drug-receptor pair, constant *Ki* is reflective of the binding affinity of the drug to a receptor. For each studied antipsychotic drug, values of binding affinities (Ki) at 12 different receptors potentially involved in iatrogenic hyponatraemia were searched in the European Bioinformatics Institute-ChEMBL [131], International Union of Basic and Clinical Pharmacology (IUPHAR, 2020) [132], Psychoactive Drug Screening Program (PDSP, 2020) [133] and BindingDB [194]. Only Ki obtained from *human* species and subset on data for ligand-receptor combinations studied in CNS tissue or cloned receptors were selected. In case of multiple Ki values for the same receptor-ligand pair, we computed the dispersion, discarded values at the tails of the distribution and reported the resulting average.

4.2.1.6. Statistical Analyses

We first developed descriptive statistics for cases and non-cases. The normality of data was also verified by means of the Kolmogorov test and

appropriate parametric and non-parametric analyses were conducted. Descriptive analysis was performed for cases and non-cases, in terms of age, female sex and use of concomitant medication associated with hyponatraemia. The Student's t-test was used to assess whether age was distributed differently between cases and non-cases, whereas the Pearson's Chi-square test was used to assess whether categorical variables (sex and the presence of concomitant medications associated with hyponatraemia) were differently distributed between cases and non-cases. Tests were two-tailed, with significance set at a p-value of 0.05.

We then performed univariate and multivariate logistic regression to study the association between receptors' occupancy and the occurrence of hyponatraemia with antipsychotic drugs. Quantitative values of occupancy were converted into a categorical variable by grouping values into two categories ["low level of occupancy" (<50%), and "high level of occupancy" (≥50%)] and were included in the regression models. In case of several antipsychotics with different receptor occupancies on the same receptor, the highest degree of occupancy was selected.

Logistic regressions used hyponatraemia cases as the dependent variable. Potential explanatory variables included categorised values of occupancies [("high level of occupancy" versus "low level of occupancy" (reference group)] of involved antipsychotic drugs at 12 different receptors investigated along with the confounding variables discussed in section 4.2.1.4.

First, univariate analyses (model 1) were carried out to calculate the unadjusted association between receptor occupancy and outcome separately for each receptor. Levels of receptor occupancies that were significantly associated with the outcome in the univariate analyses were simultaneously entered in the multivariate model (model 2). A stepwise descending procedure (model 3) was conducted to select the main variables related to hyponatraemia. In this modelling, the p-value to enter the model was ≤0.05, and the p-value to leave the model was >0.10. The validity of the models was checked using the Hosmer and Lemeshow Goodness-of-Fit Test. The association between receptor occupancy and hyponatraemia was estimated as crude and adjusted Odds Ratios with their corresponding 95% confidence interval (95% CI).

To identify antipsychotics that were reported more frequently than expected in FAERS, we performed a case/non-case comparison among the 19 drugs to measure the disproportionality of drug-associated hyponatraemia reporting for each antipsychotic. This method allows the comparison of hyponatraemia to other cases of ADRs regarding the exposure to one antipsychotic in comparison to other antipsychotics [135], quantified as reporting odds ratio (ROR) and its corresponding 95% CI [82]. The ROR for each antipsychotic–ADR combination was defined as the ratio between proportions of cases containing the suspected antipsychotic in the "case" (number of cases with one antipsychotic as the suspected drug divided by the number of cases with other antipsychotics) and in the "non-case" (number of non-cases with the same antipsychotic as the suspected drug divided by the number of non-cases with other antipsychotics) group. We only included antipsychotic drugs that had been reported three or more times to be a suspected cause of hyponatraemia. A signal of disproportionate reporting (SDR) was defined when the lower limit of the 95% two-sided CI for the ROR exceeded the threshold value of 1. All analyses were performed using counts of unique cases. Data reading, filtering, and processing were done through RStudio. All statistical analyses were using STATA® (StataCorp, College Station, TX, USA).

4.2.2. Systematic review

We conducted a systematic review of the published literature, in accordance with Preferred Reporting Items for Systemic Reviews and Meta-Analyses guidelines [195] for the evidence of hyponatraemia following antipsychotic (excluding lithium) treatment as reported in clinical trials, cohort and case-control studies. Outcome measures were incidence rates and the odds or hazard ratios (OR/HR) of hyponatraemia in both inpatients and outpatients treated with antipsychotic drugs.

4.2.2.1. Search Strategy

A search was conducted in the MEDLINE, PsychINFO, and EMBASE databases between April 2009 and 06 December 2020 to be current with the most recent literature since the last most comprehensive review by Meulendijks et. al [175] on antipsychotic associated hyponatraemia studies available in the literature. For the search, which was based on keywords

from the systematic catalogue or alphabetic index, the following terms were used: 'antipsychotic agents', 'neuroleptic agent', 'hyponatraemia', 'inappropriate ADH syndrome', 'sodium blood level', 'sodium deficiency', 'sodium depletion', and 'water-electrolyte balance'. The only limit on the search was time.

4.2.2.2. Study Selection, data abstraction and quality assessment

After duplicate removal, the search results were then screened by title and abstract. All potentially relevant publications were retrieved in full text and evaluated in detail. Bibliographies of retrieved articles were examined for further relevant publications. Studies were eligible if they included descriptions of study design and population(s) and case definitions of hyponatraemia and if the results on the outcome for subjects exposed to antipsychotics were separately identified. We excluded studies that reported polydipsia but not hyponatraemia/SIADH, or explicitly reported polydipsia. Our study eligibility criteria also excluded lithium. Any disagreements about study selection were resolved by consensus with 2 researchers (FM and VB).

For each included study, we extracted the following information: study design (study type, study duration, sample size, serum sodium cut-off values used for definition of hyponatraemia); patient characteristics (in/out patients and source, age, sex and number exposed to antipsychotics); and outcomes on antipsychotic associated hyponatraemia. Because of our broad inclusion criteria, we anticipated considerable heterogeneity, which is why we assessed and compared effect studies for study design, sample size, patient characteristics, cut-off values, definition of hyponatraemia.

The quality of the individual studies was assessed by one reviewer (FM) and independently checked by a second (VB); disagreements were resolved by consensus. Cohort studies and case-control studies were assessed with the designated Newcastle-Ottawa Scale (NOS) [196]. The NOS for cohort studies was adapted for use with cross-sectional studies in a similar manner to previous research [197]. The NOS which was adapted for cross-sectional studies uses the same star system in the main scale only. The difference is that on this scale there are five stars for the selection dimension, two stars for the comparability dimension and three stars for the outcomes dimension, which indicates the quality of the study. Each

item is scored one or two and summed for a total indicating overall study quality as either high (7–9), moderate (5–6), or low (0–4).

4.3. RESULTS

4.3.1. In silico pharmacodynamic analysis

4.3.1.1 Study population

From the FAERS we identified 138,194 ICSRs involving at least one of the 19 antipsychotics of interest with information on age and sex. Of 138,194 ICSRs, 1,520 (1.1%) were related to hyponatraemia (cases). Univariate analyses of demographics and characteristics of nested cases and non-cases population are presented in **Table 4.1**. Compared to non-cases, prevalence of hyponatraemia cases was higher in female individuals (56% vs. 53%; $p < 0.05$) and most frequently reported by physicians and patients. The mean age of cases was significantly higher than that of non-cases (55.88 vs. 47.05 years; $p < 0.0001$). Concomitant medication associated with hyponatraemia was used in 69% of the cases and in 66% of the non-cases ($p < 0.05$).

Table 4.1. Characteristics of nested cases/non-cases population in the FAERS (n=138,192).

Characteristic	**Cases (N=1,520)** [n (%)]	**Non-cases (N = 136,674)** [n (%)]	**P value**
Patient age, mean (SD), years	55.88 ± 16.65	47.05 ± 17.14	<0.0001#
Sex, females	849 (56)	72,366 (53)	0.024*
Concomitant use of medication associated with hyponatraemia	1,049 (69)	90,205 (66)	0.0460*
Reporter type			
physician	502 (33)	50,569 (37)	0.024*
pharmacist	304 (20)	17,768 (13)	
other caregivers	182 (12)	13,667 (10)	
patient/consumer	426 (28)	41,002 (30)	
unknown	106 (7)	13,667 (10)	

Reporting year			
2004-2012	684 (45)	43,736 (32)	0.074*
2013-2019	836 (55)	92,938 (68)	
Years since marketing			
<10	532 (35)	56,036 (41)	0.023*
10 to 15	289 (19)	38,269 (28)	
15 - 20	426 (28)	9,567 (7)	
>20	274 (18)	32,802 (24)	

All data except patient age are shown as number (%).
t-test
*chi-square test

4.3.1.2. Pharmacovigilance–Pharmacodynamic analysis for the association between receptor occupancies and antipsychotic associated hyponatraemia.

Univariate logistic regression analysis showed a significant and positive association between hyponatraemia reports and dopamine D_3 (OR=1.20; 95% CI=1.09-1.31) and D_4 (OR=1.17; 95% CI=1.06- 1.28) receptor occupancies, whereas significant negative association was found with histamine H_1 (OR=0.72; 95% CI=0.65-0.79) and serotonin $5HT_{1A}$ (OR=0.52; 95% CI=0.47- 0.58) and $5HT_{2A}$ (OR=0.53; 95% CI=0.48-0.60) receptor occupancies (**Table 4.2**).

Using multivariate logistic regression analysis, higher receptor occupancies for dopamine D_3 (Adj. OR=1.43; 95% CI=1.13-1.56), D_4 (Adj. OR=1.12; 95% CI=1.02-1.23), α_1 adrenergic (Adj. OR=0.70; 95% CI=0.64-0.77), and serotonin $5HT_{1A}$ (Adj. OR=0.53; 95% CI=0.47-0.58) and $5HT_{2A}$ (Adj. OR=0.59; 95% CI=0.53-0.67) were significant. No other significant association were found for the remaining 6 targets.

Table 4.2. The association between antipsychotic receptor occupancy and hyponatremia, using logistic regression analyses.

Receptor	Cases (hyponatraemia)		Non-cases (other AEs)		Crude OR (95% CI)	Adj OR (95% CI) ^	Adj OR (95% CI) ^
	n	*%*	*n*	*%*	*Model 1 (univariate)*	*Model 2 (multivariate)*	*Model 3 (stepwise)*
D_2	1,255	66.0	113,892	66.8	0.96 (0.87–1.06)	0.94 (0.85–1.03)	-
D_3	765	40.2	61,177	35.9	1.20 (1.09–1.31)	**1.43 (1.13–1.56)**	1.21 (1.09–1.34)
D_4	706	37.1	57,128	33.5	1.17 (1.06–1.28)	**1.12 (1.03–1.23)**	-
H_1	979	51.5	101,463	59.5	0.72 (0.65–0.79)	0.81 (0.65–1.08)	-
M_1	909	47.8	93,675	55.0	0.75 (0.68–0.82)	1.02 (0.86–1.29)	-
M_2	143	7.5	22,732	13.3	0.93 (0.44–1.43)	1.21 (0.95–1.31)	-
α_1	937	49.3	97,469	57.2	0.72 (0.66–0.79)	**0.70 (0.64–0.77)**	-
α_2	88	4.6	8,672	5.1	0.90 (0.73–1.12)	1.05 (0.84–1.30)	-
5-HT_{1A}	488	25.7	67,774	39.8	0.52 (0.47–0.58)	**0.53 (0.47–0.58)**	-
5-HT_{2A}	1,563	82.2	152,651	89.6	0.53 (0.48–0.60)	**0.59 (0.53–0.67)**	0.78 (0.68-0.90)
5-HT_{2C}	1,416	74.5	135,657	79.6	0.84 (0.67–1.03)	0.95 (0.81–1.18)	-

OR: Odds Ratio; AOR: Adjusted Odds Ratio; CI: Confidence Interval

For each receptor: high receptor occupancy (≥50%) versus low receptor occupancy (<50%) set as the baseline level.

D1 receptor not included as none of the antipsychotic possess high occupancy.

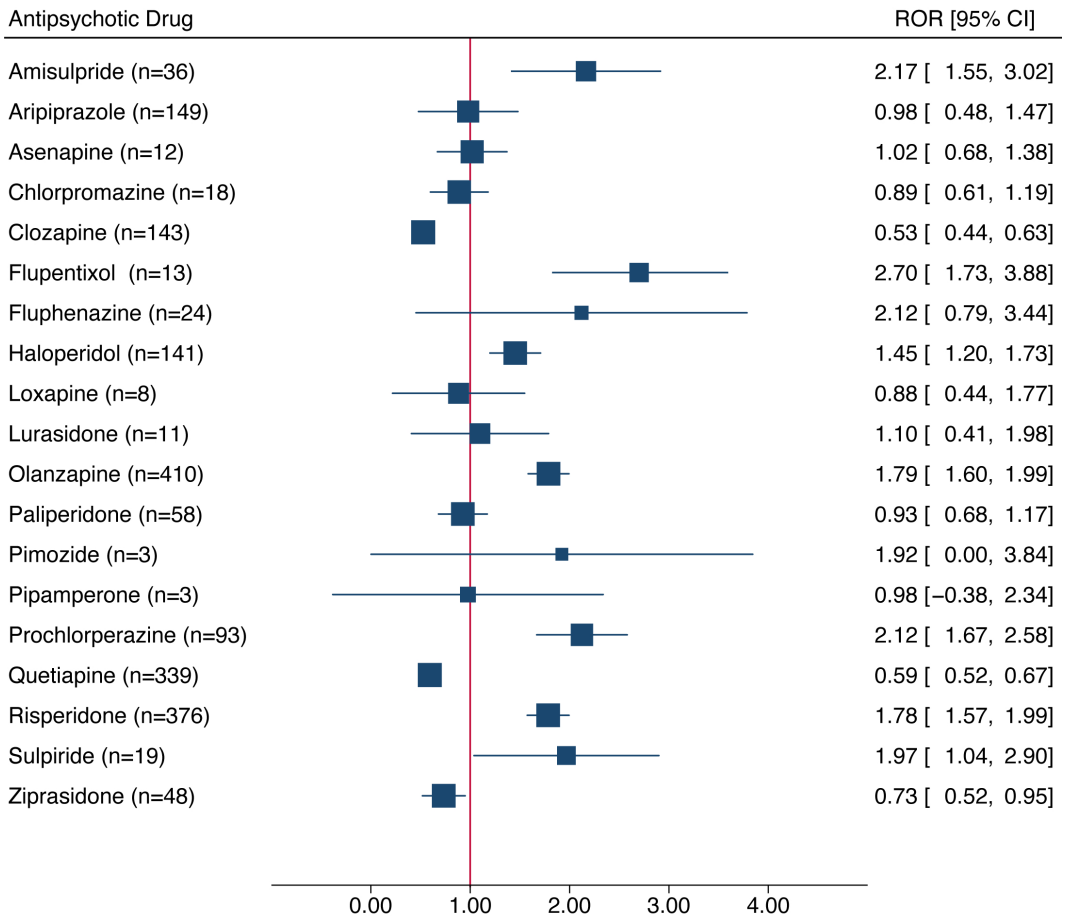
^adjusted for age, gender, concomitant use of medication associated with hyponatraemia, type of reporter and years since marketing.

Receptor targets significantly associated (D_3, D_4, α_1, $5HT_{1A}$ and $5HT_{2A}$) with hyponatraemia occurrence were then entered into a stepwise regression model while adjusting for all potential confounders. In the stepwise multivariable analysis, only dopamine D_3 (Adj OR=1.21; 95% CI=1.09-1.34) receptor occupancy found to be associated with significantly increased risk for hyponatraemia. In contrast, the occurrence of hyponatraemia decreased substantially with higher serotonin 5HT2A receptor (Adj OR=0.78; 95% CI=0.68-0.90) occupancy.

4.3.1.3. Case–Non-case Analysis

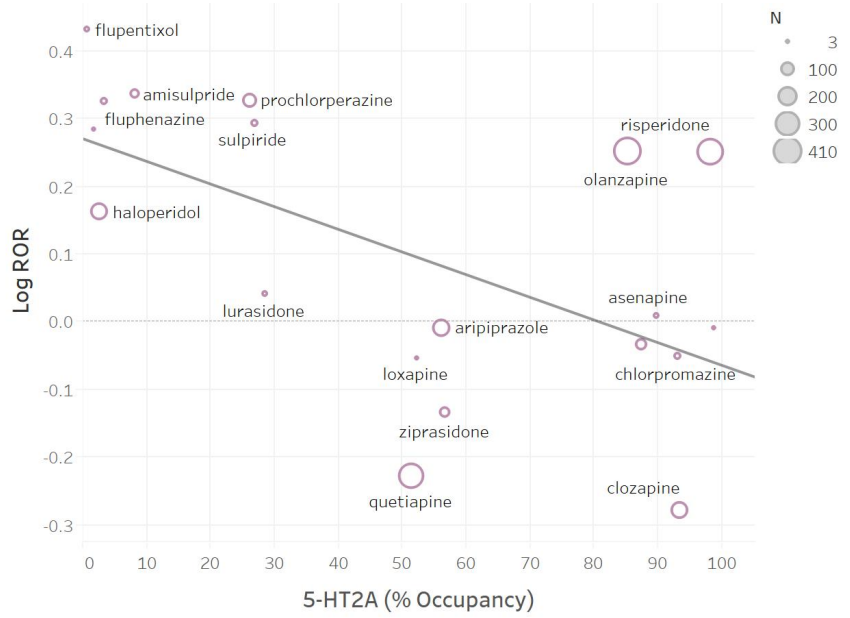
Results of disproportionality analysis for each antipsychotic drug are shown in **Figure 4.1**. For the individual antipsychotic drug, the highest RORs were found for flupentixol (n=13; ROR=2.70; 95% CI=1.73–3.88), amisulpride (n=36; ROR=2.17; 95% CI=1.55–3.02), prochlorperazine (n=93; ROR=2.12; 95% CI=1.67–2.58), and fluphenazine (n=24; ROR=2.12; 95% CI=0.79-3.44). In contrast, no SDR was found for lurasidone, asenapine, clozapine, quetiapine, ziprasidone, loxapine, chlorpromazine, aripiprazole, pipamperone and paliperidone. **Figures 4.2** and **4.3**, show the degrees of 5-HT_{2A} and D_3 receptor occupancies by antipsychotic drugs plotted against ROR, together with a line describing the logistic model.

Figure 4.1: Case–noncase analysis for the association between antipsychotic exposure and the occurrence of hyponatremia in FAERS.



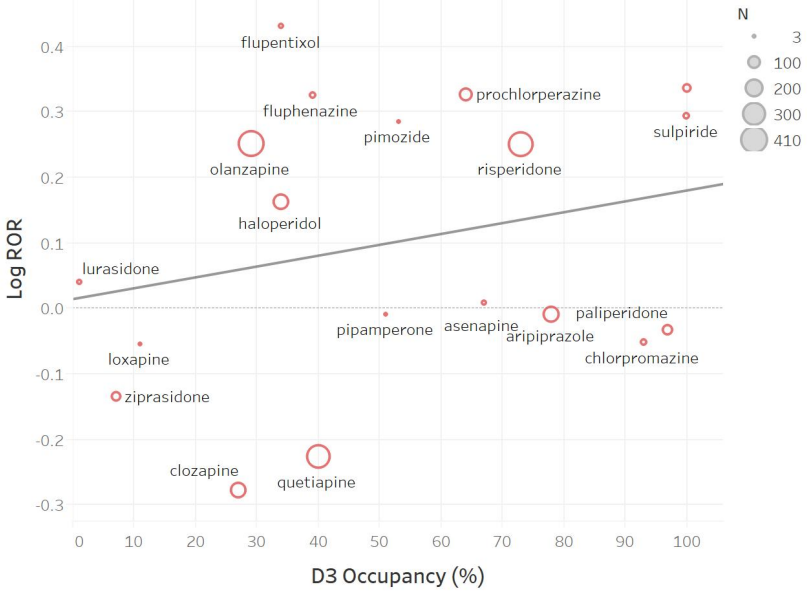
ROR: Reporting Odds Ratio; 95% CI: 95% Confidence Interval. Antipsychotics with less than 3 reports of diabetes are not presented (i.e. promazine, melperone, zuclopenthixol).

Figure 4.2. Relationship between 5-HT_{2A} receptor occupancy by antipsychotics and occurrence of hyponatremia in nested case-noncase population studied in the FAERS.



Bubble size shows sum of number of cases for each antipsychotic.

Figure 4.3. Relationship between D_3 receptor occupancy by antipsychotics and occurrence of hyponatremia in nested case-noncase population studied in the FAERS.

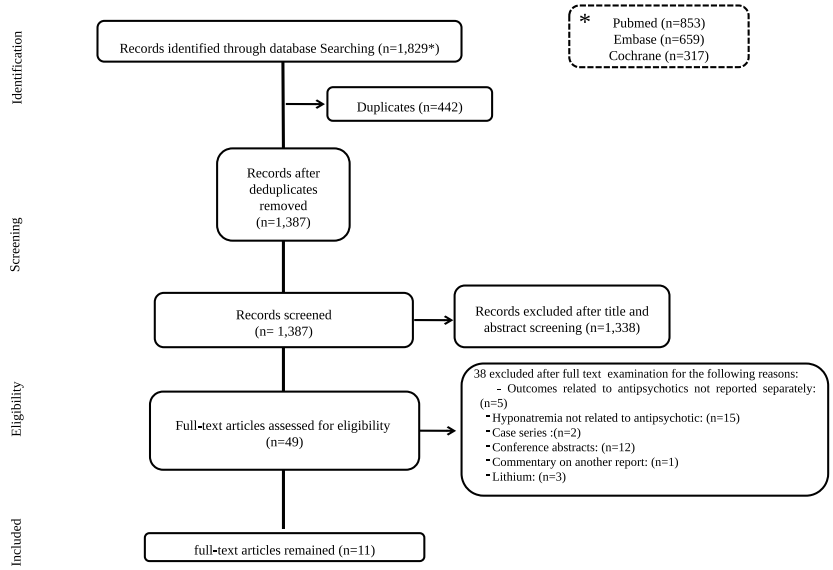


Bubble size shows sum of number of cases for each antipsychotic.

4.3.2. Systematic review of the literature

Figure 4.4 shows the flow of the systematic literature search. The search resulted in 1,343 unique titles. All titles were screened, after which 48 full-text articles were assessed for eligibility. Ultimately, 11 studies were identified which satisfied the eligibility criteria.

Figure 4.4. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) flow diagram of process of study selection.



4.3.2.1. Characteristics of the Reviewed Studies

No randomised controlled trials reporting hyponatraemia following antipsychotic drugs use were found. Eleven observational studies were eligible for the inclusion in our systematic review (**Table 4.3**); of these, 6 were case-control studies comparing hyponatraemia cases with controls (normonatraemia) for their relative exposure to antipsychotic drugs [180-184, 198], 2 were retrospective cohort studies that included control group as patients not exposed to antipsychotic drugs [199, 200], 2 were cross-sectional [201, 202]and 1 report from drug-surveillance programmes, relying on a system of enhanced monitoring of drug-related adverse events [113]. Shepshelovich et al. [202] exclusively looked for medication-induced SIADH.

Table 4.3: Studies on risk or incidence of Hyponatremia with the use of antipsychotics

Author, yr	Study Design				Patient Characteristics					Outcome					
	Duration (years)	Sample size	SNa^+ active monitoring	Cut-off SNa^+ (mEq/L)	In/ Out	Disease	Age (mean± SD)	F (%)	Exposed to AP (n)	Cases of hyponatraemia/no. exposed or no. of	Type of AP used	Type of measure	Overall	TAs	ATy APs
Case-control studies (hyponatraemia vs. normonatraemia)															
$Jun et al., 2020 [198]	5 (with ≥ 30 days follow-up for recurrent hyponatraemia)	19,173	No	<135	Out	Recurrent hyponatraemia	>65 (range: 65-75)	52.1	1,535	79/1,456^	Any	OR [95% CI]	0.83 [0.64-1.09]	-	-
Falhammar et al., 2019 [181]	9	71,741	No	<135	In	Ad	Median 76 (range: 18–103)	72	317 (newly initiated)	148/169^	Any	OR [95% CI]	1.80 [1.38–2.34];	2.94 [2.09–4.13]	1.05 [0.75-1.47]
Yamamoto et al., 2019 [183]	12	7,442	Yes	<130	In/ Out	Epilepsy	36.2 ± 14.4	46.7	504	17/487^	Any + anticonvulsant (except CBZ)	Incidence (%)	3.40	-	-
												OR [95% CI]	3.47 [2.03-5.95]	-	-
Yang and Cheng, 2017 [184]	15	2,051	No	<135	In/ Out	Psychiatric illness	54.7± 13.9	43.8	1,069	92/977^	Any	Incidence (%)	8.61	10.66	10.05

												HR [95% CI]	-	3.13 [1.83 – 5.34]	2.09 [1.36– 3.23]
Manu et al., 2012 [182]	1	924	No	<136	In	Ad	45.15 ± 19.6	46.8	642	37/605^	Any	Incidence (%)	6.11	11.84	5.29
Bun et al., 2011 [180]	1	248	Yes	<130	In	Psychiatric illness	46.45 ± 17.0	38.3	248	91/157^	Any	OR [95% CI]	1.79 [1.04-3.10]	-	-
Cohort Studies (antipsychotic users vs. non-antipsychotic users)															
Gandhi et al., 2016 [199]	9	116,016	No	≤132	Out	Ad	81 ± 7.7	66.8	58,008	86/58,008	ATyAPs	Incidence (%)	-	-	0.15
												RR [95% CI]	-	-	1.62 [1.15-2.29]
Lange-Asschenfeldt et al., 2013 [200]	3	7,113	Yes	<135	In	Psychiatric illness	Median 67 (range: 21 - 101) #	NR	4,976	199/4,976	Any	Incidence (%)	3.99	6.00	3.40
Cross-sectional															
Shpshelovich et al., 2017 [202]	6	198	No	<135	In	SIADH	66.6 ± 17.3	55.5	22	19/22	Any	Incidence (%)	86	-	-
Serrano et al.,	4	219	No	<135	In/ Out	Psychiatric illness	44.2 ± 15.7	52.5	183	13/183	Any	Incidence (%)	7	26	Clozapine = 3;

2014 [201]															others = 5
											Clozapine vs any other AP	OR [95% CI]	-	31.3 [3.9-247.0]	2.9 [0.5-18.2]
Observational multidrug surveillance program/ adverse drug reaction monitoring															
Letmaier et al., 2012 [113]	14	263,864	No	<130	In	Psychiatric illness	60.7± 15.9	55.7	189,462	5/189,462	Any	Incidence (%)	0.003	Perazine = 0.015; haloperidol = 0.007	Risperidone = 0.004

Patient characteristics: Ad = hospital admission for hyponatraemia; AP= antipsychotic; AtyAPs=atypical antipsychotics; F=females; In = inpatient; NR=Not Reported; Out = outpatient; SIADH= syndrome of inappropriate antidiuretic hormone secretion; TAs= typical antipsychotics;
Outcome: CBZ=carbamazepine; HR = hazard ratio; RR=relative risk ratio; OR = odds ratio (where provided we report only adjusted ratio)
^ number of patients without hyponatraemia (controls)
* reported as absolute number of hyponatraemia cases
$ cases and control were drawn from the population in a fully enumerated cohort
# values related only to patients who experienced hyponatraemia.

All included studies were retrospective, of which 3 [180, 184, 200] explicitly mentioned a systematic monitoring of serum sodium levels, defined as "active monitoring" in this review.

Four large-scale studies reporting hyponatraemia following antipsychotic treatment were identified: one population-based cohort study [194, 199] and 2 population-based case-control study [181, 184], using health administrative databases and 1 in a drug-surveillance program of psychiatric inpatients [113]. The RR, HR or OR of hyponatraemia associated with antipsychotic drugs was calculated in 7 studies: 5 case-control studies [180, 181, 183, 184, 198] 1 cohort study [199] and 1 cross-sectional study [201].

The quality of the included studies was moderate (mean NOS 6.9, standard deviation 2.31) (**Appendix III**). Four studies (one for cross-sectional and cohort and three for case-control studies) were classified as "high quality", and the three case-control studies were categorised as "moderate quality". Three studies (two for cross-sectional and one for cohort studies) were rated as low quality. In general, most studies with low-moderate quality had no score for comparability section and outcome assessment was unsatisfactory.

4.3.2.2. Risk or incidence rate of hyponatraemia

Because of considerable heterogeneity in study designs and characteristics of the studied populations and serum sodium (S_{Na+}) threshold values (S_{Na+} ≤130 vs. ≤135 mmol/L), incidence rates varied greatly, with the larger-scale studies or without active monitoring of serum sodium levels resulting in more modest rates. Studies with a S_{Na+} cut-off of ≤135 or ≤136 mmol/L resulted in incidence figures between 3.99% and 86%, whereas in studies using stringent a case definition of S_{Na+} ≤130 or ≤132 mmol/L or severe hyponatraemia, incidences between 0.003 % and 3.40% were reported.

The HR, OR or RR of hyponatraemia for pooled antipsychotic drugs was determined in 4 studies. The lowest risk was found by Bun et al. [180] (Adj. OR=1.79; 95% CI=1.04–3.40), whereas the higher risk was reported in adult epilepsy patients treated with antipsychotics in addition to antiepileptic drugs (carbamazepine excluded) (Adj. OR=3.47; 95% CI=2.03-5.95) [183]. One

population-based cohort study reported atypical antipsychotic use compared to non-use was associated with an increased risk of hospitalization with hyponatraemia within 30 days (RR=1.62; 95% CI=1.15–2.29) [199]. Jun et al. [198] found no association between the use of antipsychotics and recurrence of symptomatic or severe hyponatraemia in older patients (Adj. OR=0.83; 95% CI=0.64-1.09).

Several studies exclusively reported the occurrence of hyponatraemia in users of atypical or typical antipsychotic users. Use of typical antipsychotic consistently reported for increased risk of hyponatraemia as compared to atypical antipsychotic. The Swedish register-based case-control study reported user of typical antipsychotics were more likely to experience severe hyponatraemia (adj. OR=2.94; 95% CI=2.09–4.13) than those on atypical antipsychotics [adj. OR=1.05; 95% CI=0.75–1.47) [181]. Similarly, a population-level case-control study using Taiwan's claim database reported an elevated risk of hyponatraemia with typical antipsychotics [Adj. HR= 3.13 (1.83–5.34)] vs atypical [Adj. HR= 2.09 (1.36–3.23)] [184].

The same distribution was found in the AMSP (Arzneimittelsicherheit in der Psychiatrie) study, with a higher incidence of hyponatraemia with the typical antipsychotic perazine (0.015%) and haloperidol (0.007%), and lower with risperidone (0.004%) [113]. In a case-control study by [182], hyponatraemia was reported to occur less with atypical antipsychotics [5.29%; n=9/76] than with typical antipsychotics [11.84%; n=28/529], however no statistically significant difference was found.

4.4. DISCUSSION

To date, the mechanism by which antipsychotics induce hyponatraemia is not well understood. This is the first study aimed at assessing whether the degrees of antipsychotics' receptor occupancy explain the occurrence of hyponatraemia, by using one of the largest spontaneous reporting system databases, *i.e.,* FAERS. We found a statistically significant positive association with dopamine D_3 receptor degrees of occupancy and hyponatraemia

occurrences and a negative one with serotonin 5-HT_{2A} degree. These associations persisted after adjustment for potential confounding factors such as sex, age, concomitant medications and type of reporter.

The evidence for the relationship between neuroendocrine abnormalities and D3 receptors in the literature are scarce. Dopamine D_3 is a member of the D_2-like receptor family that can couple to effector mechanisms similar to the D_2 receptor subtype [203, 204]. Dopamine D_3 receptors are unique among the D_2-like receptors, exhibiting sustained high affinity for dopamine (>20-fold higher than D2 receptors), suggesting that D3 receptors, in vivo, are occupied by endogenous dopamine for extended periods, leading to high spontaneous activation of D_3 receptors [205, 206]. Accordingly, small changes in the number or function of D_3 receptors may lead to dramatic effects on synaptic transmission, suggesting that D_3 receptors could be critical modulators of normal dopaminergic function. Due to the lack of selective D_3 receptor antagonists available on the market, direct evidence on the effects of selective dopamine D_3 receptor antagonists on neuroendocrine abnormalities is lacking.

Many atypical antipsychotic drugs and some typical antipsychotic ones have high affinities for both D_2 and D_3 receptors [207]; the high affinity of endogenous dopamine for D_3 receptors has been postulated to result in only minimal or no D_3 receptor occupancy by antipsychotic drugs in dopamine rich areas [208-211].

The involvement of D_2 receptors in the regulation of neuroendocrine abnormalities has been described in several [178, 179, 212] animal models. It is generally thought that antipsychotics may stimulate ADH release in the brain by supersensitivity of D_2 receptors. Therefore, neuroendocrine dysfunction might be explained, at least in part, by inhibition of the dopamine D_3 receptor. Our FAERS analysis does not support this possibility as we did not retrieve any case where cariprazine, a D_3-preferring antipsychotic, was suspected in the occurrence of hyponatraemia, consistently with the fact that only one case of hyponatraemia associated with the use of cariprazine leading to drug discontinuation has been reported to date [213]. It must be acknowledged, however, that cariprazine has only been recently

commercialised, and we need to wait for additional observational studies using real-world data on larger scale samples to reach a definite conclusion.

Some early animal studies showed secretion of ADH through serotonin-mediated effects on central 5-HT_2 and 5-HT_{1C} receptors [137, 176, 214]. It has been speculated that the effect of various psychotropic drugs on serotoninergic transmission contribute to excess ADH secretion. However, a study we recently performed using the FAERS database [186], showed that serotonin-mediated neurotransmission may not be involved in the hyponatraemia associated with antidepressant drugs. The results of the present analysis, and our previous results, suggest that the emergence of hyponatraemia with antipsychotic is linked when D_3 receptor occupancy exceeds a certain threshold, whereas high 5-HT_{2A} occupancy provides relative protection from hyponatraemia.

Our multivariable model showed that both blockades of dopamine D_3 and serotonin 5-HT_{2A} receptors independently explain the risk of hyponatraemia. The case/non-case study we did supports thus the hypothesis that hyponatraemia induced by antipsychotic drugs results from a disruption of the fine balance between dopaminergic- and serotonergic-mediated transmission. The unbalanced inhibition of dopamine D_3 and serotonin 5-HT_{2A} receptors can explain why antipsychotics which have high D_3 and low 5-HT_{2A} occupancies, such as amisulpride, sulpiride, and prochlorperazine, were the ones we found most associated with hyponatraemia. Consistently, antipsychotics with nearly balanced antagonistic activities at D_3 and 5-HT_{2A} (pipamperone, risperidone, chlorpromazine, asenapine, paliperidone, aripiprazole, and quetiapine) or high 5-HT_{2A} antagonist property were the ones least associated with hyponatraemia.

In the systematic review we did observe considerable heterogeneity across studies, with incidence rates of hyponatraemia following any antipsychotic use diverging between 0.003 % and 86%, whereas the odds developing hyponatraemia from effect studies range between 0.83 to 3.47. Regarding the classes of antipsychotic drugs, odds ratios for typical antipsychotics (2.9-31.3) were consistently higher than for atypical antipsychotics (1.1-2.9). The

risks associated with individual antipsychotics drugs between two classes could not be established due to insufficient information.

Despite this limitation, our review clearly shows that the risk of hyponatraemia is higher with typical antipsychotics but not confined to these agents several novel aspects. It also reveals that study methodologies greatly affected outcomes, particularly, the choice (or lack) of non-exposed groups or the presence/absence of active monitoring for hyponatraemia. Timing and clinical indication for sodium level checks varied and often were unclearly reported. The commonly accepted definition of hyponatraemia is a serum sodium level lower than the arbitrarily defined threshold of 135 mEq/L, however, some studies selected a 130 mEq/L threshold, which may, in fact, be more relevant to the severity or clinical practice, considering that symptoms seldom occur at higher values. Additionally, inconsistencies in hyponatraemia case definitions compromised comparison. Studies also differed with respect to confounding factors possibly causing or contributing to hyponatraemia; some studies chose to exclude such cases [183], whereas others included them but performed multivariate regressions for statistical correction [184, 199].

4.4.1. Limitations and strengths

The use of a spontaneous reporting system database has some important implicit limitations, because reporting is influenced by factors such as the notoriety bias, selection bias and under-reporting [161]. The PV–PD analysis based on pharmacological receptors theory has its own limitations [162], for instance, receptor affinity does not directly reflect the intrinsic activity of a drug. For instance, receptor occupancy does not necessarily mean antagonism, and the interaction at the receptor level of antipsychotics is complex and only partially characterised. Moreover, some antipsychotics have high occupancy but fast dissociation which is sufficient to elicit a biological therapeutic response. Nevertheless, PV–PD analysis provides reliable estimates of putative relationship and in recent past years, studies validated pharmacological preclinical studies in humans by combining real-word data with pharmacodynamic data. We have used unbound therapeutic plasma

concentrations to estimate receptor occupancies, while a brain concentration would be the ideal approach, however, monitoring of the human brain concentration of unbound drug is experimentally not feasible.

Unlike typical case-control studies, cases and non-cases are drawn from different populations, thus, this method cannot be a substitute for the classical case-control but gives relatively reliable results if the database contains several thousand of different drugs−event combinations. RORs are influenced by potential confounders such as selective under-reporting, follow-up period bias and exposure misclassification bias. Neither the prevalence, nor the incidence rate of ADRs can be computed from the analysis of the FAERS dataset since the primary goal of such system is to signal the existence of a possible relationship between a drug or drug class and an ADR, and it does not prove causality.

As a primary limitation, our systematic review included a small number of studies, which may influence the quality and the strength of the results. Moreover, heterogeneity in study design (case-control, cohort, cross-sectional) precluded us to pool the estimates.

Despite these limitations, the present work has several strengths. The study was conducted using one of the largest pharmacovigilance databases, which includes more than 10 million reports. Furthermore, our base cohort contains a considerably higher number of reports, strengthening the statistical power of the analyses. Furthermore, to contribute in closing the knowledge gap on this issue, we used a relatively innovative approach in the field of pharmacovigilance and unlike previous studies that applied a similar approach to investigate pharmacological mechanisms of ADR using one pharmacological target in the linear regression model, we considered all relevant targets in our multivariable logistic regression analyses.

4.5. CONCLUSION

This is the first study aimed at evaluating the potential association between degrees of antipsychotics' receptor occupancy and the occurrence of

hyponatraemia, by using one of the largest spontaneous reporting system databases, *i.e.,* FAERS. Of importance, our PV–PD analysis showed that both blockades of dopamine D_3 and serotonin 5-HT_{2A} receptors independently explain the risk of hyponatraemia. The unbalanced inhibition of dopamine D_3 and serotonin 5-HT_{2A} receptors can explain why antipsychotics which have high D_3 and low 5-HT_{2A} occupancies (amisulpride, sulpiride, and prochlorperazine), were the ones we found most associated with hyponatraemia. Consistently, antipsychotics with nearly balanced antagonistic activities at D_3 and 5-HT_{2A} (pipamperone, risperidone, chlorpromazine, asenapine, paliperidone, aripiprazole, and quetiapine) or high 5-HT_{2A} antagonist property were the ones least associated with hyponatraemia. Given the fact that antipsychotics have high affinities for multiple receptors and receptor subtypes, the potential for specific adverse events varies for each different antipsychotic. Therefore, drug-specific side-effect profiles should be considered together with patient-specific risk factors when deciding on the optimal treatment for individual patients.

Our review of the current literature, based on the few, nonetheless, relatively large-scale studies published to date, shows that typical antipsychotics have a more attenuated risk profile for hyponatraemia. However, hyponatraemia can also occur with these agents, albeit less frequently. We were unable to draw conclusions regarding the risks associated with individual antipsychotic owing to insufficient information. Prospective controlled studies are needed that assess the risk of hyponatraemia more systematically in well-defined, larger-scale populations.

CHAPTER 5: STUDY 3

CHAPTER 5: STUDY III

Bullous pemphigoid induced by dipeptidyl peptidase-(DPP-4) inhibitors: a pharmacovigilance-pharmacodynamic assessment through an analysis of the VigiBase®

ABSTRACT

Objectives: To examine the signals of bullous pemphigoid (BP) with dipeptidyl peptidase-4 inhibitors (DPP-4i) in VigiBase® and the potential role of their pharmacodynamic/pharmacokinetic parameters in the occurrence of BP.

Methods: Case/non-case analyses were performed in VigiBase® to examine the signal of BP [reporting odds ratio (ROR)] for gliptins. Secondly, the authors performed linear regression analyses to explore the association between DPP-4i signals for BP and their affinities toward different target enzymes (DPP-2, DPP-4, DPP-8, and DPP-9) and their volume of distribution (Vd).

Results: A significant BP signal was found for DPP-4i. The ROR for pooled DPP-4i was 179.48 (95% CI: 166.41–193.58). The highest ROR was found for teneligliptin 975.04 (801.70–1185.87) and lowest for saxagliptin 18.9 (11.5–30.9). Linear regression analyses showed a considerable trend to significance for the linear correlation between the BP signal and gliptin affinity at DPP-4 (slope = 1.316 [−0.4385–3.21], p = 0.067, R^2 = 0.40) but not the other enzyme targets, nor for Vd.

Conclusion: The findings suggest a clinical relevance of gliptins selectivity for DDP-4 in the development of BP as a result of exposure to these drugs. Future preclinical and clinical studies are needed for a better understanding of this correlation.

5.1. INTRODUCTION

Bullous pemphigoid (BP) is an uncommon autoimmune subepithelial blistering disease that most frequently arises in elderly and is characterized by the presence of cutaneous bullae and erosive mucosal lesions. The mechanism that leads to BP is not fully understood, but most likely involves autoantibody-mediated damage to the epithelial basement membrane

zone, a complex structure that mediates adhesion, permeability, and cellular organization and differentiation [215]. Autoantibodies against two principal hemidesmosomal proteins, bullous pemphigoid antigen 180 (BP180) and bullous pemphigoid antigen 230 (BP230), are strongly linked to the clinical disease. Genetic factors and the phenomenon of epitope spreading are also considered potential contributory factors [216-219].

Autoimmune reactions triggered by exposure to certain drugs may thus be a cause of BP, as a result of drugs acting as antigens in the basement membrane zone. Even though the strength of this association is uncertain [220], a variety of drugs has been associated with the development of BP (**Appendix IV**) [221, 222]. In particular, dipeptidyl peptidase-4 (DPP-4) inhibitors, a class of anti-hyperglycemic drugs widely used in the treatment of diabetes mellitus, previously associated with hypersensitivity reactions (including anaphylaxis, angioedema) and blistering skin conditions (including Stevens-Johnson syndrome) in post-marketing reports, have emerged recently as a putative triggering factor of BP [223-226]. The association between DPP-4 inhibitors and the development of BP comes mainly from clinical reports, a few disproportionality analyses of pharmacovigilance databases and a limited number of case-controlled studies [9–13].

The only available metanalysis addressing this topic shows that DPP-4 inhibitors exposure is associated with a 3.2-fold increased risk for BP, with a variety of risks associated with each drug; however, the limited number of studies included in this analysis may have interfered with the findings, highlighting current gaps and the need to elucidate this issue better [227].

Despite the fact that an increasing number of cases of BP induced by DPP-4 inhibitor use is reported in the literature, the exact mechanism underlying this association remains unclear and needs to be elucidated. Several different theories including selectivity of drug toward different DPP-4 family enzymes [228-231], higher volume of distribution and gliptin-induced inhibition of the DDP-4/cluster of differentiation 26 (CD26) mediated helper T cell type-1component of immunity have been postulated as potential mechanisms of the association between BP and DPP-4 exposure [232, 233].

Data mining of large pharmacovigilance databases is of great importance

in the detection of the earliest possible signals; in addition, coupling information from disproportionality analyses with pharmacodynamic characteristics of drugs (such as receptor occupancy or enzyme inhibition) allows the investigation of a potential pharmacological mechanism of a given adverse drug reaction (ADR) [234].

The primary objective of the present study was to examine the potential signals of BP with DPP-4 inhibitors by performing a data-mining of cases of BP submitted to the largest and most comprehensive pharmacovigilance database, i.e., VigiBase®. Our secondary objective was to examine the potential role of pharmacological (pharmacodynamic/pharmacokinetic parameters) characteristics of different gliptins in the occurrence of BP risk as a result of exposure to DPP-4 inhibitors.

5.2. MATERIAL AND METHODS

5.2.1. Data source

We analysed the reports of suspected ADRs in VigiBase®, the WHO global Individual Case Safety Reports (ICSRs) database [235], which aimed to identify the earliest possible pharmacovigilance signals. VigiBase® contains over 18 million Individual case safety reports (ICSRs) until February 2019 (started in 1968). Drugs recorded in the reports are coded according to the latest version of the WHO Drug™ dictionary. ADRs are coded according to the latest version of the Medical Dictionary for Regulatory Activities (MedDRA®). Analyses exploiting this database may be performed via queries in VigiLyze™ [236], which is an online resource delivering useful searches and subsequent data extractions of VigiBase®.

5.2.2. Case/non-case analysis

A case/non-case analysis was performed to quantify the association between DPP-4 inhibitors exposure and BP occurrence in the VigiBase®. In this analysis, disproportionality methods are used to identify statistical associations between drug and events in pharmacovigilance databases. Such methods compare the observed count for a drug-event combination with an 'expected' count.

Cases were defined as all ICSRs corresponding to the BP (MedDRA Preferred Term 'Pemphigoid') between January 2006 to 3 February 2019.

This time period was determined by the fact that the first-ever DPP-4 inhibitor was approved in the USA in 2006. Non-cases were all reports of ADRs other than the ADR of interest (BP) during the same period. Exposure was defined as 'DPP-4 inhibitor(s)' [Anatomical Therapeutic Chemical classification fourth-level code A10BH] at the time of the ADR occurrence, where it is suspected in the occurrence of ADR. Our exposure definition also includes DPP-4 inhibitors with more limited geographical availability that do not have ATC codes (anagliptin, teneligliptin, trelagliptin, omarigliptin, and gosogliptin).

5.2.3. Pharmacological data and data sources

We investigated the potential role of pharmacodynamic/pharmacokinetic characteristics of DPP-4 inhibitors in the appearance of BP. For this purpose, we coupled pharmacodynamic/pharmacokinetic data of DPP-4 inhibitors with their corresponding reporting risks for BP in VigiBase® as a result of the DPP-4 inhibitor exposure.

Enzymatic inhibition of the DPP-4 family by DPP-4 inhibitors is based on affinity measures such as IC_{50} (concentration at which there is 50% inhibition of measured activity in vitro, dependent on substrate concentration) and Ki (enzyme–inhibitor dissociation constant, independent of substrate concentration) values for their biological targets or interactions (DPP-2, DPP-4, DPP-8, and DPP-9). For each DPP-4 inhibitor studied, values of affinity measures for DPP-2, DPP-4, DPP-8, and DPP-9 potentially involved in gliptins-induced BP were searched in the BRENDA [237], BindingDB [194], PubChem, European Bioinformatics Institute-ChEMBL [131], KEGG LIGAND [238] and DrugBank databases [239]. For the purpose of this study, we treated all affinity measure available in the databases (IC50 or Ki) as equivalent.

The profiles of affinity measures (negative log of the IC50 or Ki value in molar) for different DPP-4 inhibitors at DPP-2, DPP- 4, DPP-8, and DPP-9 are summarized in the supplemental table and can be access at https://doi.org/10.1080/14740338.2019.1668373. Values of affinity measures are expressed as an arithmetic mean of values as determined in enzyme assays. Affinity measures obtained from assays performed on human samples were selected. Where data from the human sample were not

available, we considered data from non-human sample assay as replacements. In the case of a lack of information (e.g., alogliptin for DPP-2), we used the Ki value of 4 in general. Alternatively, we also profiled fold selectivity data of each studied DPP-4 inhibitors for inhibition of DPP-4 enzyme over DPP-8/DPP-9. To make fold selectivity data levelled, we used base 2 logarithm for expressing the fold selectivity of DPP-4 enzyme vs. DPP-8/DPP-9 (**Appendix V**).

For pharmacokinetic data (volume of distribution), Integrated Database of ADMET and Adverse effects of Predictive Modelling (IDAAPM) [193], KEGG LIGAND [238] databases and individual monographs or regulatory documents were searched.

5.2.4. Data analyses

For the case/non-case analysis, the strength of an association between DPP-4 inhibitors and BP occurrence was estimated by computing measures of disproportionality, expressing as the reporting odds ratio (ROR) and its two-sided 95% confidence interval (CI). These measurements of disproportionality were based on a 2 × 2 contingency table. An ROR is considered significant when the lower limit of the 95% two-sided CI exceeds 1, and it is considered to reach a threshold value for the disproportionate reporting risk of BP. A minimum of three AE reports was the pre-requisite to consider a drug-event as clinically relevant and the same was selected for case/non-case analysis.

First, we performed a case/non-case analysis to investigate a potential BP signal for all DPP-4 inhibitors altogether. All the DPP-4 inhibitors were compared with all the other drugs stated in each report in the whole of VigiBase® during the study period. A similar analysis was carried out for each individual DPP-4 inhibitor drug (alogliptin, anagliptin, linagliptin, omarigliptin, saxagliptin, sitagliptin, teneligliptin, trelagliptin, and vildagliptin) [analysis a]. Each DPP-4 inhibitor drug was compared with all the other drugs (including the other DPP-4 inhibitor drug) found in VigiBase®. To account for the possible role of co-medication (co- suspect) as an effect modifier, stratified estimates were also computed after removing all cases where another medication (other than DPP-4 inhibitors) that are known to cause BP was also suspected in the occurrence of BP [analysis b]. Sensitivity analysis was also carried out by comparing DPP-4

inhibitors with all other drugs that are known to cause BP [analysis c].

In the same way, each anti-hyperglycaemic drug class [biguanides, α-glucosidase inhibitors (α-GIs), glinides, glucagon-like peptide 1 receptor agonists (GLP-1), sulfonylureas, sodium-glucose cotransporter 2 (SGLT2) inhibitors, and thiazolidines] excluding all kind of insulins was compared with all the other classes of drugs (including the other classes of anti-hyperglycaemic) and finally, another one for each of the anti-hyperglycaemic drug belongs to above-mentioned classes. Because most patients with diabetes mellitus receive a combination of medications from different anti-hyperglycaemic drug classes, thus, to avoid co-prescription bias we also analysed each anti-hyperglycaemic drug after excluding cases that received DPP-4 inhibitors. Finally, we also examined drugs that are known to induce the BP stratified by pharmacological class or chemical group (**Appendix IV**). Each drug was compared with all the other drugs (including DPP-IV inhibitors) found in VigiBase®.

For the pharmacodynamic analysis, we performed linear regression models including two quantitative variables: RORs estimated for each individual DPP-4 inhibitor [analysis a] and affinity measures for DPP-4 inhibitors at DPP-4 and off-targets (DPP-2, DPP-8, and DPP-9) or fold-selectivity for inhibition of DPP-4 to detect potential linear association(s) between them. RORs for BP were treated as the dependent variable and affinity measures as the independent one. For simplicity's sake of pharmacodynamic analysis, base 2 logarithms of the RORs were taken. A significant correlation suggests, while not proving with absolute certainty, that the affinity of a DPP-4 inhibitor drug for the off-target enzymes explains to some extent different level of BP reporting (quantified as ROR). Likewise, a pharmacokinetic parameter, the volume of distribution, was taken as an independent variable and RORs estimated for each individual DPP-4 inhibitor as a dependent. Statistical analyses were conducted using the software package STATA® (StataCorp, College Station, TX, USA). This investigation complied with the REporting of studies Conducted using Observational Routinely collected health Data (RECORD) statement.

5.3. RESULTS

5.3.1. Case selection

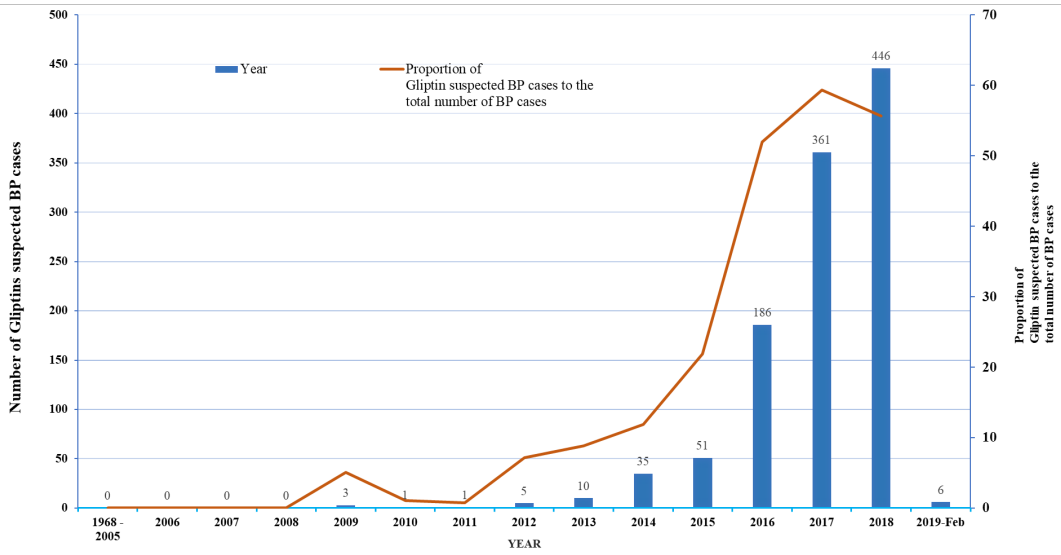
During the study period (between 2006 and 2019), 15,020,678 ICSRs were entered in the VigiBase®. Among these, 2,947 ICSRs were related to BP (cases). Of the 2,947 cases, 1,070 (36.3%) were involved at least one DPP-4 inhibitor suspect. Among 1,070 cases with DPP-4 inhibitor suspect, in 368 cases other drugs in addition to a DPP-4 inhibitor were also suspected in the bullous pemphigoid occurrence. In 35 cases, more than one DPP-4 inhibitor was involved as the suspect drug.

5.3.2. Characteristics of the cases

The number of gliptins suspected BP cases increased exponentially over time between 2006 and 2019, with a spike recorded in the year 2018 for which the highest number of cases (n = 446) were recorded (**Figure 5.1**). The proportion of gliptin suspected BP cases to the total number of BP reported to VigiBase® in the studied period had also been steadily increasing over time.

The male-to-female ratio was 0.59 and the median age was 78 years [interquartile range (IQR) 70–83, range 41–100]. More than four-fifths (931/1070 = 87%) of the cases were designated adjudicated as serious by the reporter. Of serious 931cases, 55 (6%) were with fatal outcome. The improvement of the BP upon the withdrawal of DPP-IV inhibitor (positive DE-challenge) was reported in 57% (593/1028) of the cases whereas re-occurring of BP on re-administration of DPP-4 inhibitor (positive challenge) was mentioned in only seven cases. Metformin was a co-suspect with a DPP-IV inhibitor in 36 of 1070 cases (3.36%).

Figure 5.1. Number of DPP-4 inhibitor(s) suspected Bullous Pemphigoid cases reported to the VigiBase® between 2006 and 2019. Proportions of DPP-4 inhibitor(s) suspected Bullous Pemphigoid cases to the total number of Bullous Pemphigoid cases reported to the VigiBase® are shown by a line. Legend: BP: Bullous Pemphigoid.



5.3.3. Case–noncase analysis

Comparison of DPP-4 inhibitors all together [including cases with co-suspect] showed an increase ROR for BP (179.48; 95% CI: 166.41–193.58) (**Table 5.1**). When each individual DPP-4 inhibitor [including co-suspect] compared with all other drugs in VigiBase®, the highest RORs were found for teneligliptin (975.04; 95% CI: 801.70–1185.87), followed by omarigliptin (672.31; 95% CI: 376.68–1199.97), vildagliptin (399.70; 95% CI: 362.26–441.02) and trelagliptin (240.53; 95% CI: 118.25–489.23). Of note, RORs for omarigliptin (n = 13) and trelagliptin (n = 8) were calculated from a small number of cases compared to other DPP-4 inhibitors. After removing all cases where drugs other than DPP-IV inhibitors were suspected in the occurrence of BP, the magnitude of signals of disproportionate reporting was markedly decreased. Sensitivity analysis of the comparison of the individual DPP-4 inhibitors with all other drugs known to induce BP (positive controls) or reported to be associated with the development of BP in the literature confirmed that the pooled RORs of DPP-4 inhibitors for BP (62.98; 95%: 57.66–68.79) were greater over the

studied period than for any other drug suspected in BP occurrence.

Among other anti-hyperglycaemic drug classes after exclusion of cases who were receiving DPP-4 inhibitors, significant RORs were observed only for repaglinide (ROR: 10.23; 95%: 4.25–24.63) and for the sulphonamides: glimepiride, gliclazide, torsemide and glibenclamide (Table 5.1).

Table 5.1: Reporting odds ratios (RORs) for the association between different dipeptidyl peptidase-4 inhibitor, all other antihyperglycemic drugs and bullous pemphigoid

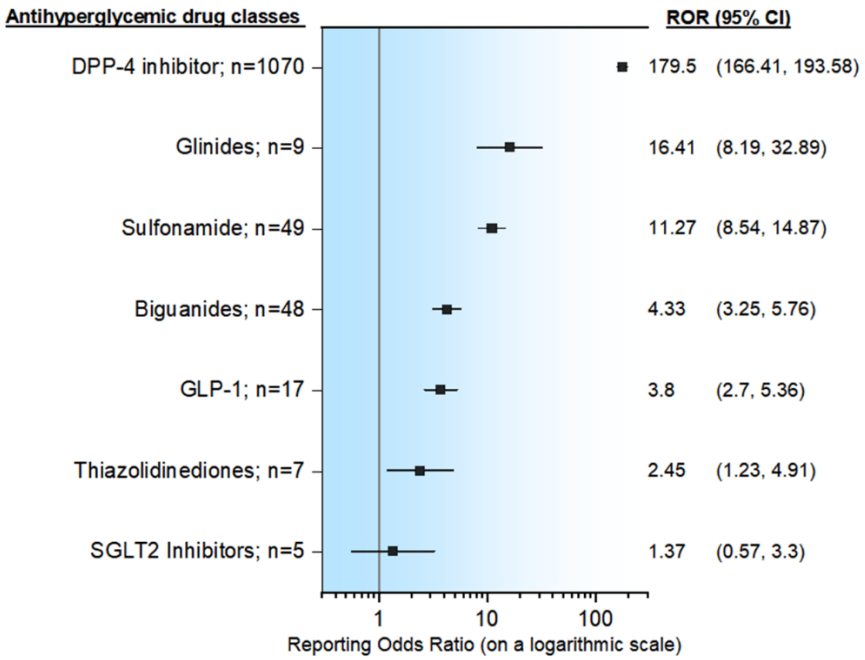
Drug	**With BP** (n)	**Without BP** (n)	**ROR (95% CI)**
Dipeptidyl peptidase-4 inhibitor [Including cases where drugs other than DPP-IV inhibitors were suspected in the BP occurrence]			
pooled	1070	47,561	179.50 (166.41– 193.58)
Alogliptin	35	1246	144.89 (103.35– 203.14)
Anagliptin	9	320	143.80 (74.07– 279.18)
Linagliptin	173	6538	143.23 (122.60– 167.33)
Omarigliptin	13	99	672.31 (376.68– 1199.97)
Saxagliptin	16	4328	18.94 (11.58– 30.99)
Sitagliptin	223	26386	46.52 (40.57– 53.36)
Teneligliptin	123	671	975.04 (801.70– 1185.87)
Trelagliptin	8	170	240.53 (118.25– 489.23)
Vildagliptin	505	7768	399.70 (362.26– 441.02)
Dipeptidyl peptidase-4 inhibitor [excluding cases where drugs other than DPP-I inhibitors were suspected in the BP occurrence**]**			
pooled	702	47929	97.69 (89.71–106.38)
Alogliptin	26	1255	106.53 (72.13–157.35)
Anagliptin	7	322	111.07 (52.48–235.07)
Linagliptin	139	6572	113.10 (95.22–134.33)
Omarigliptin	11	101	557.23 (298.75–1039.35)
Saxagliptin	11	4333	12.99 (7.18–23.49)
Sitagliptin	164	26445	33.42 (28.53–39.13)
Teneligliptin	85	709	629.23 (500.97–790.32)
Trelagliptin	6	172	178.18 (78.88–402.45)
Vildagliptin	394	7879	294.08 (263.88–327.74)
Dipeptidyl peptidase-4 inhibitor [Including cases where drugs other than DPP-IV inhibitors were suspected in the BP occurrence] vs all other drugs known to cause BP]			
DPP-4 inhibitor	1070	47929	62.98 (57.66–68.79)

all other drugs known to cause BP	934	2635050	
Biguanides [excluding DPP-4 inhibitors]			
Metformin	14	57,275	1.25 (0.74 – 2.11)
Glinides [excluding DPP-4 inhibitors]			
Repaglinide	5	2,494	10.23 (4.25– 24.63)
Glucagon-like peptide 1 [excluding DPP-4 inhibitors]			
Exenatide	11	65,119	0.86 (0.48–1.56)
Liraglutide	5		0.89 (0.37– 2.14)
Sodium-glucose Cotransporter-2 (SGLT2) Inhibitors [excluding DPP-4 inhibitors]			
Canagliflozin	2	18,589	0.55 (0.14– 2.19)
Sulfonamide [excluding DPP-4 inhibitors]			
Glimepiride	6	9,038	3.39 (1.52–7.55)
Gliclazide	8	5,895	6.93 (3.46–13.88)
Torasemide	5	3,761	6.79 (2.82–16.33)
Glibenclamide	5	9,038	5.34 (2.22 –12.85)
Thiazolidinediones [excluding DPP-4 inhibitors]			
Pioglitazone	3	16,645	0.92 (0.30–2.85)

BP: Bullous Pemphigoid; NA: Not included in the statistical analysis because the analysis was based on unique drug–event combinations with at least three occurrences.
Non-significant RORs are presented in light text.
[a] A report may contain more than one suspected DPP-4 inhibitor drug. For this reason, the total number of reports in which an individual DPP-4 inhibitor
drug (n = 1070) was mentioned as the suspected drug was higher than the total number of reports in which DPP-4 inhibitor drug was mentioned as the suspected drug (n = 1105).
[b] See **Appendix IV** for the list of drugs known to cause BP.

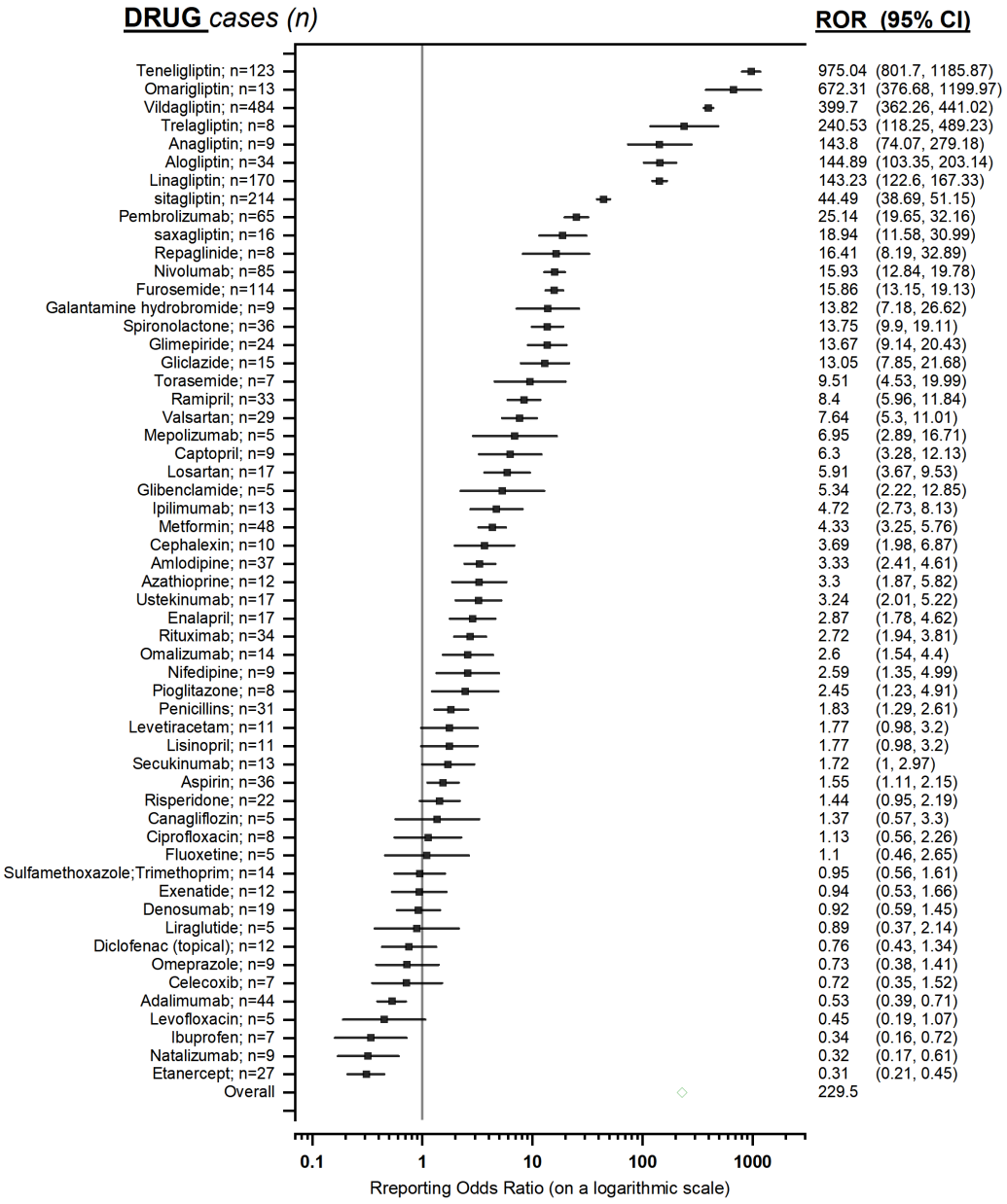
When comparing each anti-hyperglycaemic class with all other drugs in VigiBase® (including drugs from other classes of anti-hyperglycaemic drugs), RORs for pooled DPP-4 inhibitors (ROR: 179.48; 95%: 166.41–193.58) were far greater than for any other anti-hyperglycaemic drug class (**Figure 5.2**). RORs for drugs that are known to induce the BP are presented in **Figure 5.3**. ROR for furosemide (for which re-challenge evidence supports an association with BP) was (ROR: 15.8; 95% CI: 13.1–19.1).

Figure 5.2. Results of the case/non-case analysis for different classes of antidiabetic agents. (each class of antidiabetic was compared with the all the other drugs in the VigiBase®).



α-GIs: glucosidase inhibitors; GLP-1: glucagon-like peptide 1 receptor agonists; SGLT2: sodium-glucose cotransporter 2 inhibitors

Figure 5.3. Forest plot, on a logarithmic scale, showing adjusted reporting odds ratios of different drugs known to induce bullous pemphigoid (each drug was compared with the all the other drugs in the VigiBase®, including DPP-4 inhibitors)



5.3.4. Pharmacovigilance–pharmacodynamic/ pharmacokinetic assessment

Details of the regression analyses for the putative association between RORs for individual DPP-4 inhibitors obtained from case/noncase analysis and various pharmacodynamic/pharmacokinetic predictable variables are presented in **Table 5.2**.

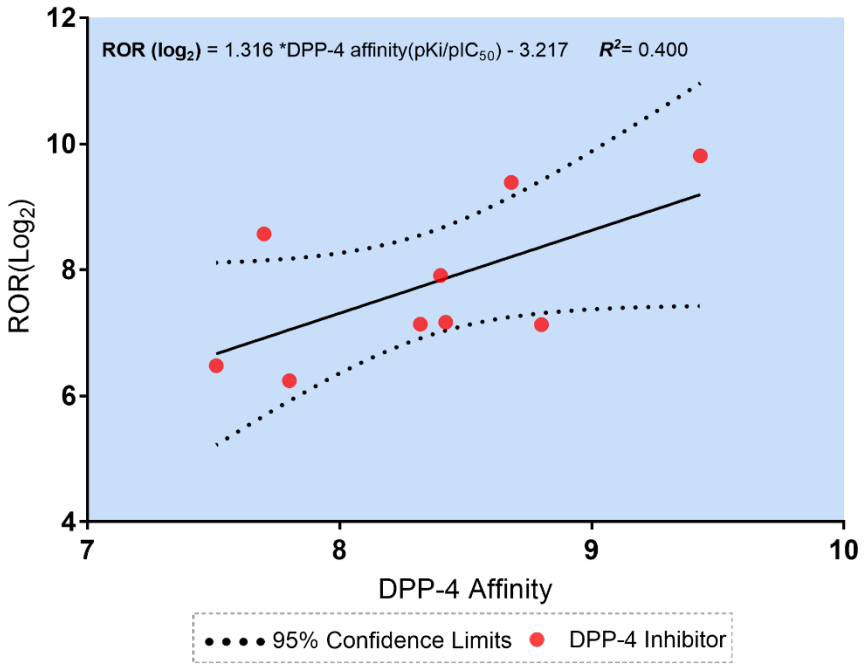
Table 5.2: Summary of Linear regression analyses

Dependent Variable	Predictor variable	Slope (95 CI%)	p-value	R^2
Reporting odds ratio (RORs)	DPP-2 affinity[#]	-4.822 ± 3.535; (-0.1233–2.756)	0.214	0.21
	DPP-4 affinity[#]	1.316 ± 0.6088; (-0.4385–3.21)	0.067	0.40
	DPP-8 affinity[#]	0.02895 ± 0.4208; (-0.9661–1.024)	0.947	0.006
	DPP-9 affinity[#]	0.06907 ± 0.3853 (-0.842–0.9801)	0.862	0.0045
	Fold selectivity for DPP-4 vs. DPP-8	0.08034 ± 0.1332 (-0.2346–0.3953)	0.565	0.049
	Fold selectivity for DPP-4 vs. DPP-9	0.04271 ± 0.1111 (-0.2199–0.3053)	0.712	0.020
	Vol. of distribution	-0.0005302 ± 0.001059 (-0.003034–0.001974)	0.632	0.034

Each linear regression model included RORs (expressed in base 2 logarithms) for the different DPP-4 inhibitors as dependent variable and either affinity measures for DPP-2, DPP-4, DPP-8, and DPP-9 targets; fold selectivity for DPP-4 (expressed in base 2 logarithms) over DPP-8, DPP-9; and volume of distribution (in litres) as predictor variable. [#]all affinity measures (pIC_{50} or pKi) available in the databases were treated as equivalent.

In general, there were no noticeable significant positive or negative correlations among all the tested predictor variables. Although affinity of DPP-4 inhibitors at DPP-4 was approaching significant association [slope = 1.316 ± 0.6088; (−0.4385–3.21), p = 0.067] and this variable accounts for 40% of the variance in the out- come (R^2 = 0.40) (Figure 5.3), except DPP-4 inhibitors affinity at DPP-2, and volume of distribution of drug showed non- significant linear relationship at conventional significance level. In contrast, there were non-significant inverse relationship between DPP-4 inhibitors affinity at DPP-2 [slope = – 4.822 ± 3.535; (−0.123–2.756), p = 0.214, R^2 = 0.21], volume of distribution of drug [slope = –0.0005302 ± 0.001059 (−0.003034–- 0.001974), p = 0.632, R^2 = 0.034] and RORs.

Figure 5.4. Linear regression plot between Dipeptidyl Peptidase-4 inhibitors affinities at DPP-4 and reporting odds ratio (log2) for bullous pemphigoid.



Legend: ROR: reporting odds ratio; DPP-4: Dipeptidyl Peptidase-4

5.4. DISCUSSION

This is the first study aimed at evaluating the potential role of pharmacological parameters of different gliptins in the occurrence of BP as a result of exposure to DPP-4 inhibitors, by using the largest SRSs system database, i.e., VigiBase®.

Previous disproportionality analyses of European [240, 241] and Japanese [223] pharmacovigilance databases investigating DPP-4 inhibitors-induced BP have found a significant association of it with DPP-4 inhibitors. In the present study using a worldwide pharmacovigilance database, the association was systematically examined for all currently clinically approved DPP-4 inhibitors between 2006 and 2019 with an enormously higher number of reports (15,024,872), strengthening the statistical power of the analyses.

Results obtained from case/non-case analyses of VigiBase® suggest there is a persistent increase in BP reporting risk as a result of DPP4 inhibitor exposure. The increased reporting risk of BP signals with DPP-4 inhibitors

observed with a very large sample size in VigiBase® is in accordance with case-control studies [226, 242, 243]. Moreover, recently, Kridin et al. in a meta-analysis on four population-based observational studies, have found that DPP-4 inhibitor exposure is associated with a 3.2-fold increased risk for BP (pooled OR: 3.16; 95% CI: 2.57–3.89) [227].

Our analysis showed that the magnitude of signals of disproportionate reporting for BP with teneligliptin and omarigliptin was greater than for any other DPP4 inhibitors. It is pertinent to note that omarigliptin and teneligliptin were discovered and marketed firstly in Japan and later approved in Argentina, Korea, and India. As of June 2017, omarigliptin and teneligliptin have been suspected in 45 BP cases of the 695 non-cases and 1 BP case of 19 non-cases, respectively, in the Japanese Adverse Drug Event Report (JADER) database [223]. Analysis of VigiBase® data showed a persistent increase in reporting of BP for these two drugs over a two-year period. However, the high magnitude of signal of disproportionate reporting points to an increased possibility for confounding as an alternative explanation.

In VigiBase®, BP cases were reported relatively more frequently for sitagliptin and vildagliptin than for other gliptins. In case-control studies addressing this issue (pooled vildagliptin- tin-specific OR: 10.16; 95% CI: 6.74–15.33), vildagliptin has been particularly implicated as the greatest risk factor in developing BP [241, 242, 244]; similarly, BP was reported with vildagliptin at disproportionately higher rates compared to any other individual DPP4i in previous disproportionality analyses of pharmacovigilance databases [240, 241]. In our analysis, in contrast, sitagliptin demonstrated a weaker association with BP in comparison to most DPP-4 inhibitors. This finding is also consistent with that obtained in a meta-analysis (pooled sitagliptin-specific OR: 1.29; 95% CI, 0.46–3.57) [227]. Of importance, in our dataset, is the fact that the ROR for saxagliptin was lower than that of all other DPP-4 inhibitors.

To date, there is little evidence, even in case reports, for the association between saxagliptin use and BP. Previously, saxagliptin was suspected in four cases of BP reported to JADER [223], whereas in only one in the French pharmacovigilance database [241]. This could be explained by the fact that sitagliptin and vildagliptin (not saxagliptin) are the most

prescribed gliptins worldwide. Like any pharmacoepidemiology studies, valid results from disproportionality analyses rely on accurate classification of event and drug exposure. Underreporting of ADRs due to 'no exposure misclassification' bias (patients exposed to the drug of interest may be more (or less) at risk for the adverse reaction than those exposed to other drugs) may explain a decrease in the number of reports of the comparator drug in case/non-case analyses, and henceforth in ROR [245]. Future studies adjusting disproportionality analyses on national dispensing data might unveil the true association of saxagliptin with BP.

It is worth mentioning that results obtained from the sensitivity analyses with other anti-hyperglycaemic drugs restricted to reports without DPP-4 inhibitors exposure confirmed stable signals of disproportionate reporting for all DPP-4 inhibitors, thus suggesting a class effect. Overall, suspected cases of BP for diabetes medications other than DPP4 inhibitors were substantially lower than the rate of suspected BP cases associated with D-PP4 inhibitor exposure. Furthermore, BP is reported relatively more frequently in association with gliptins than with other medicinal products reported in the literature as a plausible cause in the occurrence of BP.

Previously, Arai et al. in the analysis of JADER described that after exclusion of cases received DPP-4 inhibitors, significant RORs disappeared for case subjects receiving all the other anti-hyperglycaemic drugs [223]. In our analysis of diabetes medications other than DPP4 inhibitors, repaglinide and sulphonamide oral anti-hyperglycaemic drugs significant associations with BP. Sulphonamide derivatives are common inducers of pemphigoid [246, 247]. Previous evidence regarding differences between anti-hyperglycaemic drugs in their potential for eliciting BP is inconclusive. Due to sample size issues, or as a result of 'group analysis' of all the antidiabetic drugs apart from DPP-4 inhibitor group, most studies were unable to demonstrate risks associated with distinct anti-hyperglycaemic drug classes [220, 248-250]. However, no association with the risk of development of BP was found for sulfonylureas, or any other classes of anti-diabetes drugs in a recent Finnish registry-based retrospective controlled study [250]. With the emergence of new antidiabetic therapies and their increasing use, there is a need for ongoing investigation and surveillance to further clarify the potential association between the newer

diabetes medications and BP.

To date, the mechanism by which DPP-4 inhibitors induce BP is not well understood. By coupling RORs with the affinity values of DPP-4 inhibitors for each possible biological substrate, we investigated the potential mechanisms related to BP. Gliptins have pluripotent biological actions and they mainly interact with serine proteases of the DPP-4 family. The DPP-4 family includes four key enzymes, DPP-4, FAP (fibroblast activation protein), DPP-8, and DPP-9 [251]. Gliptin molecules have varying affinities toward the DPP-4 substrate. In general, the peptidomimetics (such as vildagliptin and saxagliptin) have lesser selectivity toward DPP-4 compared to DPP8/9. The lesser the relative selectivity toward DPP-4 and the greater the relative inhibition of DPP8/9, the greater the possibility of side effects (allergic skin manifestations, etc.) [230, 252, 253]. However, it is important to note that the potential for adverse effects associated with off-target inhibition of DPP-8 and DPP-9 by non- selective DPP inhibitors has been reported based on a single preclinical study [230]. Specific functions of DPP-8 and DPP-9 are unclear; however, unlike DPP4, DPP8/9 are intracellular proteases responsible for T-cell activation [254].

We found that the correlation between DPP-4 affinity and the BP reporting signal with DPP-4 inhibitors showed a considerable trend toward significance ($p = 0.067$). Of note, this trend toward significant association, albeit low coefficient of determination (R^2) of the linear regression model between DPP-4 affinity and RORs, underscores the potential role of DPP-4 and downplays the role of DPP8/9 in gliptin-induced BP.

Both teneligliptin and omarigliptin exhibit >10,000-fold selectivity for DPP-4 enzyme over other targets (i.e., DPP-8 and DPP-9) [255, 256]; in VigiBase® the magnitude of the disproportionate reporting for suspected BP cases for both drugs was considerably greater than for any other gliptins (**Table 5.1**). Conversely, it is worth mentioning that the magnitude of the disproportionate reporting for moderately selective gliptins, namely vildagliptin (270-fold vs. DPP-8; 32-fold vs. DPP-9) and saxagliptin (31-fold vs. DPP-8; 77-fold vs. DPP-9) [257-260], was considerably lower than for teneligliptin and omarigliptin; this suggests that DDP-4 selectivity plays a potential role in the development of BP.

Initially, it was thought that the inactivation of DPP-4 by a gliptin could

provoke immune dysfunctions associated with DPP-4/CD26 inactivation (a marker of activated T-cell) [261]. Several lines of evidence from animal studies with DPP-4- knockout and wild-type mice indicate now that DPP-4 enzyme activity is not a prerequisite for the T-cell activation and proliferation [262-265]. Nevertheless, metabolic benefit aside, the interconnected immunological effects of long-term DPP-4 inhibition are not understood fully at this stage.

We found no significant correlation with the remaining biological targets. The apparent volumes of distribution among the gliptins range from 70 to 1200 L. It is also possible that the intracellular accumulation of drug may cause intracellular inhibition of DPP-8 or DPP-9. Nonetheless, the distribution of the drug is strongly influenced by protein binding, a variable that depends on a variety of factors. There is also emerging evidence that the immuno-pathophysiology of DPP-4 inhibitor-induced BP differs from typical BP [243, 266] and interaction between genetic predisposition and drug intake may contribute to the manifestation of the disease [267].

Alternatively, one might hypothesize that different structural forms of DPP-4 inhibitors may play a role in developing BP. DPP-4 inhibitors are structurally diverse and they can be categorized into several groups based on the skeleton [268]. Despite structural heterogeneity, they share common binding interactions with the DPP-4, with exception of vidalaglitin and saxagliptin that possess an electrophilic trap such as nitrile group to form a covalent bond with Ser630 of the catalytic triad in the active site [269]. It has also been postulated that these DPP-4 inhibitors possessing the electrophilic trap are unstable and have a low selectivity against DPP-8 and DPP-9 that may result in multi-organ toxicities [230]. However, it remained unknown as to whether structural differences in gliptins and their binding characteristics might result in different susceptibility toward BP. Since most of the DPP-4 inhibitors have been reported to be associated with BP, it appears unlikely that the chemical structure of the DPP-4 inhibitor has clinical relevance in the development of BP.

5.4.1. Limitations

The use of a SRS database has some important implicit limitations, reporting being influenced by such factors as the notoriety bias, selection bias, and under-reporting, which precludes making causal inferences

except in unusual circumstances [270]. As shown in Figure 5.1, the observed increasing trend in reporting of gliptin suspected BP cases may be attributed to a notoriety effect following the publication of a clinical case report and case-control studies. This effect may lead to a bias that is responsible for an increase in the ROR for the drug in question. The actual risk and incidence rates cannot be determined from the analysis of SRS since the primary goal of such system is to signal the existence of a possible relationship between a drug or drug class and an ADR, and it does not prove causality. It is important to recognize that pharmacodynamic comparisons among the different gliptins based on the reported affinity data are difficult because DPP-4 enzyme assays are not standardized across the studies. From a combined data and methods limitation perspective, we took both Ki and IC50 affinity measures as equivalent. IC50 values are influenced by the concentration of substrate used and the ratio of substrate concentration to its Ki for that enzyme [271]. In most literature reporting affinity/selectivity data, methodology is incomplete and generally includes non-physiological conditions; thus, we were unable to determine Ki values for biological targets. Nonetheless, in the absence of assay information, these two affinity measures can be mixed without any loss of quality if corrected by a factor of 2, (which is the conversion factor most frequently used by databases) through comparing the IC50/Ki values in databases for the same protein-ligand systems [272]. The PV–PD approach is based on pharmacological receptors theory that has its own limitations, for instance, target affinity does not directly reflect the intrinsic activity of a drug. The PV–PD approach allowed us to determine, to some extent, a positive linear relationship between the ROR of BP and the affinities for DPP-4, whilst not for other targets. These results, which appear at a first glance counterintuitive, are due to the fact that we could analyse only one biological target at a time. A more proper PD evaluation should comprise all possible targets at the same time; the applicability of such an approach, however, is increasingly limited as the pharmacological complexity of the mechanisms of action increases.

5.5. CONCLUSION

Our case/non-case analyses and sensitivity analysis of VigiBase® suggest that BP reported at a disproportionate rate. There is a gradually increasing trend of gliptin suspected BP cases in VigiBase®. With regard to the presented results, the risk of BP with DPP-4 inhibitor appears to be greater than any other drugs known to cause this disorder. This risk is higher than for other different anti-hyperglycaemic drug classes and other medicinal products reported in the literature as a plausible cause in the occurrence of BP. This disproportionality should only be considered exploratory in the context of signal detection, as it does not allow quantification of the true risk. The correlation between DPP-4 affinity and the BP reporting signal with DPP-4 inhibitors showed a considerable trend toward significance, suggesting clinical relevance of DDP-4 selectivity in the development of BP. Future pre-clinical and clinical studies aimed at evaluating better this correlation, including the potential mechanism of gliptin-induced BP, are needed.

CHAPTER 6: STUDY IV

CHAPTER 6: STUDY IV

Changes in Anthropometric Parameters after Anti-TNF-alpha Therapy in Inflammatory Bowel Disease: A Systematic Review and Meta-analysis

ABSTRACT

Background: Tumour necrosis factor-alpha (TNF-α) inhibitors have been widely used for the treatment of moderate-to-severe inflammatory bowel diseases (IBD). TNF-α also plays an important role in the regulation of weight homeostasis, metabolism, and linked to variation in anthropometric responses. This relationship in IBD patients has yet to be determined.

Objectives: To evaluate the effects of TNF-α inhibitors on changes in anthropometric measures, in IBD patients (adults and children) through a systematic review and meta-analysis.

Methods: Multiple database searches identified studies involving children and adults with IBD and treated with TNF-α inhibitors, reporting at least one primary outcome measure. Where possible, data were combined for meta-analysis. The primary outcomes included weight, body mass index (BMI), waist circumference, height, height/velocity, fat and lean mass. Secondary outcomes included surrogate markers of disease activity. A random-effects model was used to estimate the standardised mean difference (SMD).

Results: Twenty-three cohort studies (total 1167 participants) met inclusion criteria. Meta-analysis was performed on 13 studies out of 23 included. In children, after 6 to 29.3 months of anti-TNF-α therapy, there was a small but statistically significant effect on weight (SMD=0.31; 95% CI [0.12-0.49]; P=0.001) with a mean gain in z-score of 0.30 (SE = 0.12). In adults, after 2 to 22.4 months of treatment, there was a moderate effect on BMI (SMD=0.72; 95% CI [0.17-1.26]; P=0.010) [mean gain=1.23 kg/m2 (SE = 0.21)]. A small but statistically significant increase in BMI-z score was found in children (SMD=0.28; 95% CI [0.03-0.53]; P=0.026) [mean change: 0.31 (SD = 0.14)] after 12 to 29.3 of therapy. In a meta-analysis of 4 studies there was negligible but statistically significant increase in height (SMD=0.16; 95% CI [0.06-0.26]; P=0.002) [mean change: 0.17 z-score (SE = 0.05)]. Negligible effect on fat mass (SMD=0.24 95% CI [-0.19-0.66]; P=0.272) was found in a meta-analysis of 5 studies. Noteworthy, despite the high heterogeneity among the studies that have addressed the issue, these results were also consistently supported by findings from studies not included in the meta-analysis and reviewed in the systematic review. Unfortunately, due to the lack of data, we were not able to perform moderator analysis on observed heterogeneity.

Conclusion: Anti-TNF-α treatment appears to be associated with an increase in body weight, BMI, and other anthropometric parameters. Given the different course of the IBD in children and adults, this association should be considered before initiating biologics for undernourished, overweight, and obese patients.

6.1. INTRODUCTION

Twenty years ago, infliximab was the first anti-tumour necrosis factor (TNF)-α monoclonal antibody approved for the treatment of moderate to severe inflammatory bowel diseases (IBD) [273, 274]. Elevated levels of TNF-α causally linked muscle metabolism and provoke cachexia and sarcopenia [275, 276]. TNF-α is also a powerful regulator of lipid and glucose metabolism, exerts complex and diverse effects from gluconeogenesis, loss of adipose tissue and proteolysis through regulation of enzymes involved in lipid metabolism, such as lipoprotein lipase, hormone-sensitive lipase, adipose triglyceride lipase and acetyl-CoA carboxylase [277].

The inhibition of TNF-α and the subsequent reduction of the general inflammatory state may concurrently trigger adipogenesis, which in turn may improve constructive metabolism in muscles. Hence, the control of inflammation leads to an improvement in growth in children, while in adults leads to better general clinical conditions.

Many studies have demonstrated statistically significant increases in body mass index (BMI) and/or body weight after anti-TNF-α treatment in IBD [273, 274, 278]. Evaluating the impact of anti-TNF-α therapy on anthropometric parameter changes in patients in IBD is of particular importance as the increase in lean mass is beneficial (muscle representing the protein reserve of the body and contributing to an improved immune function), This is especially true in patients with aggressive IBD in which lower body mass index (BMI) values may result from malnutrition accompanied with severe inflammation [279]. Nevertheless, about 15–40% of patients with IBD are obese and an additional 20–40% are overweight [280-284]; therefore, the potential involvement of adipose tissue in intestinal inflammation and therapeutic outcomes has gained increasing attention [280]. The increase in fat mass can have significant implications also in terms of augmented risk of obesity-related chronic diseases and the suboptimal response to therapy [285]. Pharmacokinetic studies have identified high body weight as a risk factor for suboptimal response and the odds of reaching a good response and achieving remission were lower in obese IBD patients who were treated with anti-TNF agents. High body weight is thought to associated with increased

clearance, shorter half-life, and lower serum trough drug concentrations of an anti-TNF-α agent [286-288].

The relationship between anti-TNF-α therapy and changes in anthropometric indices in IBD-patients has not yet been determined. In order to address this challenge, we performed a systematic review and meta-analysis of the studies on anti-TNF-α in adults and paediatric IBD patients reporting changes in anthropometric parameters. We also analysed other clinical outcomes pertinent to the pathophysiology of IBD such as measurements of body composition, and biochemical parameters correlating disease activity indices.

6.2. MATERIAL AND METHODS

6.2.1. Literature Search

We followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines. We submitted our Protocol at the International Prospective Register of Ongoing Systematic Reviews (revision pending) [289]. We searched PubMed/MEDLINE, EMBASE, and the Cochrane database up to September 30th, 2019 with no language restriction. The search strategy was adapted as needed for each database. In brief, we used three terms: TNF-α inhibitors, anthropometric parameters, and Inflammatory Bowel Diseases. We combined these terms with the Boolean operator "AND".

6.2.2. Eligibility Criteria

Inclusion criteria: any study that assessed at least one anthropometric parameter following the anti-TNF-α therapy in IBD patients and reported changes in those measures either for at least 2-time points (baseline and follow-up) or stratified results by anthropometric cut-off values. Conference proceedings/abstracts with relevant information on body changes were also included.

Exclusion criteria: studies were excluded if they (1) did not report values for baseline and follow-up; (2) report the effect of anthropometric measures (e.g., BMI or weight) on treatment outcome rather than vice versa; (3) included patients treated with parenteral or enteral nutrition or patients who received pharmacological treatment aimed to treat or prevent

metabolic disorders. Case reports, case series, review articles, meta-analysis, book chapters, and unpublished thesis were not included. Studies on pregnant women were also excluded.

Additional articles were identified through the reference lists of articles included in our systematic review. We did not contact authors for unpublished data.

6.2.3. Study selection

The titles and abstracts of retrieved references from the final search were imported into EndNote and duplicates eliminated. The titles and abstracts were screened, and papers deemed highly unlikely to be relevant were disregarded. Full-text versions of the remaining articles were obtained and assessed for eligibility based on our pre-specified eligibility criteria as described in section 2.2. The entire search process was conducted by two independent reviewers (VB and EI). Discrepancies between review authors were resolved by discussion with a third review author (FM) to achieve a decision.

Two authors (VB and EI) assessed the risk of bias of studies included in the systematic review by using the Newcastle Ottawa Scale (NOS) [290]. The NOS is divided into three domains evaluating group selection, comparability of the cohort, and ascertainment of the outcome of interest. The scoring sheet allowed a maximum total score of 9 points (highest quality level). Disagreement was resolved by consensus and consultation with the expert group (CC and FM).

6.2.4. Outcome measures

Primary outcomes were changes (from baseline) in the following anthropometric measures: weight (Kg), BMI (Kg/m^2), waist circumference [(WC), cm], height (cm), height/velocity (cm/years), fat mass [Bioelectrical impedance analysis (BIA) %], lean mass [Dual-Energy X-ray Absorptiometry (DXA), Kg].

Secondary outcomes included surrogate markers of disease activity [C-reactive Protein (CRP, mg/dL), erythrocyte sedimentation rate (ESR, mm/h)], phase angle (PA, degrees °) and disease severity index scores, e.g., the Crohn's Disease Activity Index (CDAI) and the Mayo score.

6.2.5. Data extraction and Synthesis

Extracted data from all included studies were compiled into an electronic summary table. The following pertinent information was extracted: change-from-baseline outcomes *i.e.,* weight, BMI, waist circumference, and other anthropometric measures. For paediatric patients, values of weight, BMI and height were mostly reported as z-score because of their growth variability. Information on the surrogate markers and disease severity index scores were also collected. Further parameters of interest such as study type (blinding/design), study duration, number of subjects, number of patients naïve to biological treatment, sex distribution, age, medication type, dose, and concomitant treatment were also included.

6.2.6. Statistical Analysis

All statistical analysis was conducted in ProMeta 3 software. For each outcome, change between baseline and follow-up after treatment commencement with a TNF-α inhibitor were analysed. Where possible, the effect of anti-TNF-α treatment on each different anthropometric measure was assessed in separate meta-analysis. We considered the mean difference (MD) and their corresponding standard deviations (SD) if reported in the primary study. If values were available as median, they were converted in mean (SD), provided that follow a normal distribution. For the missing correlations between baseline and follow-up, a correlation coefficient of 0.7 was imputed as recommended by Rosenthal [291]. Those studies with insufficient information to compute mean difference were excluded from the meta-analysis and the main findings of individual studies were summarized separately. Standardised mean differences (SMD) were based on Cohen's *d* with corresponding 95% confidence interval (CI) and were considered small ($d = 0.2$), medium ($d = 0.5$), and large ($d \geq 0.8$) as per Cohen's classification scheme [292]. $P < 0.05$ was considered statistically significant. A random-effects model was used to account for both within-group variability and between-study heterogeneity. The between-study heterogeneity index was I^2. Results were considered heterogeneous when homogeneity was unlikely ($p<0.10$). Forest plots were produced as a means of visualization. Possible publication bias was identified by visual assessment of a funnel plot.

For the body weight, BMI, height, and fat mass, the SMD represents the

effect estimate between baseline and follow-up. Positive effect estimate indicates that indice(s) was greater after treatment commencement while negative effect estimate indicates that indice(s) was lower after TNF- α inhibitor commencement.

6.3. RESULTS

6.3.1. Study Characteristics

The study selection and screening are presented in the PRISMA flow-chart (**Figure 6.1**). Of the 1016 articles retrieved (340 results were from PubMed, 117 from Cochrane and 559 from EMBASE), 23 met the inclusion criteria. Only 13 of the 23 included studies reported pre- and post-treatment changes in anthropometric measures, giving sufficient data to determine effect estimate.

Figure 6.1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) flow diagram of process of study selection.

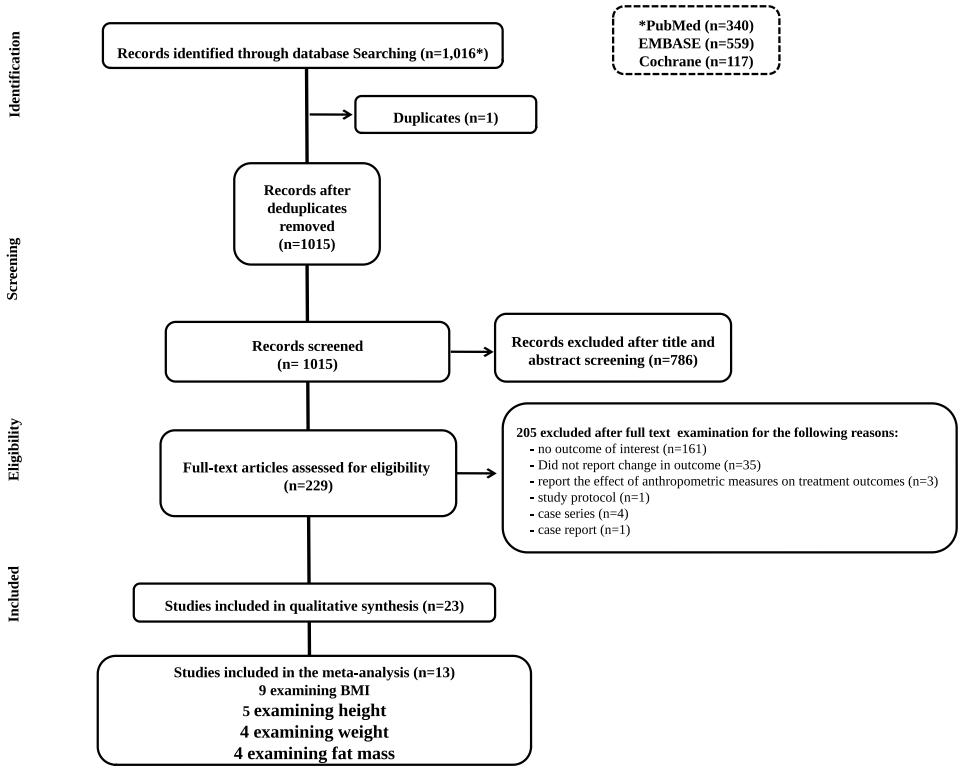


Table 6.1 provides characteristics of studies evaluated in the systematic review and meta-analyses. Of the 23 studies, 21 were observational (6 were retrospective and 15 prospective)

Table 6.1. Characteristics of studies evaluated in the systematic review and meta-analysis.

Study	Study duration (mo)	Type of study design	Sample size at baseline	Male (%)	Paediatric pts (Y/N)	Age (years) [mean ±SD]	Disease characteristics	Treatment n (%)	Dose (mg/kg)	Primary outcomes	Secondary outcomes	Meta-analysis (Y/N)
Adams 2017 [293]	6	RO	90	38 (42)	N	median 35 (IQR 26-50)	CD: 85%; UC: 15% Sarcopenic= 41 Normal Muscle= 49 (Overweight or obese: 34.5%; normal weight: 42.2%; underweight: 23.3%)	IFX 37 (41); ADA 43 (48); CZP 10 (11)	NA	Weight	CRP, ESR, activity (HBI)	N
Amato 2011 [294]	mean 22.4 (range 1-95)	RO	54	21 (39)	N	median 45 (range 20-69)	CD: 80%; UC: 20%	IFX 40 (74); ADA 14 (26)	NA	BMI	activity (CDAI, Mayo score)	Y
Assa 2013 [295]	60	RO	102	66 (65)	Y	13.5± 3.9	CD: 100% Disease location at diagnosis (Paris classification): L1 (distal ileum)	IFX 84 (82); ADA 18 (18)	IFX: NA; ADA: induction: 160 mg/1,73, 80	BMI	CRP, ESR	N

							40%; L2 (colonic) 33%; L3 (ileocolonic) 27%; L4a/b (upper GI disease) 17%. Presence of perianal disease: 35%		mg/1,73; maintenance: 40 mg/1.73			
Borrelli 2004 [296]	6	PO	18	NA	Y	median 13 (range 6-18)	CD: 100% 17%: involvement of the upper GI tract	IFX 18 (100)	5	Weight, height	CRP, ESR, activity (PCDAI)	Y
Branquinho 2014 [297]	36	PO	62	18 (29)	N	37.3± 13.8	CD: 74%; UC: 26% Underweight: 16.1%; overweight or obese: 12.9%	IFX 62 (100)	NA	BMI	NA	N
Csontos 2016 [298]	3	PO	40	24 (60)	N	33.4± 12.9	CD: 82.5%; UC: 17.5% - CD: disease type (Montreal classification): inflammatory (B1) 57.6%; penetrating (B3)	IFX 16 (40); ADA 24 (60)	IFX: 5; ADA: 160 at week 0, 80 at week 2; mainte	Weight, BMI, fat mass, lean mass	CRP, activity (CDAI, partial Mayo score)	Y

							42.4%; stricturing (B2) 0%. Location (Montreal classification): small bowel involvement (L1 + L3) 72.7%; colon involvement 27.3% - UC: location (Montreal classification): left sided (E1+E2) 14.3%; pancolitis (E3) 85.7%		nance: 40 eow.			
DeBoer 2016 [299]	12	PO	72	42 (58)	Y	15.1± 2.6	CD: 100% Location of disease: ileal 5.6%; colonic 28.5%; ileocolonic 69.4%; iso-upper 84.7%; perianal 37.5% Tanner stage: II 23.6%; III 25%; IV 30.6%;	IFX 72 (100)	NA	Weight, BMI, height	CRP, ESR, activity (PCDAI)	Y

							V 20.8%					
DeBoer 2018 [300]	12	PO	75	46 (61)	Y	14.1± 3.3	CD: 100% Disease location: ileal 5%; colonic 26%; ileocolonic 69%. Disease descriptor: isolated upper 91%; perianal 35% Tanner stage: I 15%; II 23%; III 19%; IV 25%; V 19%	IFX 75 (100)	NA	BMI, height, lean mass	CRP, ESR	Y
Dos Santos 2017 [301]	6	PO	23	11 (48)	N	42±12	CD: 100% Location of CD: upper GI tract 7.5%; ileum 26.1%; colon 17.4%; ileum and colon 49%. Phenotype of CD: non-stricturing and non-penetrating (B1) 52.2%; stricturing (B2)	IFX 23 (100)	5	Weight, BMI, waist circumference, fat mass, lean mass	activity (HBI), phase angle	Y

							30.4%; penetrating (B3) 17.4%					
Emerenziani 2017 [302]	3	PO	12	7 (59)	N	45±8	CD: 100% Ileal involvement: 75% CDAI 220-450 (moderate disease): 58%; CDAI > 450 (severe disease): 41%	IFX 12 (100)	5	Lean mass,	Phase angle, CRP	N
Franchimont 2005 [303]	1	PO	20	12 (60)	N	NA	CD: 100% Disease location: upper GI tract 15%; ileum only 20%; ileum and colon 35%; colon only 45%; anal 45% Disease type: inflammatory 45%; structuring 10%; fistulizing 45%; extraintestinal manifestations	IFX 20 (100)	NA	Weight, fat mass	CRP, activity index (CDAI)	Y

							35%					
Gouldthorpe 2013 [304]	Median 18	RO	71	48 (68)	Y	median 14.4 (range 3.95-20.1)	CD: 100% Disease classification (Montreal): -Location: ileocolonic 63.4%; colonic 31%; ileal 5.63%. + upper GI 57.7% -Behaviour: inflammatory 83%; fibrostenotic 7%; penetrating 10%. + perianal 39.4% Children on maintenance IFX (Severe disease: 55.9%; moderate disease. 44.1%)	IFX 71 (100)	5	Weight, height	NA	N
Griffin 2015 [305]	12	PO	74	47 (64)	Y	median 14 (range 5-21)	CD: 100% Disease location: ileal	IFX 74 (100); of these, 4 switched	NA	BMI, height, lean mass	CRP, ESR	Y

							5%; colonic 27%; ileocolonic 68% Disease descriptor: isolated upper 84%; perianal 38% Tanner stage: I-II 39%; III-IV 43%; V 18%	to ADA, 1 to CZP				
Haas 2017 [285]	median 29.3	RO	69	37 (54)	Y	NA	CD: 85%; UC: 12%; IC 3% -CD: distribution (Paris classification): small intestine (L1) 16.9%; colon (L2) 10.2%; small intestine and colon (L3) 83.1%; isolated upper disease (L4a) 40.7%; inflammatory (B1) 72.3%; stricturing (B2) 10.2%; penetrating (B3)	IFX 63 (91); ADA 32 (46); CZP 8 (12)	NA	Weight, BMI	NA	Y

							20.3%; penetrating and stricturing (B2B3) 2.7%; perianal disease (P) 20.3% -UC: distribution: proctitis or left-sided 2.5%; extensive 12.5%; pancolitis 62.5%					
Kierkus 2012a [306]	9.3	open label single arm trial	33	NA	Y	median 14.2 (IQR 12.1-16.5)	CD: 100%	IFX 33 (100)	5	Weight, BMI, height	CRP, activity (PCDAI)	N
Kierkus 2012b [307]	2.3	open label single arm trial	66	29 (44)	Y	14.06 ±3.59	CD: 100% Involved region: small intestine 43%; colon 91%; upper GI tract 32%	IFX 66 (100)	5	BMI	CRP, ESR, activity (PCDAI)	N
Koutroubakis 2009 [308]	3.5	PO	22	14 (64)	N	38.6	CD: 86%; UC: 14% -CD: localization:	IFX 22 (100)	induction: 5; maintenance: 5-10	BMI	CRP, activity (CDAI, SCCAI)	Y

							ileum 21.1%; colon 26.3%; ileum and colon 52.6%. Disease type: inflammatory 63.2%; penetrating 36.8% -UC: localization: extensive colitis 100%					
Malik 2011 [309]	12	PO	36	22 (61)	Y	14.7	CD: 100% Disease location: most commonly panenteric (ileocolonic and upper GI tract, L3+L4 42%. + perianal disease 42%. Tanner stage: I 19%; II 14%; III 14%; IV 6%; V 25%; NA 22%	ADA 36 (100)	18 pts: 80 at wk 0 + 40 at wk 2; 9 pts 24/m^2; 2 pts: 160 at wk 0 + 80 at wk 2; 7 pts: other regimens; mainte	Height velocity	activity (PCDAI)	N

									nance: 33 pts: 40 eow; 3 pts: 24/m2 eow			
MirandaBautista 2015 [310]	36	RO	128	64 (50)	N	43.55 ±12.82	CD: 72%; UC: 25%; IC: 3% - CD localization (Montreal classification): ileal (L1) 32.3%; colonic (L2) 9.7%; ileocolonic (L3) 58.1%; upper GI tract infection (L4+) 13.8%. Behaviour (Montreal classification): non stricturing and non-penetrating (B1) 39.8%; stricturing (B2) 17.2%; penetrating (B3)	IFX 104 (81); ADA 10 (8); IFX + ADA: 14 (11)	IFX 5; ADA 160 at wk 0 + 80 at wk 2	BMI	NA	N

							43%; perianal disease 36.2% - UC: extension (Montreal classification): proctitis (E1) 3.1%; proctosigmoiditis (E2) 12.5%; left colitis (E3) 40.6%; pancolitis (E4) 43.8%					
Parmentier-Decrucq 2009 [311]	2	PO	21	8 (38)	N	32±8	CD: 100%	IFX 21 (100)	5	BMI, fat mass	NA	Y
Vadan 2011 [312]	13.5	PO	30	17 (57)	N	33.3± 13.87	CD: 100% Disease location: ileum 3.3%; colon 63.3%; ileum+colon 33.3% Disease behaviour:	IFX 30 (100)	5	Weight, BMI	NA	N

							inflammatory 60%; structuring 40% 30 patients (100%) with moderate/severe flares of disease 43.3% of patients with severe nutritional risk defined by the NRI[a]					
Van Hoeve 2019 [313]	12	PO	42	21 (50)	Y	NA	CD: 62%; UC: 38% -CD (Paris classification): disease location: L1 19%; L2 19%; L3 62%. Upper GI involvement: L4a 62%; L4b 4%. Disease behaviour: B1 81%; B2 19%. Perianal disease modifier: 12%.	IFX 42 (100)	8	Weight, BMI, height	CRP, ESR	Y

							Growth: G0 69%; G1 31% -UC (Paris classification): disease extent: E1 6%; E2 25%; E3 19%; E4 50%. Disease severity: S0 69%; S1 31%					
Wiese 2008 [314]	6	PO	7	1 (14)	N	41.1	CD: 100% (ileal or ileocolonic) Of the 7 patients, 5 had active disease defined by CRP > 1.0 and 6 had active disease defined by HBI > 5	IFX 7 (100)	5	BMI, fat mass, lean mass,	CRP, activity (HBI)	Y

PO: prospective observational; RO: retrospective observational; IQR: interquartile range; CD: Crohn's disease; UC: ulcerative colitis; IBD: inflammatory bowel disease; IC: indeterminate colitis; IFX: infliximab; ADA: adalimumab; CZP: certolizumab pegol; NA: not available: week; eow: every other week; mo: months; pts: patients.
Primary outcomes with bold analysed using meta-analysis methodology.

and 2 were open-label single-arm trials (yielding a total of 1167 patients aged between 1 month to 85 years).

The average age among paediatric studies (n=658) ranges from 13 to 20 years [range: 1 month – 20 years]. For adults (n=509) average age ranges between 32 to 45 years [range: 18–85]. Of the 1167 patients, 1053 (90.2%) had a diagnosis of CD; 96 (8.1%) of UC, 2 (0.16%) of unclassified-IBD and 4 (0.33%) of indeterminate colitis. With respect to the type of medication used, 22 studies reported data on infliximab (n=989; 84.7%), 7 on adalimumab (n=159; 13.6%), and 3 on certolizumab Pegol (n=19; 1.6%). Concerning concomitant therapy, 79 (6.68%) patients were treated with corticosteroids, 188 (16.1%) patients with aminosalycilates, or nonsteroidal anti-inflammatory drugs, 453 (38.8%) patients with immunomodulators and 4 (0.33%) patients with antibiotics. Eight studies enrolled paediatric patients (under 18 years). The mean percentage of male patients was 51.27%. The period from the baseline to the last follow-up varied considerably among the studies with a mean follow-up period of 15 months (range:1-29.3).

The quality of the included studies was moderate (mean NOS 5.5, standard deviation 0.51) (**Appendix VI**). All the studies included in the review were rated as moderate quality (NOS score 5-6). The quality issue most often found was in the comparability domain.

6.3.2. Primary outcomes

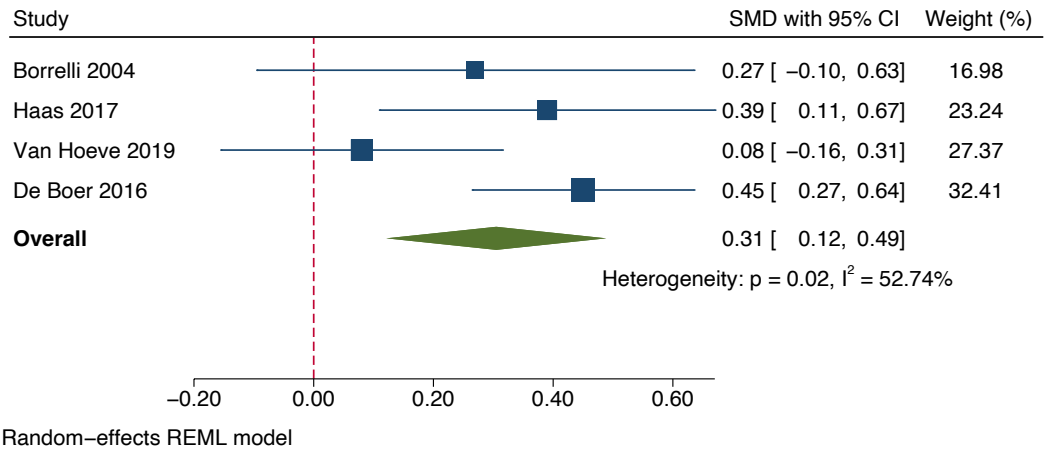
6.3.2.1. The effect of TNF-a Inhibitors on Body weight

Eleven of 23 studies reported information on body weight changes. Of these, 6 studies involved paediatric patients (164 participants) [285, 296, 299, 300, 304, 313] and 5 studies involved adults [293, 298, 301, 303, 312]. For paediatrics, SMD calculation was possible for 4 of the 6 studies; these 4 studies were included in the meta-analysis [285, 296, 299, 313]. The analysis revealed that patients' weight was significantly increased in children (SMD=0.31; 95% CI [0.12-0.49]; P=0.001) after the commencement of anti-TNF-α therapy (duration ranges from 6 to 29.3 months). **Figure 6.2** illustrates the effects of anti-TNF-α pre- and post-treatment on weight in paediatrics. The weighted pooled mean increase in

weight z score was 0.30 (SE = 0.12). The between-study heterogeneity was significant (P=0.096, I2 = 52.74 %). Funnel plot (**Appendix VII, figure a**) showed no potential publication bias. The remaining two studies that were excluded from analysis support these findings (**Table 6.2**). Briefly, in one study children with CD following maintenance therapy of infliximab significantly increased weight z-score by 0.51(P < 0.001) [304]. Similarly, in a study by Kierkus *et al,* there was a significant increase in body weight by 5.6 kg after 50 weeks of treatment [306].

Concerning adults, SMD calculations were not possible due to insufficient information as the values on change in body weight for at least 2-time points were not reported. Although there was marked heterogeneity in the way in which the studies included physical frailty patients, all the studies nevertheless reported a significant increase in body weight following anti-TNF-α treatment (**Table 6.2**).

Figure 6.2. Forest plot showing the change in body weight between baseline and after treatment commencement with a TNF-α inhibitor in paediatric patients.



Standardized mean difference (SMD) estimates were based on Cohen's d with corresponding 95% confidence interval (CI) and were considered small (d = 0.2), medium (d = 0.5), and large (d ≥ 0.8) as per Cohen's classification scheme [20]. P < 0.05 was considered statistically significant.

In the study by Adamas *et al.*, there was a trend toward statistical difference for body weight change among patients with normal muscle mass (Δ1.86 kg, P = 0.07), but not in those with sarcopenia (Δ1.14 kg, P= 0.4) after 6 months of anti-TNF-α therapy [293]. Vadan *et al* reported that

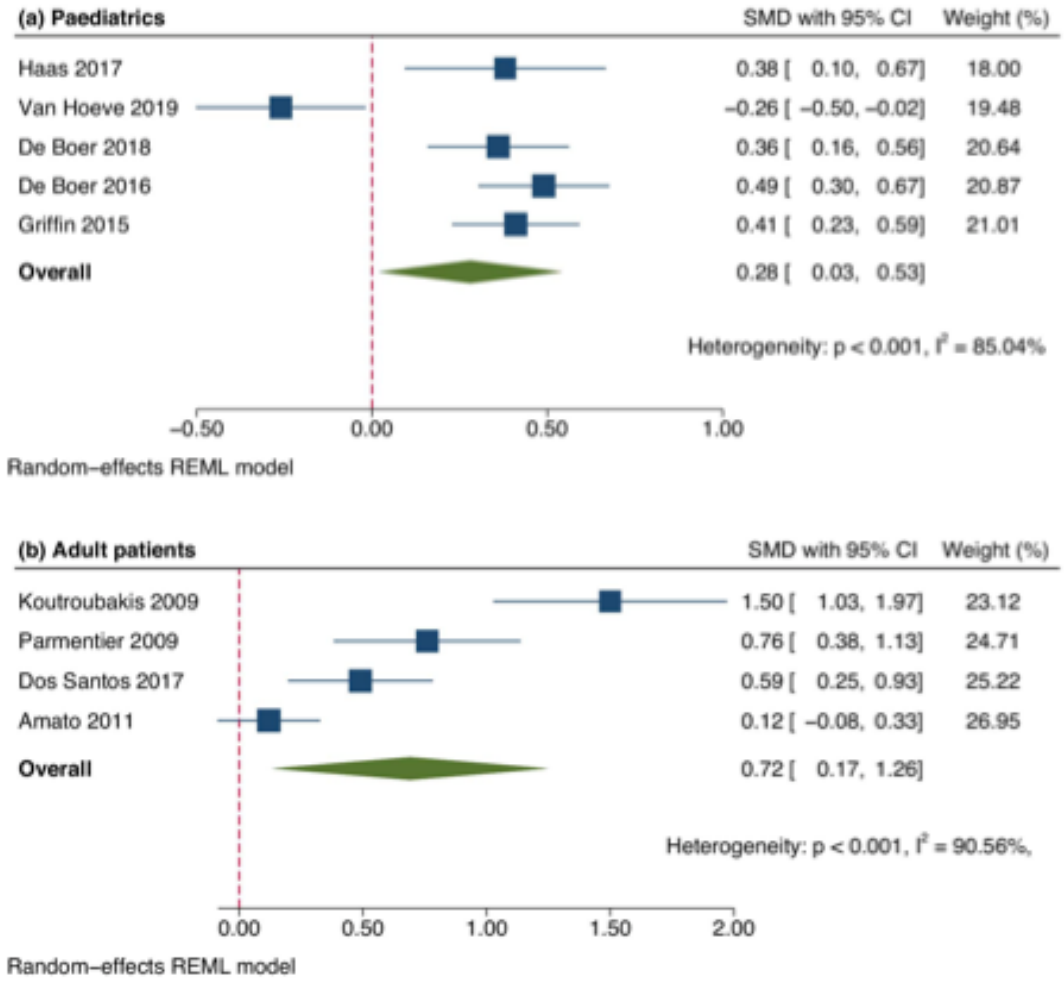
undernourished patients had a significantly higher increase in body weight compared to well-nourished patients at 30th and 54th week after anti-TNF-α treatment [312]. A significant increase in body weight was also found in the other three prospective studies, after a mean duration of treatment of 3.33 months (range: 1 to 6) [298, 301, 303].

6.3.2.2. The impact of TNF-a Inhibitors on BMI

In total, 19 studies examined the effect of anti-TNF-α on BMI. Of these, 9 were eligible for meta-analysis. For paediatrics, the analysis of 5 studies (281 participants) [285, 299, 300, 305, 313] revealed significant effect of anti-TNF-α on BMI (SMD=0.28; 95% CI [0.03-0.53]; P=0.026) with a weighted pooled mean change in BMI z-score of 0.31 (SD = 0.14 **(Figure 6.3a)**. The duration of anti-TNF-α therapy ranged from 12 to 29.3 (mean 15.46). Significant between-study heterogeneity was detected I^2 =85.04 (P<0.001). Funnel plots (**Appendix VII, Figure b**) showed potential publication bias.

For adults, 4 studies with 120 participants were included in the meta-analysis [23,29,38,42]. The duration of anti-TNF-α therapy in included studies ranged from 2 to 22.4 months (mean 8.47). The overall effect was nonsignificant (SMD=0.72; 95% CI [0.17-1.26]; P=0. 1) with an average BMI gain of 1.23 kg/m^2 (SE = 0.21) **(Figure 6.3b)**. Statistically significant between-study heterogeneity was observed (I^2=90.56, P<0.001). Funnel plots (**Appendix VII, Figure c**) indicate potential publication bias. We explored sources of heterogeneity using stratification and repeated the analysis using random effects as an additional sensitivity analysis. A sensitivity analysis was conducted by excluding the conference abstract [294]. The gain in BMI remains significant (SMD=0.93, 95% CI [0.42-1.43], P<0.0001) with considerable unexplained heterogeneity (I^2 = 79.53, P=0.008).

Figure 6.3. Forest plot showing the change in BMI between baseline and after treatment commencement with a TNF-α inhibitor in paediatric (a) and adult (b) patients.



Standardized mean difference (SMD) estimates were based on Cohen's d with corresponding 95% confidence interval (CI) and were considered small (d = 0.2), medium (d = 0.5), and large (d ≥ 0.8) as per Cohen's classification scheme [20]. P < 0.05 was considered statistically significant

Table 6.2. Summary of post anti-TNF-α treatment changes in anthropometric measures in studies *excluded* from meta-analysis.

Outcome	Author, year	Baseline	Endpoint	Variation [presented as change (Δ) unless otherwise specified]	Duration of anti-TNNF therapy
Body Weight (kg)	Adams 2017 [293]	NR	NR	Entire cohort: 1.5 (P= 0.06) Sarcopenic: 1.14 (P= 0.4) Normal muscle: 1.86 (P=0.07)	6 months (IFX, ADA, CZP)
	Csontos 2016 [298]	63.4 (58.82 – 79.40) §	63.7 (58.49 – 82.65) §	**Overall:** NR; Significant increase (P < 0.001) **Stratified by disease severity:** Mild: 3.54 (3.59) * Moderate: 1.87 (2.60) * Severe: 0.98 (2.67) *	3 months (IFX, ADA)
	Dos Santos 2017 [301]	62.6 (9.5) *	68.4 (13.2) *	NR; Significant increase (P=0.006)	6 months (IFX)
	Franchimont 2005 [21]	63.6 (3.6) ¶	64.4 (3.5) ¶	NR; Significant increase (P=0.013)	1 month (IFX)
	Gouldthorpe 2013 [304]	Weight-for-age SDS: −0.77 §	Weight-for-age SDS: +0.48 §	NR; Significant increase (P < 0.05)	44 months (IFX)
	Kierkus 2012a [306]	43 (36.2 – 50.7) ⋆	48.6 (42 – 53.5) ⋆	NR	10 months (IFX)
	Vadan 2011 [312]	NR	NR	Stratified by BMI category: Patients baseline BMI <18.5= 11.2 (3.58) (P =0.002) * Patients baseline BMI >18.5= 6.58 (2.32) *	13.5 months (IFX)
	Assa 2013 [295]	BMI for age and sex SDS – z scores: -0.8 *	BMI for age and sex SDS – z scores: -0.4 *	NR; significant increase (P=0.04)	60 months (IFX, ADA)

	Branquinho 2014 [297]	21.4 (3.07) *	22.8 *	NR; non-significant increase at 1year, significant increase at 3 years (P=0.026)	36 months (IFX)
	Csontos 2016 [298]	21.75 (19.20 – 26.55) §	22.5 (20.17 – 27.02) §	Overall: NR; significant increase (P < 0.001) **Stratified by disease severity** Mild: 1.16 (1.19) * Moderate: 0.63 (0.88) * Severe: 0.34 (0.91) *	3 months (IFX, ADA)
	Kierkus 2012a [307]	17.9 (16.4 – 19.5) ·	18.9 (16.9 – 20) ·	NR; significant increase	10 months (IFX)
	Kierkus 2012b [306]	17.5 (15.4 – 19.4) §	18 (16.7 – 20) §	NR; significant increase	2.5 months (IFX)
	Miranda Bautista 2015 [310]	23.9 (4.6) *	NR	1.44 (3.5) (P < 0.001) *	36 months (IFX, ADA)
	Vadan 2011 [312]	Patients baseline BMI <18.5= 17.31 (1.22) * Patients baseline BMI >18.5= 21.03 (2.1) *	Patients baseline BMI <18.5= 21.46 (1.61) * Patients baseline BMI >18.5= 23.51 (2.22) *	NR; significant increase (P =0.01)	13.5 months (IFX)
	Wiese 2008 [314]	24.45 *	26.66 *	2.21 (P=0.03) *	6 months (IFX)
Height (cm)	Gouldthorpe 2013 [304]	Height-for-age SDS: −0.33 §	Height-for-age SDS: 0.86 §	NR; significant increase (P < 0.05)	44 months (IFX)
	Kierkus 2012a [306]	154.3 (142 – 164.5) ·	158.5 (152 – 168.5) ·	NR; significant increase	10 months (IFX)
Height velocity (cm/y)	Malik 2011 [309]	2 (0 – 5.8) £	4.2 (0 – 10.3) £	NR; non-significant increase (P=0.11)	12 months (ADA)
Fat mass	Parmentier-Decrucq 2009 [311]	TAF (cm3): 212 (47) *	TAF (cm3): 251 (50) *	NR; significant increase (P=0.027)	2 months (IFX)
	Csontos 2016 [298]	visceral fat area: 95.65 cm3 BFMI: 4.57 Kg/m^2	visceral fat area :85.00 cm3 BFMI: 4.76 Kg/m^2	NR; non-significant increase	
	Dos Santos [301]	BFMI: 5.5±2.3 kg	BFMI: 6.8±2.3 kg	NR; significant increase	

Lean mass	Csontos 2016 [298]	FFMI: 17.64 (3) * SMI: (kg/m2): 9.81±1.83	FFMI: 18.14 (3.08)) * SMI: 10.05±1.90	NR; significant increase (P < 0.001 **Stratified by disease severity (FFMI):** Mild: 1.02 (0.74) * Moderate: 0.46 (0.68) * Severe: - 0.05 (0.61) * Differences within mild and severe disease activity subgroups P=0.005	3 months (IFX, ADA)
	DeBoer 2018 [300]	LegLM: - 0.76 (1.04) *	LegLM: -0.27 (1.01) *	NR; significant increase (P < 0.001)	12 months (IFX)
	Dos Santos 2017 [301]	LMI: 17.5 (2.2) *	LMI: 18.1 (2.3) *	NR; significant increase (P=0.000)	6 months (IFX)
	Emerenziani 2017 [302]	FFM: 41.7 (3.7) *	FFM: 44.6 (4.2) *	NR; significant increase	3 months (IFX)
	Griffin 2015 [305]	Muscle CSA (mm2) – z score: -0.81 (1.10) *	Muscle CSA (mm2) – z score: -0.35 (1.06) *	Muscle CSA (mm2) – z score: 0.46 (0.78) * (P < 0.01)	12 months (IFX, ADA, CZP)
	Wiese 2008 [314]	DXA (Kg): 39.16 *	DXA (Kg): 40.03 *	DXA (g): 872.33 (P=0.4) *	6 months (IFX)
WC (cm)	Dos Santos 2017 [301]	88.1 (6.7) *	93.9 (7.7) *	NR; significant increase (P=0.002)	6 months (IFX)

* Mean (sd); ¶ Mean (SEM); § Median (IQR); ⋆ Median (range); £ Median (10^{th} – 90^{th} centiles) , [a] NRI = 1.519 x serum albumin (g/L) + 41.7 x (current/usual body weight)

CD = Crohn's disease; UC = Ulcerative colitis; IC = Indeterminate colitis; IFX = Infliximab; ADA = Adalimumab; CZP = Certolizumab; WC = Waist Circumference; CDAI = Crohn's Disease Activity Index; HBI = Harvey Bradshaw index; SDS = standard deviation scores; TAF = Total Abdominal Fat; FFMI = Fat Free Mass Index; LegLM = Leg Lean Mass; LMI = Lean mass Index; FFM = Fat Free Mass; Muscle CSA = Muscle cross-sectional area; DXA = Dual-energy X-ray absorptiometry; NRI = Nutritional Risk Index.

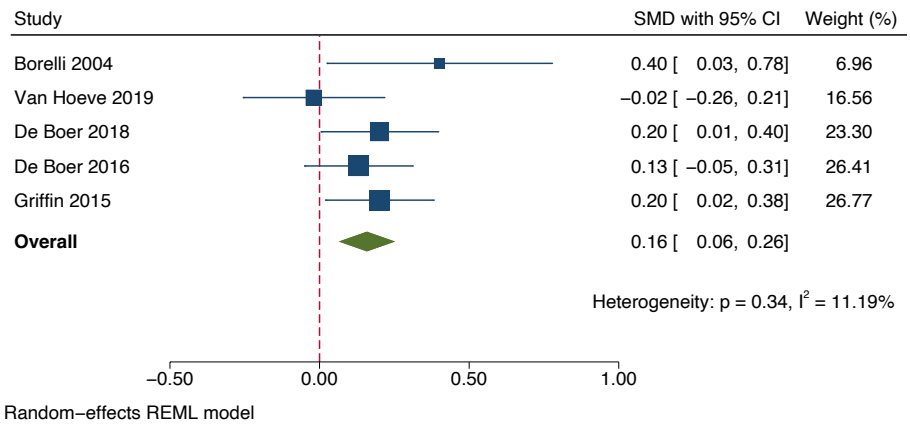
For the remaining 10 studies that were not included in the meta-analysis, the main findings are summarized in **Table 6.2**. Concerning children, in the study by Assa *et al*, the clinical response was associated with an improvement in BMI z scores (−0.8 to −0.4; p=0.04) [295]. Likewise, Kierkus *et al* reported a significant increase in BMI in children with severe CD treated with infliximab [41, 28]. For the adults, Csontos *et al*. reported significant BMI gain (from 23.81 ±7.19 kg/m^2 at baseline to 24.52 kg/m^2 ±7.34 after 3 months, P<0.001) [298]. In a retrospective cohort of 128 patients who received at least three doses of infliximab or two doses of adalimumab, a significant increase in mean BMI was observed (0.74 kg/m^2 at 1-year follow-
up and 1.44 kg/m^2 at 3-year follow-up) [310]. Vadan *et al*. evaluated 30 patients with CD undergoing infliximab therapy and observed a significant increase in BMI among underweight (from 17.31 ± 1.2 kg/m^2 to 21.46 kg/m^2± 1.61) and normal-weight subjects (from 23.24 ± 2.27 kg/m^2 to 23.51 kg/m^2± 2.22) after 54-week of treatment [312]. In the study by Wiese *et al.,* seven patients experienced a gain in BMI by 2.21 kg/m^2 (P = 0.03) after 6 months of infliximab treatment [314]. Branquinho *et al* reported no significant change in BMI after induction treatment with infliximab, whilst an increase was noted at the 1-year (from 21.4 kg/m^2 to 22.7 kg/m^2; P=0.049) follow-up which became statistically significant after three years of therapy (from 21.4 kg/m^2 to 22.8 kg/m^2; P= 0.026) [297].

6.3.2.3. The impact of TNF-a Inhibitors on height

For children, data on changes in height were reported in 8 studies. Of these, 5 studies with 269 patients [296, 299, 300, 305, 313] were included in the meta-analysis **(Figure 6.4)**. The overall effect was significant (SMD=0.16; 95% CI [0.06-0.26]; P=0.002). The weighted pooled mean increase in height z-score in was 0.17 (SE = 0.05). The between-study heterogeneity was nonsignificant (I^2 = 11.19; P=0.342). The funnel plot (**Appendix VI, Figure d**) showed no significant publication bias. Findings from other paediatric studies not included in the meta-analysis confirmed a considerable increase in height after a treatment duration ranging from

9.3 to 18 months **(Table 6.2)** [304, 306, 309].

Figure 6.4. Forest plot showing the change in height between baseline and after treatment commencement with a TNF-α inhibitor in paediatric patients.

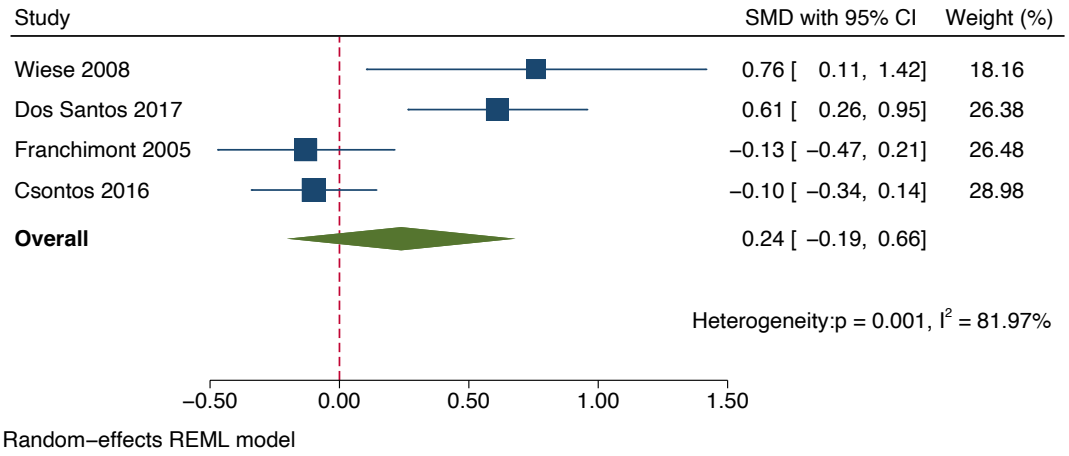


Standardized mean difference (SMD) were based on Cohen's d with corresponding 95% confidence interval (CI) and were considered small (d = 0.2), medium (d = 0.5), and large (d ≥ 0.8) as per Cohen's classification scheme [20]. P < 0.05 was considered statistically significant

6.3.2.4. The impact of TNF-a Inhibitors on fat mass

Five studies reported changes in fat mass in adults [298, 301, 303, 311, 314]; of these, 4 were eligible for the inclusion in the meta-analysis **(Figure 6.5)**. We found an overall increase in fat mass (%) (SMD=0.24 95% CI [-0.19-0.66]; P=0.272) with a considerable heterogeneity (I^2 = 81.97, P=0.001). The funnel plot (**Appendix VI, Figure e**) indicated a risk of publication bias.

Figure 6.5. Forest plot showing the change in fat mass between baseline and after treatment commencement with a TNF-α inhibitor in adult patients.



Standardized mean difference (SMD) were based on Cohen's d with corresponding 95% confidence interval (CI) and were considered small (d = 0.2), medium (d = 0.5), and large (d ≥ 0.8) as per Cohen's classification scheme [20]. P < 0.05 was considered statistically significant

Other related reported outcomes were the Body Fat Mass Index (BFMI; kg/m^2), the visceral fat area (cm^2), and the total abdominal fat (cm^3); however, finding among studies are not consistent (Table 6.2). In a study by Parmentier *et al*, there was a significant increase in the total abdominal fat (212 cm^3 ± 47 vs 251cm^3±50; P=0.027) after 8 weeks of induction treatment with infliximab [311]; whereas in a study by Csontos *et al* fat parameters did not change significantly at week 12 (visceral fat area, cm^2: 95.65 vs 85.00; P=0.730; BFMI, Kg/m^2: 4.57 vs. 4.76; P= 0.120) [298]. In the study by Dos Santos *et al,* there was a significant increase in the FMI, *i.e.,* fat mass (kg)/ squared height (5.5±2.3 vs 6.8±2.3; P=0.000) [301].

6.3.2.5. *The impact of TNF-a Inhibitors on lean mass*

Six studies with 231 patients examined the effect of anti-TNF-α treatment on lean mass [298, 300-302, 305, 314]. Computation of SMD was not possible due to insufficient information and the main findings of individual studies are summarised here.

Data on changes in lean mass were available in 6 paediatric studies (n= 231 patients) [298, 300-302, 305, 314], not eligible for the inclusion in the

meta-analysis. Briefly, the period of observation ranges from 3 to 12 months. Csontos *et al* found a significant increase in the Skeletal Mass Index (P=0.003) and the fat-free mass (FFM) index (P<0.00) at week 12, along with a significant increase in the food intake [298]. Similarly, a significant increase in the Lean Mass Index (LMI) was also reported in a study by Dos Santos *et al* (17.5kg/m^2±2.2 vs ± 18.1kg/ ±2.3; P<0.001) [301]. Emerenziani *et al* reported a nonsignificant increase in FFM among patients started infliximab compared to patients on conventional treatment (41.7kg ±3.7 vs. 44.6kg ±4.2; P <0.05) [302]. Similarly, in a study by Griffin *et al,* the PCDAI decreased after 10-week induction treatment with subsequent gains in muscle area after 12 months (z-scores: -0.81 ± 1.10 vs. -0.35 ± 1.10; P< 0.01) [305].

All studies observed lean body mass values after anti-TNF-α therapy in a period of observation ranging from 3 to 12 months. In brief, Csontos *et al* found a significant increase in the Skeletal Mass Index (P=0.003) and the fat-free mass (FFM) index (P<0.00), in IBD patients at week 12, along with a significant increase in the food intake [298]. Similarly, a significant increase in the Lean Mass Index (LMI) was also reported in a study by Dos Santos *et al* (17.5kg/m^2±2.2 vs ± 18.1kg/ ±2.3; P<0.001) [301]. Emerenziani *et al* reported a non-significant increase in FFM in patients on infliximab therapy compared to patients on conventional therapy (41.7kg ±3.7 vs. 44.6kg ±4.2; P <0.05) [302]. Similarly, in a study by Griffin *et al,* the paediatric CD activity index decreased during the 10-week induction with subsequent gains in muscle area z scores after 12 months (-0.81 mm^2 ± 1.10 vs. -0.35 mm^2 ± 1.10; P< 0.01) [305]. In a prospective cohort study of 75 patients (age: 5 to 21 years) with CD, leg lean mass score increased significantly (- 0.76 kg ±1.04 vs. -0.27 kg ±1.01; P<0.001) following 12 months of anti-TNF-α therapy [300]. In contrast, no significant change in the lean body mass (LBM) per DXA value was observed by Wiese *et al* (from 39.16 Kg at baseline to 40.03 Kg; Δ=0.87kg; P= 0.44) after 6 months of IFX treatment, in 7 CD patients [314].

6.3.2.6. *The impact of TNF-a Inhibitors on Waist Circumference*

Only one study examined change in WC after the commencement of anti-TNF-α therapy [301]. A significant increase in WC (from 88.1±6.7 at baseline to. 93.9±7.7 cm; P<0.05), was found in adults with moderate to severe CD after 6 months of anti-TNF-α therapy.

6.3.3. Secondary outcomes

Twenty-one studies reported secondary outcomes, *i.e.,* laboratory markers of disease activity, disease severity index scores, and changes in phase angle (PA) (**Appendix VIII**). In all studies, the efficacy of treatment in reducing disease activity was confirmed by a significant reduction in both, surrogate markers of disease activity (*i.e.,* ESR and CRP) and severity index scores, regardless of IBD type (CD, UC), and population (children, adults). A limited number of studies have reported on the effect of anti-TNF-α treatment on PA. There are only two studies have examined the influence of anti-TNF-α treatment on PA in IBD, which present conflicting findings. PA (degrees) remained unchanged (6.2 vs. 6.8; P=0.94) in the study by Dos Santos *et al* [301], whereas Emerenziani *et al* found a significant increase in the mean PA scores (from 4.6±0.3 to 6.2 ±0.4; P< 0.05) [302] (Table 6.2).

6.4. DISCUSSION

Evidence addressing the relationship between anti-TNF-α agents and variations in body composition is of primary importance in the assessment of safety and efficacy outcomes of this pharmacological approach. Previous studies revealed contradictory results concerning the effects of anti-TNF-α therapy on body composition in rheumatological patients [315-317].

This is the first systematic review aimed at evaluating the impact of anti-TNF-α therapy on anthropometric variations in IBD patients, both in adults and children. In doing so we took care to consider all aspects that were revealing of disease activity indices. The goal was to determine whether the weight gain was due to an increase in fat or muscle mass, to improve knowledge on any potential effect related to anti-TNF-α therapy. To

maximize comparability and minimize potential bias, studies with the possible confounding effect of parenteral or enteral nutrition, or patients who received pharmacological treatment aimed to control, or prevent metabolic disorders were thus excluded.

We found evidence for a statistically significant impact of TNF-α Inhibitors on BMI both in adults (SMD=0.72, 95% CI [0.17-1.26]; P=0.010) and children (SMD=0.28, 95% CI [0.03-0.53]; p=0.026). The SMD was larger for adults than children. Furthermore, there was a small but statistically significant effect on body weight (SMD=0.31, 95% CI [0.12-0.49]; P=0.001) and height (SMD=0.16, 95% CI [0.06-0.26]; P=0.002).

Relatedly and noteworthy, despite the high heterogeneity among the studies that have addressed the issue, these results were also consistently supported by findings from studies not included in the meta-analysis and reviewed in the systematic review. Unfortunately, due to the lack of data, we were not able to perform moderator analysis on observed heterogeneity. Such heterogeneity might be attributed to the variations of in study patients, gender, the remitting and relapsing nature of the IBD, disease severity, type of anti-TNF-α, and concomitant treatment.

BMI and body weight changes were the main outcomes mostly reported. There was a meaningful increase in BMI from the baseline in all studies; this effect was more evident in studies dealing with long-term follow-up, especially after three years of therapy, showing an increase in BMI of +1.4 Kg/m^2 [300, 310]. In line with this, we found an overall increase in BMI of +1.23±2.3 Kg/m^2, from the baseline after a therapy duration ranging from 22.4 to 2 months. Similarly, we noted an increase in weight both in adults and children, after a mean duration of 6 and 12.4 months of treatment, respectively. In line with this, increased waist circumference was evident after infliximab therapy (88.1±6.7 vs. 93.9±7.7 cm; P<0.05) in adults with moderate to severe CD [301]. Of importance, responders had significant improvements in body weight and BMI, compared to non-responders, which may reflect early discontinuation of treatment in non-responders and switch to an alternative treatment. Factors such as age, disease duration, smoking, or other medication did not show significant association with BMI, suggesting that anti-TNF-α therapy may play a significant role in body changes by ameliorating the disease status [295, 297, 298, 310].

The adult patients involved in our analysis were normal-weighted (BMI ranged from 21.9 to 24.4 Kg/m^2), except for only three studies in which <30% of the cohort were underweighted [295, 297, 311]; therefore, the increase in body parameters from the baseline raises concerns over cardiometabolic diseases and the inferior response of anti-TNF treatments in IBD patients [43]. On the contrary, paediatric patients were underweighted (BMI-z scores ranged from -1 to -0. 1), suggesting that the beneficial impact of the increase in these parameters limited to paediatric clinical setting.

At the end of follow-up (range: from 6 to 36 months), all included studies reported significant increase in weight and height in children. It would have been interesting to understand if the weight and height gain is only anti-TNF-α dependent or due to the normal growth of children over time. Reported data suggest that patients <10 years of age had the most weight gain; this may reflect the faster growth velocity seen in early puberty and/or greater impact of anti-TNF agents seen in this population. Additional studies with a larger cohort may help clarify whether anti-TNF effects on weight and height would differ from the normal weight gain of children over time.

Although the observed increase in weight and BMI during the anti-TNF-α treatment can probably be attributed to the decline in the intensity of the inflammatory response and improved nutrient absorption and utilization, an intrinsic anti-TNF-α therapy effect cannot be ruled out. Anti TNF-α therapy itself may increase abdominal fat tissue in IBD patients, likely through blockade of the TNF- α induced lipolytic effect, a mechanism that may contribute to weight and BMI gain we detected. Moreover, as an activator of NF-κB, TNF-α has a remarkable effect on metabolic pathways. As a consequence, anti-TNF-α therapy may prevent the activation of NF-κB [318], influencing the nutritional status and body composition as well.

Skeletal muscle and adipose tissue are cytokine-producing organs, thus playing an important role in the maintenance of metabolic homeostasis [319, 320]. As nutritional status assessments based on BMI, and body weight do not provide sufficient information concerning body composition, we attempted to examine changes in fat and lean mass from the baseline. However, only eight studies reported body composition changes,

suggesting that the effect of anti-TNF-α treatments on body composition in IBD patients still lacks adequate attention.

We found no significant increase in fat mass (SMD=0.24, 95% CI [-0.19-0.66]; P=0.272) likely due to the overall short period of observation (mean: 6 months; range: 1-6 months) which probably did not allow the Authors to detect substantial changes, with highly significant heterogeneity between studies (I^2 = 81.97, P=0.001). Although not all studies reached the statistical significance, findings from data not included in meta-analysis showed an overall increase in the total abdominal fat (P=0.027) [311], in the BFMI (Kg/m^2) 5.5±2.3 vs 6.8±2.3; P=0.00 [42]; 4.57 vs. 4.76; P= 0.120, and in visceral fat area (cm^2 95.65 vs 85.00; P=0.730) [298].

While we found some partially conflicting results regarding the effects of anti-TNF-α therapy on fat mass, data on changes in lean mass in paediatric studies [298, 300-302, 305, 314] consistently showed it significantly increased after anti-TNF therapy. Drilling down the studies, all but one reported a significant increase in lean body mass values from the baseline, as confirmed by skeletal mass (P=0.003), FFM (P<0.00), lean mass (LMI) indexes, muscle area z scores (P< 0.001), and leg lean mass score (P=0.001) in a period observation ranging from 3 to 12 months. Of importance, the FFM was significantly increased in patients on infliximab therapy compared to patients on conventional therapy, in the study by Emerenziani *et al.* [302]. Only in one study no significant change in lean body mass value was detected in CD patients after 6 months (P= 0.44), likely due to the small sample size, *i.e.,* 7 patients [314].

A significant proportion of children with CD has growth impairment at diagnosis [321, 322]. Whereas TNF-α is known to be implicated in the suppression of the growth hormone axis and long bone growth [323-325] evidence regarding growth benefits during anti-TNF-α therapy is still wanting [326] with no systematic data available yet. We now describe a small but statistically significant overall increase in height of (SMD=0.16 [95% CI [0.06-0.26]; P=0.002) from the baseline in paediatric patients with CD (along with an improvement in BMI and weight). All studies confirmed this substantial increase, after a treatment duration ranging from 9.3 to 18 months. The finding that growth (including height velocity) was more likely to improve in responders suggests that growth improves

as a result of better disease control by anti-TNF- α therapy [296, 309]. Some studies have suggested that in different chronic conditions, phase angle (PA) can be considered a promising tool to assess nutritional status [327-329]; reduced PA values are indeed associated with unfavourable disease progression and poor prognosis. More recently, the PA has been assessed in IBD paediatric patients during clinical remission [330]. Unfortunately, limited data on PA changes from the baseline were available in our analysis; also, findings from the only two studies reporting PA values showed conflicting results. Emerenziani *et al* found a significant increase in PA (from 4.6±0.3 to 6.2 ±0.4; P=< 0.05), along with a substantial increase in FFM index [302], whereas PA remained unchanged (6.2 versus 6.8; P=0.94) in the study by Dos Santos *et al* after 24 weeks of IFX therapy [301]. The lack of substantial improvement may be explained by the fact that PA decreases when FM increases and LM decreases; although patients gained both FM and LM, however, FM gain was even more substantial.

6.4.1. Limitations

The main limitation of this study is that we could only include 13 of the 23 identified studies in the meta-analysis as the required data were not reported in the remaining studies. The absence of published randomised controlled trials on this issue has forced us to include only observational studies. The limited data on potential co-variates such as disease duration, disease severity, other medications, smoking, physical activity, and dietary changes did not allow us to carry out meta-regressions where we could explore the effects of TNF-α inhibitors on body changes in more detail. Moreover, it should be pointed out that the vast majority of the patients included in the study (90.2%) had a diagnosis of CD and only a few patients had UC. This could be an additional bias for the interpretation of the results. CD is more often associated with weight loss and growth impairment than UC, so it would be interesting to understand if, with a higher proportion of UC patients, we would have the same results.

6.5. CONCLUSION

Our analysis revealed an increase in main anthropometric parameters (body weight, BMI, and height) among IBD patients treated with TNF-α inhibitors which were greater the longer the follow-up and in responders compared to non-responders. The potential effect of TNF-α inhibitors on anthropometric measures could be a consideration in the care of overweight and obese IBD adults given the concerns that weight gain may be a risk factor for developing metabolic disorders and increases the likelihood of anti-TNF therapy failure. In contrast, IBD was found to be associated with impaired weight gain in children, in which anti-TNF-α agents could even exert positive improvements in weight and linear growth. Further prospective studies are warranted to provide stronger evidence of the role of biological therapy on body changes, especially on fat and lean mass in IBD patients.

CHAPTER 7: STUDY 5

CHAPTER 7: STUDY V

Anthropometric changes following Anti-TNF-alpha treatment in children with Inflammatory Bowel Disease: a retrospective case series analysis combined with data from the Real-World pharmacovigilance study using the FDA Adverse Event Reporting System (FAERS) database

ABSTRACT

Background: Evidence that Anti-Tumour necrosis factor (TNF)-α therapy is associated with excess weight gain in children with inflammatory bowel diseases (IBD). has been suggested by some studies while others yielded conflicting results.
Objectives: we took advantage of real-life data on a huge number of patients to determine whether weight gain in children with IBD on maintenance anti-TNF- α therapy does occur and with which frequency, and evaluate the impact of the treatment on glycolipid profile and serum trough levels. To characterise better adverse events related to 'real-life' use of anti-TNF-α inhibitors in children, we also analysed the safety signals of anti-TNF-α inhibitor-associated adverse events related to body-changes in a large international pharmacovigilance database.
Methods: Paediatric patients with IBD, treated with anti-TNF-α inhibitors for at least 24 months were retrospectively reviewed. Anthropometric data (expressed as z scores of weight and BMI) and glycolipid measures were collected at the time of anti-TNF initiation, 12 and 24 months. A nested case/non-case analysis was performed using the Food and Drug Administration Adverse Event Reporting System (FAERS) database, focusing on events related to body-changes following the use of anti-TNF-α inhibitors. The risk was expressed as a measure of disproportionality using the reporting odds ratio while adjusting for potential confounders. We also applied the Weibull proportional hazards models to time-to-event data in FAERS for anti-TNF-α inhibitors to examine the expression patterns of event.
Results: Sixteen patients were included with a median age of 9.5 years and a median duration of treatment of 26 months. At baseline, mean weight was -2.88 (SD 1.7), while mean BMIz was -0.14 (1.3). Compared to baseline, there was a significant increase in body weight z score after 2 years (mean gain 2.06 ±0.38 $p < 0.0001$). Changes in body weight between the first and second year of treatment were statistically significant (1.99 ± 0.36; $p<0.001$). Variations in BMIz was not statistically significant at any time point. At 1-year, there was a significant increase in total cholesterol and low-density lipid. At 1-year follow-up, the difference in the mean BMIz of patients with subtherapeutic trough levels (Δ -0.83 ± 1.13) and patients with therapeutic trough levels was statistically significant (Δ 1.36 ± 0.55; $p<0.05$). Disproportionate reporting of body-change events in FAERS was found for anti-TNF-α in general (pooled aROR: 1.10 (1.04; 1.15). Sensitivity analysis showed stronger disproportionality for paediatrics [pooled aROR: 3.14 (2.47; 3.18)] than in adults [pooled aROR: 1.02 (0.97; 1.08)]. Among paediatrics, the strongest association with body-change was observed for etanercept [aROR:5.20 (3.77; 7.21)]. Time-to-onset analysis revealed that anti-TNF-α treatment-associated weight changes in IBD had a wear-out failure-type profile.
Conclusion: Anti-TNF-α agents appear to cause an excess weight gain in paediatric IBD patients. Given the potential of anti-TNF-α treatment to induce excess weight gain and dysmetabolism, continuous attention for this side effect with appropriate counselling regarding lifestyle modifications is warranted.

7.1. INTRODUCTION

Tumour Necrosis Factor (TNF)-α inhibitors have revolutionized the treatment of inflammatory bowel disease (IBD), as several anti-TNF-α agents such as infliximab (IFX) and adalimumab (ADA) have been proven effective in inducing and maintaining corticosteroid free remission in both adults and children with Crohn's disease (CD) and ulcerative colitis (UC).

In line with the complex gluco-metabolic effects exerted by TNF-α, that include gluconeogenesis, loss of adipose tissue, proteolysis, and regulation of enzymes involved in lipid metabolism, previous studies have demonstrated significant increases in body mass index (BMI) and/or body weight after anti-TNF-α treatment in IBD patients [331].

Despite modest weight gain is a desirable effect in IBD subjects because they are at a high risk of malnutrition, some observational studies suggest that these therapies themselves may also be associated with excess weight gain [332] and might lead to obesity, that in turn associated with an increased risk of cardiovascular disease [333] and metabolic syndrome [334]. It is also worthy of mentioning that, up to 40% of IBD patients are obese and an additional 20–40% are overweigh: In immune-mediated inflammatory diseases (IMIDs), excess body weight has been associated with more severe disease activity [335], inferior quality of life, and suboptimal response to anti–TNF-α treatment [336, 337]. In view of all the above-mentioned considerations, the potential association between anti–TNF-α treatment and negative anthropometric changes needs to be clarified.

We report here the results of a retrospective case series analysis on changes in body weight after 24-months of treatment with anti-TNF-α therapy in paediatric IBD patients. We also investigated changes in levels of glycolipid parameters, trough levels of IFX as well as their association with weight change. In order to map a broader profile of anti-TNF-α therapy in a real-world setting, we concurrently, carried out a comprehensive analysis to quantify the association between TNF-α therapy and the rate of body-related changes, by using one of the largest and most

comprehensive pharmacovigilance databases, i.e., the US Food and Drug Administration Adverse Event Reporting System (FAERS) Database. We also applied time-to-onset analysis to body-changes.

7.2. METHODS

7.2.1. Retrospective case series analysis

7.2.1.1. Patients and study design

We carried out a detailed retrospective case series analysis of paediatric IBD patients at our tertiary referral centre using an existing prospectively recorded inpatient electronic health records. The study was performed in accordance with the Declaration of Helsinki. The study was approved by Research and Ethics Committee at King Fahad Central Hospital, Jizan. As a retrospective review, there was a waiver of requirements for informed consent. No identifier was used, and privacy and confidentiality of patients were completely protected.

The main source of the data was the patient's medical records from both patients' charts and the hospital electronic medical system. The medical records of all patients seen in the Division of Paediatric Gastroenterology and Hepatology were electronically searched to identify patients with IBD who received anti-TNF-α therapy. Paediatric patients were defined as those who were less than 18 years of age at the time of first anti-TNF-α therapy and have a follow-up of at least 24 months.

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Two reviewers (AMM, SA) abstracted data through medical records. Two reviewers (FM, VB) validated the abstracted data. Relevant data collected include age, gender, weight, height, type of IBD, duration and extent of disease, clinical activity indices [Paediatric Crohn's Disease Activity Index (PCDAI) and the Paediatric Ulcerative Colitis Activity Index (PUCAI)],

length of follow-ups, dates and dosing of anti-TNF-α therapy, concomitant medications and adverse events. In addition, physical assessment, IFX trough levels and laboratory data including fasting blood glucose, serum total cholesterol (TC), triglycerides, high-density lipid (HDL), and low-density lipid (LDL) levels were collected from the medical records.

We collected data at the time of anti-TNF-α therapy initiation, at 12 and 24 months. A two-year follow-up was defined as the end of the study period, discontinuation of anti-TNF-α therapy or intestinal surgery was defined as the end of the study period. Patients were excluded if they had insufficient data or follow-ups (i.e., <24 months after anti-TNF-α initiation). Those patients who switched to other anti-TNF-α agents were also excluded.

The age and sex standardized body weight and body mass index (BMIz) were calculated by using SAS macro [338] at each time point based on the Centers for Disease Control 2000 (CDC) published data [339]. These z scores account for differences in the rate of change in weight and BMI based on age and gender. Weight and BMI z-scores at each time point are reported as mean with standard deviation. We have used the CDC established method of classifying children and adolescents as overweight/obese or normal weight [340]. In the absence of a clear definition of excess weight gain, we present data by the number of patients who by BMIz (a) changed from ‘‘normal’’ to ‘‘overweight/ obese’’ (b) changed from ‘‘overweight’’ to ‘‘obese’’ or (c) ≥ 0.5 change in BMIz during the study period.

Current practice at the time for anti-TNF-α dosing was as follows: for IFX, to give all children an initial three-dose course at week 0, week 2, and week 6, followed by maintenance: 5 mg/kg/dose every 8 weeks thereafter. For ADA, initial dose: 80 mg divided into 2 injections (40 mg each) on day 1, then 40 mg administered 2 weeks later (day 15); and then on day 29, begin maintenance dose: 20 mg every other week. Based on published literature, a trough level ≥ 5 μg/mL during maintenance was considered subtherapeutic [341]. Weight-based dosing refers to the practice of administering appropriate doses of IFX based on the actual weight of the patient. Dose optimization was defined as an increase in weight-based dose or drug interval shortening in response to reactive

therapeutic drug monitoring (TDM) to maintain clinical remission.

7.2.1.2. Data analysis

Changes in body weight, body weight z-score, BMIz, disease activity scores and glycolipid parameters were stated as descriptive statistics, with comparisons from baseline values by paired t-tests or Wilcoxon signed-rank sum tests, as appropriate. Further analyses were performed to explain the relative variation of weight, BMIz and glycolipid parameters, disease activity scores using the Spearman correlation coefficient. All the analysis protocol had been finalized before any data were viewed.

7.2.2. Pharmacovigilance Study

7.2.2.1. Data source and study design

Data were obtained from the FAERS. As a spontaneous reporting system, FAERS allows for disproportionality analysis, by which to detect and quantify the associations between target drugs and suspected adverse events. The FAERS receives approximately 1.5 million adverse events, product complaints, and user error reports from health care practitioners, consumers, companies, and other sources, concerning drugs, vaccines, and medical devices for human use. Adverse events are recorded in the FAERS using the Medical Dictionary for Regulatory Activities (MedDRA®) preferred terms. Due to the increase in the type and number of products the agency regulates, the awareness of the importance of these reports, and a larger population, the number of safety reports sent is continuously expanding, and the database is largely used to detect novel drug-related safety events, to identify possible mechanisms of adverse events, to explore potential drug-drug interaction related to adverse events and to the discovery of promising new concomitant uses of drugs [186, 342-344]. This study was designed as a nested case-control study. The base cohort consisted of all adverse events involving any anti-TNF-α medication [Anatomical Therapeutic Chemical (ATC) code L04AB, as suspect, interacting or concomitant drug. The study period covered the first quarter of 2015 to the first quarter of 2020.

7.2.2.2. Data Acquisition and Data Processing

Adverse events recorded in the FAERS were downloaded from the Food and Drug Administration (FDA) website [345]. The database consists of seven datasets, namely patient demographic and administrative information (file descriptor DEMO), drug and biologic information (DRUG), adverse events (REAC), patient outcomes (OUTC), report sources (RPSR), start and end dates of drug therapy (THER), and indications for use/diagnosis (INDI). These seven datasets were joined by unique identification numbers for each FAERS report and a relational database was built. Data extraction was restricted to reports without missing values for age and gender. Duplicate records were detected and deleted accordingly as previously described [57].

7.2.2.3. Definition of cases and controls

The basis for our analyses was the MedDRA Preferred Terms describing the adverse events of interest *i.e.,* 'weight-related events' and 'obesity'. A custom list of weight-related event terms was created for data mining, combining different Preferred Terms that contain a range of Lowest Level Terms (LLTs) reflecting the same medical concept expressed by synonyms and lexical variants [346]. After a review of all LLTs in MedDRA, 19 different LLTs were selected. Additionally, the LLT 'waist circumference increased' was also selected because of the relevance to the medical concept. **Appendix IX** summarizes the list of 'LLTs' used to define our custom list of terms called "Body Changes".

Cases were defined as all Individual Case Safety Reports (ICSRs) where at least one LLT from our customized list has been coded as an adverse event (outcome of interest). Non-cases (controls) were all other events reported in the database during the same period (*i.e.,* all reports without the outcome of interest).

7.2.2.4. Potential confounding factors

Potential confounding factors retrieved from the case reports included age, sex of the patient and exposure to medications (concomitant) that are associated with clinically significant weight gain. Concomitant use of medication associated with weight-related events, defined as reporting one of the drugs summarised in **Appendix X**, as a concomitant or interacting

drug for an ADR, was taken into account when recorded.

7.2.2.5. Time-to-onset analysis

Time-to-onset duration from the FAERS database was calculated from the time of the patient's first prescription to the occurrence of the adverse events. For duplicate entries of the same anti-TNF-α agents in the same case, we adopted the most recent entry to identify the beginning of the time of a subject's first prescription. Before analysis potentially miscoded entries were deleted. The median duration, quartiles, and The Weibull shape parameter (WSP) were used to evaluate the time-to-onset data [347].

7.2.2.6. Statistical Analyses

Descriptive analysis was performed for cases and non-cases, in terms of age, female sex and the use of concomitant medications associated with body changes. Between-group differences for the continuous variables were analysed by the Student's t-test while categorical variables (sex and the presence of concomitant medications associated with body changes) analysed by Pearson's Chi-square test. Tests were two-tailed, with significance set at a p-value of 0.05.

To identify signals of disproportionate reporting for body changes related adverse events in association with TNF-α inhibitors, we used the reporting odds ratio (ROR) as a measure of disproportional reporting, which estimates the frequency of an event of interest with the tested drugs compared with the other drugs. Signals of disproportionate reporting were detected when the number of reports was higher than three and ROR – 95% CI was greater than one. Apart from the crude ROR (cROR), the adjusted ROR (aROR) was calculated using multivariate logistic regression analysis adjusted for potential confounding factors as, i.e., age, gender, indication, concomitant treatments and reporting reporter type. For each TNF-α inhibitor, the cROR and aROR were calculated to compare the proportion of body changes events with the given individual TNF-α inhibitor to the proportion of body changes events with the other TNF-α inhibitor. The homogeneity between the stratified aROR was tested using the Breslow-Day test. If RORs were not significantly different, they were pooled to calculate Mantel–Haenszel–adjusted ROR. Significance of the adjusted

ROR could be assessed using the Cochran–Mantel–Haenszel test.
Given the high potential for confounding by age and indication in SRS data, we stratified analysis by indications related to IMIDs and age to mitigate confounding by these covariates, combining stratum-specific estimated into Mantel–Haenszel–adjusted ROR.
The WSP test is used for statistical analysis of time-to-onset data and can describe the non-constant rate of incidence of adverse events (i.e., the risk of increase or decrease over time). The scale parameter α of the Weibull distribution determines the scale of the distribution function. The shape parameter β of the Weibull distribution determines the shape of the distribution function. In the analysis of the SRS, the shape parameter β of the Weibull distribution was used to indicate the hazard without a reference population. when the shape parameter β was <1 and it's 95% CI was <1, the hazard was considered to have decreased over time (early failure-type profile); when the shape parameter β was equal to or nearly 1 and its 95% CI included the value 1, the hazard was estimated to constantly occur over time (random failure type profile); and when the shape parameter β was >1 and it's 95% CI excluded the value 1, the hazard was considered to increase over time (wear-out failure-type profile) [348, 349]. All the analysis protocol had been finalized before any data were viewed. All analyses were performed using counts of unique cases. Data reading, filtering, and processing were done through RStudio [350], statistical analyses with STATA ® (StataCorp, College Station, TX, USA).

7.3. RESULTS

7.3.1. A retrospective case series analysis

7.3.1.1. Demographics, clinical characteristics and medication use

We identified 37 potential subjects younger than 18 years of age with IBD who were treated with anti-TNF-α therapy between January 2016 and December 2019. Of these patients, 16 had at least 2 years of anti-TNF-α therapy follow-up and were included in this case series. Enrolled patients, 8 boys and 8 girls, were ages 7 to 14 years (median age 9.5 years) at the diagnosis of IBD, with a median of 28 months (range 6–60) from IBD

diagnosis to start of anti-TNF-α therapy.

Median follow-up was 26 months (range 24–34), with data available beyond 30 months for 2 patients. **Table 7.1** summarizes the general and disease-related characteristics along with concomitant medications in patients with CD and UC. In all, 11 of 16 patients (69%) received IFX as the first biological agent, whereas the remaining 5 subjects (31%) received ADA. Throughout the study period, concomitant immunomodulatory agents were used in 7 (44%) patients. Use of corticosteroids at baseline, 12 months, and 24 months was 31%, 37%, and 56%, respectively (data not shown).

After two months of induction therapy, 5 (31.2%) achieved and sustained clinical remission through the remaining study period. During follow-up, 7 (43.7%) subjects had their IFX dose increased based on reactive TDM of IFX; 4 of these dose escalations were achieved by decreasing the dosing interval from every other week to weekly administration. Remaining three patients had the dose increased. Three patients on IFX discontinued during the study period; 1 due to secondary loss of response at 26th month, 1 to serious infection at 30 month and 1 required surgery at 24th month. One patient was diagnosed with type 1 diabetes and excluded at 24-month follow-up analysis.

Table 7.1: Demographic characteristics of paediatric patients who received anti-TNF-α therapy (n = 16)

Male sex, *n (%)*	8 (50)
Median age at diagnosis, *years [range]*	9.5 [7-14]
Age categories, *n (%)*	
<10 years,	8 (50)
10–15 years	2 (12.5)
>16 years	6 (37.5)
Duration of disease at start of anti-TNF-α, *months [range]*	28 [6-60]
Type of inflammatory bowel disease, *n (%)*	
Crohn's disease	12 (75)
Ulcerative Colitis	4 (25)
Crohn's disease distribution (Paris classification), *n (%)*	
L1 Small intestine	2 (12.5)
L2 Colon	6 (37.5)
L3 Small intestine and colon	1 (6.25)
L4a Isolated upper disease	1 (6.25)

P Perianal disease	3 (18.75)
Ulcerative Colitis disease distribution, *n (%)*	
Pancolitis	
Proctitis	
Treatment at inclusion, *n (%)*	
Infliximab	11/16 (68.75)
Adalimumab	5/16 (31.25)
Concomitant medications, *n (%)*	
Steroids	11/16 (68.75)
Aminosalicylates	13/16 (81.25)
Immunomodulators	7/16 (43.7)
Azathioprine	10/16 (62.5)
Methotrexate	2/16 (13.5)
Antibiotics	13/16 (81.25)
Enteral nutrition	2/16 (13)

7.3.1.2. Change in anthropometers over time

Table 7.2 summarizes the changes in clinical and anthropometric characteristics, lipid profile, and glucose over time of the included patients. At the start of anti-TNF-α therapy, the mean weight z-score was -2.88 ± 1.74, and the mean BMIz was –0.14 ± 1.32. According to BMIz classification, at baseline, 9 (72.5%) patients were classified as normal weight, 4 (15.9%) underweight, 1(5.8%) overweight and 2 (5.8%) obese **Table 7.3**.

After 2 years of therapy, we observed a significant improvement of the activity scores assessed by the PCDAI (–26.1±6.29; $p<0.005$) and PUCAI (-19± 13.87; $p<0.10$). A decrease was also observed in systemic inflammation assessed by erythrocyte sedimentation rate (ESR, –6.81± 7.5; $p < 0.05$), and C-reactive protein (CRP, –19.5 ± 4.9; $p < 0.05$).
Compared to baseline, there was a significant increase in body weight z-score after 2 years (mean gain 2.06 ±0.38 $p < 0.005$). Changes between the baseline to the first year was not statistically significant. However, weight change between first and second year of treatment was statistically significant (1.99 ± 0.36; $p<0.005$). Variations in BMIz was not statistically significant at any time point. Similar results were obtained for weight and BMI in a subanalysis of patients with sex (data not shown).

Table 7.2: Change in anthropometry, glycolipid profile and inflammatory markers over time

	Baseline (n=16) Mean ± SD	**1-year post maintenance (n=16)** Mean ±SD	**2-year post maintenance (n=15)** Mean ±SD	**Baseline vs. 1-year post maintenance** Δ Mean ±SD	**1-year vs 2-years post maintenance** Δ Mean ±SD	**Baseline vs. 2-years post maintenance** Δ Mean ±SD
Weight, *kg*	30.6 ± 9.66	31.02 ± 9.05	41 ± 11.26	0.46 ± 0.56	-9.9± 2.1	10.4 ±2.27***
Weight z-score	-2.88 ± 1.74	-2.82 ± 1.62	-0.82 ± 1.32	0.06 ± 0.13	1.99 ± 0.36 ***	2.06 ±0.38 ***
BMIz	-0.14 ± 1.32	-0.06 7± 1.25	0.12 ± 0.88	0.082 ±0.1	-0.22 ± 0.19	0.27; 0.33
PCDAI, (n=12)	45.56 ± 17.61	39 ± 16.04	19.4 ± 4.64	-6.58 ±1.87**	-19.58± 5.91 ***	-26.1 ±6.29 ***
PUCAI, (n=4)	24.7 ±16.9	27.25 ± 15	15.75 ±11	-7.5 ±3.92	-11.5 ±12.23	-19 ± 13.87 *
ESR, *mm/h*	55.68 ± 35.31	35.5 ± 25.5	49.5 ± 30.58	-20.1 ±4.85 ***	14 ±6.1 *	-6.81± 7.5*
CRP, *mg/L*	25.75 ± 20.18	8.1 ± 8.04	6.74 ± 8.99	-19.5 ±4.9 ***	-1.87±1.25	-19.5 ± 4.9 **
TGs, *mmol/L*	1.13 ± 0.51	1.05 ± 0.44	1.178 ± 0.78	-0.08 ± 0.16; 0.6	0.126±0.19	0.04±0.22
TC, *mmol/L*	3.13 ± 1.18	3.87 ± 1.22	4.14 ± 1.31	0.7 ±0.29 *	0.27±0.29*	1.01±0.33**
HDL, *mmol/L*	3.28 ± 1.69	3.89 ± 1.21	3.72 ± 1.75	0.60 ±0.47	-0.167±0.39	0.44±0.52
LDL, *mmol/L*	2.20 ± 0.68	2.63 ± 0.53	3.61 ± 0.85	0.43±0.09 **	0.978±0.23***	1.41±0.25**
Glucose, *mmol/L*	5.24 ± 0.70	4.7 ± 0.88	5.09 ± 0.77	-5.2±0.30	0.37±0.3	-0.14±0.28

* $p < 0.10$, ** $p \leq 0.05$, *** $p \leq 0.005$

BMIz: age and sex standardized body mass index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoproteins; LDL: low-density lipoproteins; PCDAI: Paediatric Crohn's Disease Activity Index; PUCAI: Paediatric Ulcerative Colitis Activity Index; TC: Total Cholesterol; TGs: triglycerides.

Table 7.3: weight categories using BMIz score from baseline to 2-years

Baseline weight category n (%)	**Weight category at 2-year**	**At 2 year, BMIz score Δ >0.5**
Underweight n = 4 (15.9%)	Normal weight, n = 4	*n=4* –2.2 to 0.03 (Δ 2.19) –0.1 to 1.31 (Δ 1.45) –1.3 to –0.25 (Δ 1.09) –2.2 to –1.21 (Δ 0.99)
Normal weight n = 9 (72.5%)	Normal weight, n = 7 Overweight, n = 2	*n=3* –1.9 to –0.31 (Δ 1.58) 0.1 to 0.94 (Δ 0.80) 0.5 to 1.20 (Δ 0.69)
Overweight n = 1 (5.8%)	Normal weight, n = 1	-
Obese n = 2 (5.8%)	Normal weight, n = 1 Obese, n = 1	-

Table 7.3 summaries the weight categories using BMIz score from baseline to the last follow-up. All the patients who were ‘‘underweight’’ at baseline (n=4) were normalized at 2-year follow-up. Of the 12 patients who were of ‘‘normal,’’ ‘‘overweight,’’ or ‘‘obese’’ at baseline, 10 (83%) gained excess weight using Δ ≥ 0.5 criteria. Of the 9 patients who were initially ‘‘normal’’ weight at baseline, 2 were ‘‘overweight’’ at last follow-up. Of the 3 patients who were ‘‘obese/overweight’’ at baseline, 1 remained so at 2-year follow-up, while the other 2 patients loosen weight to ‘‘normal’’. We also assessed the possible influence of concomitant corticosteroids treatment to excess BMI gain. Of the six children that had not been exposed to corticosteroids for at least 12 months, 4 were categorized as normal weight at 1 year, while 1 child become overweight at last follow-up using CDC classification.

7.3.1.3. Change in glycolipid profile over time

At 1 year there was a significant increase in TC and LDL from baseline by a mean (± SD) of 0.7 ±0.29 ($p<0.10$) and 0.43±0.09 ($p<0.05$), respectively (**Table 7.2**). At 2 years the increases reached 0.27±0.29 ($p<0.10$) and 0.978±0.23 ($p<0.005$), respectively; the 2-year values were different from both the baseline and the 1-year values ($p< 0.05$ and $p <0.10$ for the TC, $p < 0.05$ and $p < 0.005$ for the LDL from baseline and 1 year, respectively). Two-year change in body weight z-score, was positively correlated with 2-year change in LDL ($r = 0.55$, $p = 0.02$) while negatively with disease activity score ($r = –0.52$; $p=0.04$). Two- year changes in LDL were positively correlated with 2-year changes in serum glucose ($r = 0.38$; $p=0.047$) (**Table 7.4**).

Table 7.4: Spearman correlation analysis of 2-year changes of body weight, Body Mass Index, glycolipid profile and disease activity scores

	Δ Weight	**Δ BMI**	**Δ TC**	**Δ LDL**	**Δ TG**	**Δ Glucose**	**Δ Disease activity**
Δ Weight z	-	-0.71	-0.12	0.55	-0.25	0.17	-0.52
Δ BMIz	-0.71	-	-0.28	-0.45	-0.01	-0.19	0.23
Δ TC	-0.12	-0.28	-	0.29	0.35	0.25	0.10
Δ LDL	0.55	-0.45	0.29	-	0.36	0.38	-0.52
Δ TG	-0.25	-0.01	0.35	0.36	-	0.50	0.23
Δ Glucose	0.17	-0.19	0.25	0.38	0.50	-	0.08
Δ disease activity	-0.52	0.23	0.10	-0.52	0.23	0.08	-

Values in bright red indicates significant negative correlation
Values in bright blue indicates significant positive correlation

7.3.1.4. Infliximab Trough Levels and anthropometric Characteristics

Infliximab trough levels were available for 7 (66.8%) patients during maintenance therapy as a result of reactive TDM. The median duration from IFX initiation to the first trough level measurement was 14 months (range: 11–16). All 7 patients had subtherapeutic trough levels. Mean 1st trough level of IFX was 2.2 ±1.58 μg/mL (range:0.6-4.9) (data not shown). Of note, in patients with subtherapeutic trough levels, 2 patients were obese, 1 overweight and five were normal weight with a gain of at least $\Delta \geq 0.5$ from baseline to 1 year. At 1-year follow-up, the difference in the mean BMIz of patients with subtherapeutic trough levels (Δ -0.83 ± 1.13) and patients deemed to have therapeutic trough levels (those who had not undergone reactive TDM) was statistically significant (Δ 1.36 ± 0.55; $p<0.05$) (data not shown).

7.3.2. Pharmacovigilance Study

7.3.2.1. Study population

From the FAERS we identified 272,921 reports involving at least one anti-TNF-α agent with information on age and sex. Of 272,921 reports, 2,713 (1%) were related to body-changes (cases). Univariate analyses of demographics and characteristics of nested cases and non-cases population are presented in **Table 7.5**.

Compared to non-cases, the prevalence of cases was higher in female individuals (75.6% vs. 67.9%; $p < 0.001$) and frequently reported by physicians and patients. The mean age of cases was significantly lower than that of non-cases (49.07±17 vs. 51.1 ±18 years; $p < 0.001$). Concomitant medications associated with weight gain were used in 18.4% of the cases and 8.4% of the non-cases ($p < 0.001$). The indications for anti-TNF-α use for different IMIDs also differed significantly among cases and non-cases ($p<0.05$).

Table 7.5. Characteristics of cases and non-cases in FAERS

	Cases (n=2,713)	**Non-cases (n=270,208)**	**P-value**
Age, yrs.			
Mean (SD)	49.07 (17.02)	51.10 (17.53)	<0.001
Median (IQR)	52 (39-61.53)	53.94 (39.73-63.90)	
≤ 17 years	177	13,062	
Gender, n (%)			
Male	661 (24.4)	86,721 (32.1)	<0.001
Female	2,052 (75.6)	183,487 (67.9)	
Indication for Anti-TNF, n (%)			
Rheumatologic diseases	1,390 (51.2)	143,021 (53.0)	<0.05
Psoriatic forms	233 (8.6)	26,187 (9.7)	
Inflammatory Bowel Diseases	727 (26.8)	67376 (24.9)	
Other	363 (13.4)	33,624 (12.4)	
Concomitant treatment related with the risk of weight gain, n (%)	498 (18.4)	22,716 (8.4)	<0.001
Reporter type, n (%)			
Patient	1623 (59.8)	144,240 (53.4)	<0.001
Medical Doctor	566 (20.1)	66,435 (24.6)	
Pharmacist	127 (4.7)	16,144 (6.0)	
Other	368 (13.6)	39,526 (14.6)	
Unknown/not available	29 (1.1)	3,863 (1.4)	

IQR: interquartile range; SD: standard deviation.

7.3.2.2. Disproportionality analysis

The number of cases, crude and the aROR for body-changes for anti-TNF-α agents, stratified by age are presented in **Table 7.6**. Ranked by the absolute number of reports, the highest number of body-changes events were reported for ADA use 59% (n = 1,602), followed by IFX 16% (n = 433), and eternacept 30% (n = 819). In 463 cases, more than one anti-TNF-α agent was recorded as suspect.

According to univariate analysis, the use of IFX crude RORs: 1.58 (1.42; 1.75) and adalimumab 1.23 (1.14; 1.33) were associated with a higher reporting rate of events related to body-changes. After adjustment for

gender, other drugs associated with weight changes and reporter type, disproportionality was found for anti-TNF-α in general (pooled aROR: 1.10 (1.04; 1.15). For the individual agents, IFX has by far the highest aROR 1.69 (1.51; 1.88) followed by etanercept 1.14 (1.04; 1.24) and adalimumab 1.10 (1.01; 1.20). No events were observed for golimumab and certolizumab. The trend of signals was consistent with reporting frequency over the study period (data not shown).

To inspect effect modification by age, age-specific RORs were calculated for all anti-TNF-α with at least 30 cases. The model assessing age showed a stronger disproportionality for paediatrics [pooled aROR: 3.14 (2.47; 3.18)] than in adults [pooled aROR: 1.02 (0.97; 1.08)]. Among 2,713 cases in the study population, 177 cases (6.5%) concerned paediatrics (until 17 years of age). In this specific population, the strongest association with body-change was observed for eternacept [aROR:5.20 (3.77; 7.21)]. Cases for adults yielded SDR for IFX [aROR:1.48 (1.30; 1.67)], albeit they were diluted due to a large number of background reports. In contrast, the association did not reach the signal threshold for the remaining anti-TNF-α agents in adults.

Subset analysis of cases with selected IMID indications among paediatric did not reach the signal threshold for IBD (**Table 7.7**). Briefly, IFX, ADA, and eternacept were associated with an SDR for body-change in rheumatological disorders, however, the confidence intervals were wide.

Table 7.6: Crude and adjusted reporting odds ratios (ROR) for the association between different anti-TNF-α agents stratified by age groups.

Anti-TNF-α agent	Overall			Age					
	n ~	**cROR (95% CI)**	**aROR (95% CI)**	**≤17 years**			**≥18 years**		
				n	**cROR (95% CI)**	**aROR (95% CI)**	**n**	**cROR (95% CI)**	**aROR (95% CI)**
Infliximab	433	1.58 (1.42; 1.75)	1.69 (1.51; 1.88)	109	2.98 (2.20;4.06)	2.34 (1.67; 3.30)	324	1.39 (1.24; 1.56)	1.48 (1.30; 1.67)
Adalimumab	1,602	1.23 (1.14; 1.33)	1.10 (1.01; 1.20)	123	3.72 (2.71; 5.17)	4.40 (3.12; 6.28)	1,479	1.16 (1.07; 1.25)	1.01 (0.92; 1.10)
Etanercept	819	0.97 (0.89; 1.05)	1.14 (1.04; 1.24)	108	4.24 (3.14; 5.77)	5.20 (3.77; 7.21)	711	0.86 (0.79; 0.94)	1.00 (0.91; 1.10)
Golimumab	144	0.86 (0.73; 1.02)	0.84 (0.71; 1.00)	0	-	-	144	0.89 (0.75;1.05)	0.88 (0.74; 1.04)
Certolizumab	178	0.95 (0.81; 1.10)	0.91 (0.78; 1.06)	0	-	-	178	0.99 (0.85; 1.15)	0.97 (0.83; 1.13)
Pooled	-	1.12 (0.86-1.37)	1.10 (1.04; 1.15)	-	3.49 (2.48; 4.13)	3.14 (2.47; 3.18)	-	1.01 (0.96; 1.06)	1.02 (0.97; 1.08)

aROR: Adjusted Reporting Odds Ratio; cROR: Crude Reporting Odds Ratio; CI: confidence interval; IBD: inflammatory bowel disease; rheumatological diseases.
Non-significant RORs are presented in italic
A significant signal was defined as ROR > 1 and an α threshold of 0.05 and presented with bold.
Adjusted ROR was calculated using multivariate logistic regression analysis, with adjustment for sex, drugs known to cause weight gain and reporter type.
~ A case may contain more than one suspected anti-TNF-α agents. For this reason, the total number of cases in which an individual anti-TNF-α agents (n = 3,176) mentioned as the suspected drug, was higher than the absolute number of cases in which anti-TNF-α agent was mentioned as the suspected (n = 2,713)

Table 7.7: Crude and adjusted reporting odds ratios (RORs) for the association between different anti-TNF-α agents in paediatric stratified by IMIDs indication.

	Cases (n)	**Non cases (n)**	**ROR (95% CI)**	**aROR (95% CI)**
Rheumatological disorders				
Infliximab	81	198	**59.19 (37.51; 96.52)**	**15.82 (8.86; 28.81)**
Adalimumab	88	1,238	**10.18 (6.26; 17.51)**	**7.73 (4.44; 14.08)**
Etanercept	98	2,284	**8.21 (4.25; 18.41)**	**12.66 (6.15; 29.74)**
Golimumab	0	59	-	-
Certolizumab	0	215	-	-
Inflammatory bowel diseases				
Infliximab	27	3,054	0.84 (0.50; 1.42)	1.34 (0.75; 2.37)
Adalimumab	30	2,776	1.26 (0.75;2.13)	0.80 (0.45; 1.44)
Etanercept	0	3	-	-
Golimumab	0	31	-	-
Certolizumab	0	115	-	-
Psoriatic forms				
Infliximab	0	9	-	-
Adalimumab	0	81	-	-
Etanercept	3	430	-	-
Golimumab	0	1	-	-
Certolizumab	0	2	-	-
Other diseases				
Infliximab	1	1,245		
Adalimumab	5	796	1.93 (0.56; 6.42)	1.77 (0.44; 6.85)
Etanercept	7	757	**4.35 (1.31; 16.65)**	**3.77 (1.02; 15.45)**
Golimumab	0	33	-	-
Certolizumab	0	52	-	-

CI: confidence interval; IBD: inflammatory bowel disease.
-significant RORs are presented with bold.
-A significant signal was defined as ROR > 1 and an α threshold of 0.05
-Adjusted ROR was calculated in an adjusted multivariate logistic regression analysis, with adjustment for sex, drugs known to cause weight gain and reporter type.
- Not included in the statistical analysis because the analysis was based on unique drug–event combinations with at least three occurrences

7.3.2.3. Time-to-onset analysis

Forty-six combinations having complete information for the date of starting treatment and the onset of adverse events in paediatric patients were

analysed. Overall, the median value for body-changes events caused by anti-TNF-α inhibitors was 8 (range:1-60) months and the upper limit of 95% CI of the shape parameter was >1, suggesting a random failure type profile (**Appendix XI**). When restricting the analysis to the cases in which anti-TNF-α indicated for IBD, shape parameter β was >1 and it's 95% CI excluded the value 1, suggesting wear-out failure-type profile.

7.4. DISCUSSION

Anti-TNF-α therapy has improved our ability to induce and sustain clinical and endoscopic remission in IBD and an increasing number of IBD specialists worldwide are adopting an early anti-TNF-α based regimen into real-life clinical practice [351]. Despite the evidence on the efficacy and safety of early biologic treatment in adult patients with moderate-to-severe CD, relevant data are relatively scarce in children and adolescents. Our case series analysis provides the first data on changes in anthropometric and glucometabolic changes in IBD children treated with anti-TNF-α inhibitors. Furthermore, this is the first study aimed at quantifying the association of anti-TNF-α therapy with body-changes, using the large spontaneous reporting system database FAERS.

Our case series analysis showed a significant gain in body weight z-score after 1 and 2 years (mean 2.06) of treatment, in parallel with a decrease in disease activity resulting from anti-TNF-α therapy. These findings sustain the scant evidence on paediatric patients with IBD available thus far [295]. A recent meta-analysis of limited studies demonstrated a significant increase in BMI and weight gain in children following anti-TNF-α treatment in IBD [352] Haas et al [353] reported 10/69 (17%) patients had excess weight gain at last follow-up (24-month). Recently, a post hoc analysis of clinical trials in 1273 IBD patients revealed that IFX therapy at any dose increased the body weight (+ 3.3 kg on 5 mg/kg dose, + 3.7 kg on 10 mg/kg dose) when compared to azathioprine monotherapy (+ 2.3 kg) after 1 year of treatment [354]. Similar outcomes were reported by a

small study (n = 57) from Sweden with a significant increase in weight after initiation of IFX therapy at week 6 (+ 2 kg) which continued to 12 months (+ 3 kg) [355].

The risk of body-changes in children treated with anti-TNF-α inhibitors is further supported by the analysis of the FAERS database as significant disproportionality in reporting of events related to body-changes with the use of anti-TNF agents was observed in the paediatric setting (and not for adults). However, when stratifying results by indications, the disproportionate rate of reporting was found with rheumatological disorders and not with IBD, suggesting that the tendency to gain weight differs by the underlying IMIDs. These preliminary results are in line with the limited number of studies reporting significant weight gains (ranging from 2.2 to 4.8 kg) in adults with rheumatologic diseases, in particular RA and psoriasis [356, 357].

This might be attributed to the difference in the tissue source of inflammatory cytokines, as well as the energy intake and the physical activity, thus resulting in a different tendency to gain weight according to several IMIDs. Although modest weight gain is desirable in IBD subjects because they usually are at a high risk of malnutrition, some patients clearly might have excessive weight gain that poses a health hazard due to the increased risk of cardiovascular comorbidities and the development of type 2 diabetes. In our sample, 3 patients were overweight/obese at baseline using BMI-z score. Furthermore, it is worthy of mentioning that CD and UC can exist in overweight and obese patients and that the traditional description of malnourishment in paediatric IBD may no longer represent the majority of cases. As weight gain appears to be stronger in those with normal and overweight IBD subjects, this group might benefit from counselling about this side effect.

An intriguing unexpected finding of our analysis was a significant increase in LDL and TC over time (**Table 7.2**). We studied the correlation between changes in weight and LDL and TC changes at 2 years, and we found a correlation between the 2-year changes in weight gain and LDL **(Table**

7.4). Data on glucose and lipid profile in IBD patients are rather limited. Only one study has examined IBD treated with IFX in the adult population; Koutroubakis et al. [358] reported IFX alters the lipid profile of patients as TC, HDL cholesterol and apo-A1 levels were significantly increased after the IFX treatment compared with baseline. There have been conflicting data among studies in the adult rheumatology and dermatology literature with regard effect on markers of metabolic syndrome after treatment [359-361]. However, a recent meta-analysis on the effect of TNF-α inhibitors on lipid profile in rheumatoid arthritis found that long-term anti-TNF-α treatment is associated with increased levels of HDL (+0.27 mmol/l, $p<0.0001$) and TC (+0.27 mmol/l, $p=0.03$), whereas LDL levels remained unchanged [362]. Thus, our findings support the preliminary evidence which suggests that weight gain induced by anti-TNF-α treatment might alter lipid profile in paediatric patients.

The clinical value of TDM is increasingly being recognized also in children, with the growing body of evidence linking serum anti-TNF drugs to clinical outcomes in IBD [363]. In the absence of adequate response, the dosage of the trough level of the drug has been suggested as a first step (reactive approach) [364]. A meta-analysis reported a positive correlation between obesity and inferior efficacy of anti-TNF agents in UC patients but not in CD patients [336]. More recently, a randomized clinical trial involving patients with luminal CD naive for biological therapy who started treatment with IFX or ADA identified obesity or overweight as a predictor of therapeutic failure to anti-TNF [365]. To evaluate whether the response to TNF-α inhibitors could be influenced by BMI we analyse the data available for patient underwent reactive TDM. We found a significant difference in the mean BMI-z of patients with subtherapeutic trough levels and patients deemed to have therapeutic trough levels. Our findings showed that excess weight gain is a negative prognostic factor in children with IBD treated with anti-TNF-α and a treatment effect modifier, providing additional data to the conflicting body of evidence on the potential negative impact of obesity in patients with IBD [365-367].

It should be noted that to accurately measure the effect of anti-TNF-α therapy on weight gain, it would be helpful to have drug levels at regular treatment intervals. However, at our centre, we do not follow proactive TDM; thus, in our analysis TDM data was available for only 7 patients. Additional prospective studies with a proactive TDM approach may help clarify whether anti-TNF-α effects on weight change the trough levels of weight-adjusted anti-TNF-α therapy.

Finally, to examine the onset profiles of anti-TNF-α-associated body-changes, time-to-onset analyses were conducted using the FAERS dataset. The median time-to-onset duration was 8.5 months for IBD patients. The WSP test revealed that anti-TNF-α -associated body-changes in IBD had a wear-out failure-type profile, suggesting that the risk of weight gain increases with time. Our findings imply that anti-TNF-α therapy could manifest an increase in weight as soon as by week 3.

Limitations

The main limitations of our observational study are its small, retrospective, single-centre nature, and our lack of a control comparator group; therefore, there was no standardized approach to recording patient data. Serum IFX trough concentrations were not always performed routinely as we analysed only those patients had trough concentrations taken based on a reactive strategy. Consequently, only maintenance therapy trough levels were recorded and compared. Another limitation is that IFX dose change was initially based on weight/weight gain and not trough levels, limiting our ability to determine the impact of excess weight gain on IFX trough concentrations. Children gain weight naturally during growth that potentially confounds the relationship between IFX and weight gain. Lastly, our patients were largely Arabs and centred in the Middle East, so our results may not be generalizable to other ethnicities and regions.

The use of a spontaneous reporting system database has some important implicit limitations, reporting being influenced by factors such as the notoriety bias, selection bias and under-reporting [368]. Unlike typical

case-control study, cases and non-cases are drawn from different populations, thus, this method cannot be a substitute for the classical case-control but gives relatively reliable results if the database contains several thousand of different drugs−event combinations.

7.5. CONCLUSION

To our knowledge, we are the first to report changes in weight and glucometabolic markers among paediatric IBD patients treated with anti-TNF-α drugs. Our findings suggest that anti-TNF-α agents could cause an excess weight gain in some paediatric patients with IBD; in particular, patients that are of normal or overweight by BMI-z at baseline seem to be at greater risk. This weight gain was correlated with an increase in TC, LDL and lower serum of IFX through levels.

Through the analysis of FAERS, this study confirmed the associations between anti-TNF-α therapy and adverse events related to body changes; however, age and indication related changes in associations were observed. Moreover, anti-TNF-α treatment-associated weight changes in IBD had a wear-out failure-type profile, suggesting that the risk of weight gain increases with time.

Given the potential of anti-TNF-α treatment to induce excess weight gain, dysmetabolism and widely distributed time-to-onset profile in IBD, we can infer that sustained and continuous attention for this side effect with appropriate counselling regarding lifestyle modifications are warranted in younger IBD patients receiving this treatment.

CHAPTER 8: GENRAL DISCUSSION

CHAPTER 8: GENERAL DISCUSSION

Despite each experimental chapter has its discussion, this section is intended to discuss in a more integrated and broader manner all the research studies presented in this thesis.

This dissertation has proposed and investigated different research questions in order to demonstrate a research framework. A research framework proposed in the present thesis aggregates evidence on important drug-related research questions using RWD, each of which can be informed by the methods and tools of clinical pharmacology research. Our research framework includes the method of scientific data analysis employed to investigate and evaluate how drugs are used in a population, as well as the relationship of the resulting data to an outcome. The outcome could be a benefit (a cure for a disease) or a risk (causing an adverse drug reaction or death). Understanding the patient characteristics and other factors that contribute to the risk provides evidence to improve health and demonstrate best practice.
Our research framework focussed around three main topics:

1. Methods for continual monitoring for unwanted effects and other safety-related aspects of the drugs (**Study I-III & V in Chapter 3, 4, and 5 and 7**).
2. Application of principles of epidemiology to the study of drug use and outcomes in the given sample of a population.
3. Synthesis of best possible evidence by performing SR/MA that provides an estimate of the probability of beneficial effects in populations, or the probability of adverse effects in populations, and other parameters relating to drug use benefit.

The scope of a postmarketing safety reporting system is quite broad. The system can cover all medicines used in the population, and it can receive reports of AEs/ADRs occurring in, any member of the

population. SRS databases compared to other data sources used to study drug safety questions, such as data from clinical trials, registries, and electronic healthcare data, each of which is relatively expensive to operate. In **Chapter 3, 4, and 5** and **6**, we described the use of SRS for signal evaluation and predicting plausible pharmacodynamic mechanism of given ADRs.

The availability of inpatient and other databases can allow clinical pharmacology researcher to define and create datasets tailored to a sample of distinct clinical characteristics. In an appropriately performed study, these data can be a valuable primary or adjunctive resource in helping to define the effectiveness or safety of various medications or interventions. The availability of clinical and sometimes laboratory data elements in these records affords the researcher versatility to test clinically important hypotheses related to common or rare medical conditions that were previously limited by insufficient data. When combined, these data can be used to infer a longitudinal picture of a person's medical and treatment history. Academic clinical pharmacologists can utilize these databases to publish important findings that consider the diagnosis of less common outcomes after a pharmaceutical exposure, temporal trends of a particular disease, descriptions in variations of therapeutic practices, reports on therapeutic or drug toxicity monitoring, and comparative effectiveness studies. The datasets are often representative of a large geographic area (nationally or regional) and thus the data provide an opportunity to examine differences by region, including variations in treatment practices for a specific illness. For instance, in **chapter 7 (study V)**, we demonstrate the impact of anti-TNF-alpha therapy in children with IBD on glucometabolic and anthropometric outcomes by using inpatient hospital records. We construct the of longitudinal histories and the exposure, occurrence of outcome events, and the presence of confounding factors were measured over time. We then supplement the finding of our longitudinal study by using SRS.

Our research framework also includes the systematic collection and analysis of research studies on a research question of interest, i.e., SR/MA. Besides the importance of SR/MA discussed in chapter, it offers many advantages from the training perspective of an academic clinical pharmacologist. It allows researcher familiarizing with an area in which one probably hope to conduct primary-level research, enables with the knowledge and skills required for complex bibliographic retrieval and verification of information and developing an advanced statistical skill. Further, because SR/MA is a synthesis of data that have already been collected, it is not necessary to obtain external funding or recruit participants.

8.1. CONTRIBUTIONS

1. The major contribution of this dissertation research is that it develops a framework to train and equip researcher to search, analyse and combine evidence on important drug-related clinical research questions from multiple RWD sources.
2. The studies carried out in the context of the proposed framework have contributed to the knowledge of the safety profile of drugs. So, the results provided useful information regarding the association of drug-AE and pharmacological mechanism of important AE.
3. The proposed framework requires obtaining and measuring signal scores using the most appropriate methods for SRS. We developed a data-driven and regression-based method to acquire signal scores adjusted for confounders from FAERS, which are proven to be better or comparable with other existing methods such as stratified analysis or nested-case/non-case.
4. It is novel that data-driven and regression-based methods have been developed to identify confounders in SRS and estimate drug-ADR associations while adjusting for these confounders.

5. To the best of my knowledge, this is the first time that OpenFDA API was used for data acquisition and subsequent filtering.
6. The method to combine ADR data from SRS data sources with pharmacodynamic data can be generalized to integrate evidence from other data sources. For example, using regional administrative healthcare databases.
7. Under the research framework, the dissertation provides the evidence on an important and one of the most pressing issues of the long-term safety of anti-TNF-alpha using longitudinal analysis, real-world safety data and published evidence. For the first time, the evaluation has been conducted on multiple streams of information. Moreover, the methods can be generalized to study other ADE of interest and prioritize ADR signals when signals are demonstrated consistently in multiple data sources, resolve conflict information when signals are demonstrated in one data source but not in the other data source, and detect ADR signals that are not able to be detected using individual data sources.

8.2. FUTURE PERSPECTIVES

With the growing appreciation of several important drug-related problems, it is recommended that future studies using large EHR databases by complex pharmacoepidemiological methods should also include in the research framework. Research by using EHR databases such as Health Search Longitudinal Patient Database (LPD)-Italy from IQVIA™, PEDIANET-Italy and Arianna would allow future research on drug utilization, safety, and effectiveness; pharmacovigilance and signal detection; health economics research including cost-effectiveness; studies using case-control, cohort, longitudinal, self-controlled, and other designs; and a variety of methodologic studies. New collaborations should be made to enhance the capacity of the academic centres to address important questions in medication use. Such collaborations can bring together large groups of patients for the

study, increasing the size of populations available for research, as well as their diversity and representativeness.

8.3. FINAL CONCLUSIONS

This dissertation has proposed and investigated a research framework for clinical pharmacology if this would produce reliable evidence to answer given research question by exploiting and combining evidence from different data sources, namely pharmacovigilance and observational studies. 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 research presented in this dissertation has produced several novel findings that provide new insights that demonstrate that integrating ADR signals from safety databases with pharmacodynamic data has the potential to predict pharmacological mechanism behind drug-event.
- In this thesis, antipsychotics and antidepressants have been compared and ranked based on their tendency to cause hyponatremia; an ADR could be considered detrimental for the underlying conditions of these patients.
- Autoimmune blistering diseases, like bullous pemphigoid associated with the use of gliptins and in this thesis, we demonstrated clinical relevance of gliptins selectivity for DDP-4 in the development of BP as a result of exposure to these drugs. No previous study has studied this association so far, even though a safety alert regarding bullous pemphigoid and gliptins use was released by the regulatory agencies.
- In this thesis, the association between anti-TNF and anthropometric measures in IBD patients were studied using different approaches. Despite several confounders, this safety

information should prompt clinicians as well as researchers to be aware of this effect especially at-risk patients and maybe take preventive measures. At the same time, further studies should be designed.

In conclusion, completing safety profiles over the market life of a drug is a constant challenge in the field of clinical pharmacology and is critical for patient safety. A detailed analysis of the SRS databases still contributes to the never-ending process of knowledge acquisition regarding the safety profile of medicines. This dissertation developed a research framework including technical and statistical methods for examining ADEs and/or beneficial effect of drugs that might occur among new or established users of pharmaceuticals. This work provides a template to clinical pharmacologist in establishing a continuous learning system for other priority conditions and drug classes, other populations, and other databases which could potentially unveil drug safety profiles and novel AEs.

CHAPTER 9: REFERENCES

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1. Donaldson, M.S., J.M. Corrigan, and L.T. Kohn, To err is human: building a safer health system. Vol. 6. 2000: National Academies Press.
2. Coloma, P.M. Mining Electronic Healthcare Record Databases to Augment Drug Safety Surveillance. (2012) Erasmus University Rotterdam. Available from http://hdl.handle.net/1765/41031
3. Formica, D., et al., The economic burden of preventable adverse drug reactions: a systematic review of observational studies. *Expert opinion on drug safety*, 2018. **17**(7): p. 681-695.
4. Kohn, L.T., J. Corrigan, and M.S. Donaldson, To err is human: building a safer health system. Vol. 6. 2000: National academy press Washington, DC.
5. Patel, P.B. and T.K. Patel, Mortality among patients due to adverse drug reactions that occur following hospitalisation: a meta-analysis. *European journal of clinical pharmacology*, 2019 **75**(9):1293-1307
6. Patel, T.K. and P.B. Patel, Mortality among patients due to adverse drug reactions that lead to hospitalization: a meta-analysis. *European journal of clinical pharmacology*, 2018. **74**(6): p. 819-832.
7. Gandhi, T.K., et al., Adverse drug events in ambulatory care. *New England Journal of Medicine*, 2003. **348**(16): p. 1556-1564.
8. Avery, A.A., et al., Investigating the prevalence and causes of prescribing errors in general practice: the PRACtICe Study. General Medical Council , 2012. 227 p.
9. Avery, A.J., et al., The prevalence and nature of prescribing and monitoring errors in English general practice: a retrospective case note review. The British journal of general practice : *The journal of the Royal College of General Practitioners*, 2013. **63**(613): p. e543-e553.
10. Assiri, G.A., et al., What is the epidemiology of medication errors, error-related adverse events and risk factors for errors in adults managed in community care contexts? A systematic review of the international literature. *BMJ open*, 2018. **8**(5): p. e019101.
11. Dechanont, S., et al., Hospital admissions/visits associated with drug–drug interactions: a systematic review and meta-analysis. *Pharmacoepidemiology and drug safety*, 2014. **23**(5): p. 489-497.
12. Zheng, W.Y., et al., Drug-drug interactions and their harmful effects in hospitalised patients: a systematic review and meta-analysis. *European journal of clinical pharmacology*, 2018. **74**(1): p. 15-27.
13. Espinosa-Bosch, M., et al., Prevalence of drug interactions in hospital healthcare. *International journal of clinical pharmacy*, 2012. **34**(6): p. 807-817.
14. Moura, C.S., F.A. Acurcio, and N.O. Belo, Drug-drug interactions associated with length of stay and cost of hospitalization. *Journal of Pharmacy & Pharmaceutical Sciences*, 2009. **12**(3): p. 266-272.
15. Bucşa, C., et al., How many potential drug–drug interactions cause adverse drug reactions in hospitalized patients? *European Journal of Internal Medicine*, 2013. **24**(1): p. 27-33.
16. Kaufman, D.W., et al., Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. *JAMA*, 2002. **287**(3): p. 337-344.
17. Qato, D.M., et al., Changes in prescription and over-the-counter medication and dietary supplement use among older adults in the United States, 2005 vs 2011. *JAMA internal medicine*, 2016. **176**(4): p. 473-482.
18. Midão, L., et al., Polypharmacy prevalence among older adults based on the survey of health, ageing and retirement in Europe. *Archives of Gerontology and Geriatrics*, 2018. **78**: p. 213-220.
19. Palmer, K., et al., Association of polypharmacy and hyperpolypharmacy with frailty states: a systematic review and meta-analysis. *European Geriatric Medicine*, 2019. **10**(1): p. 9-36.
20. Pedrós, C., et al., Adverse drug reactions leading to urgent hospital admission in an elderly population: prevalence and main features. *European journal of clinical pharmacology*, 2016. **72**(2): p. 219-226.
21. Sachs, A.N., et al., Pediatric information in drug product labeling. Jama, 2012. **307**(18): p. 1914-1915.
22. Allen, H.C., et al., Off-Label Medication use in Children, More Common than We Think: A

Systematic Review of the Literature. *The Journal of the Oklahoma State Medical Association*, 2018. **111**(8): p. 776.
23. Yackey, K., et al., Off-label Medication Prescribing Patterns in Pediatrics: An Update. *Hospital pediatrics*, 2019. **9**(3): p. 186-193.
24. Bourgeois, F.T. and T.J. Hwang, Improving the study of new medicines for children with rare diseases. *JAMA pediatrics*, 2018. **172**(1): p. 7-9.
25. Mahmood, I., Developmental pharmacology: impact on pharmacokinetics and pharmacodynamics of drugs, in Fundamentals of Pediatric Drug Dosing. 2016, Springer. p. 23-44.
26. Alghamdi, A.A., et al., Prevalence and Nature of Medication Errors and Preventable Adverse Drug Events in Paediatric and Neonatal Intensive Care Settings: A Systematic Review. *Drug safety*, 2019. **42**(12):1423-1436.
27. Lowrance, W.W. and J. Klerer, Of Acceptable Risk: Science and the Determination of Safety. *Journal of The Electrochemical Society*, 1976. 123(11): p. 373C-373C.
28. Lewis, J.A., Statistical principles for clinical trials (ICH E9): an introductory note on an international guideline. *Statistics in Medicine*, 1999. **18**(15): p. 1903-1942.
29. FDA-ASCO PUBLIC WORKSHOP: 2020 Clinical Outcome Assessments in Cancer Clinical Trials Fifth Annual Workshop. July 22 - 17, 2020.
30. Stegall, M.D., et al., The importance of drug safety and tolerability in the development of new immunosuppressive therapy for transplant recipients: The Transplant Therapeutics Consortium's position statement. *American Journal of Transplantation*, 2019. **19**(3): p. 625-632.
31. Shader, R.I., Safety Versus Tolerability. *Clinical Therapeutics*, 2018. **40**(5): p. 672-673.
32. Rate Outcomes, in Sample Sizes for Clinical, Laboratory and Epidemiology Studies. 2018. p. 83-98.
33. Rothwell, P.M., External validity of randomised controlled trials:"to whom do the results of this trial apply?". *The Lancet*, 2005. **365**(9453): p. 82-93.
34. FDA drug safety communication: FDA warns about prescribing and dispensing errors resulting from brand name confusion with antidepressant Brintellix (vortioxetine) and antiplatelet Brilinta (ticagrelor) [safety announcement]. U.S. Food and Drug Administration: Silver Spring, MD; July 30, 2015.
35. Franco, J.V.A., S.A. Terrasa, and K.S. Kopitowski, Medication discrepancies and potentially inadequate prescriptions in elderly adults with polypharmacy in ambulatory care. *Journal of family medicine and primary care*, 2017. **6**(1): p. 78.
36. Schlendorf, K., et al., Thrombolytic therapy for thrombosis of continuous flow ventricular assist devices. *Journal of cardiac failure*, 2014. **20**(2): p. 91-97.
37. Farina, E.K., K.G. Austin, and H.R. Lieberman, Concomitant dietary supplement and prescription medication use is prevalent among US adults with doctor-informed medical conditions. *Journal of the Academy of Nutrition and Dietetics*, 2014. **114**(11): p. 1784-1790. e2.
38. Tsai, H.H., et al., Evaluation of documented drug interactions and contraindications associated with herbs and dietary supplements: a systematic literature review. *International journal of clinical practice*, 2012. **66**(11): p. 1056-1078.
39. Chung, W.-H., et al., Medical Genetics: a marker for Stevens–Johnson syndrome. *Nature*, 2004. **428**(6982): p. 486.
40. Ferrell, P.B. and H.L. McLeod, Carbamazepine, HLA-B*1502 and risk of Stevens–Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. *Pharmacogenomics*, 2008. **9**(10): p. 1543-1546.
41. Franklin, J.M., et al., Nonrandomized real-world evidence to support regulatory decision-making: Process for a randomized trial replication project. Clinical Pharmacology & Therapeutics, 2020. **107**(4):817-826.
42. Garrison Jr, L.P., et al., Using real-world data for coverage and payment decisions: The ISPOR real-world data task force report. *Value in health*, 2007. **10**(5): p. 326-335.
43. Charter, R., et al., The assessment of value for medical devices: using real world evidence (RWE) to quantify unmet needs in diabetes management. *Value in Health*, 2016. **19**(7): p. A703.
44. Li, G., et al., Registry-based randomized controlled trials-what are the advantages, challenges, and areas for future research? *Journal of Clinical Epidemiology*, 2016. **80**: p. 16-

24.
45. Ball, R., et al., The FDA's sentinel initiative—a comprehensive approach to medical product surveillance. *Clinical Pharmacology & Therapeutics*, 2016. **99**(3): p. 265-268.
46. Ko, C.Y., et al., The American college of surgeons national surgical quality improvement program: achieving better and safer surgery. The Joint Commission. *Journal on Quality and Patient Safety*, 2015. **41**(5): p. 199-AP1.
47. Yue, L.Q., Leveraging Real-World Evidence Derived from Patient Registries for Premarket Medical Device Regulatory Decision-Making. *Statistics in Biopharmaceutical Research*, 2018. **10**(2): p. 98-103.
48. Makady, A., et al., Policies for use of real-world data in health technology assessment (HTA): a comparative study of six HTA agencies. *Value in Health*, 2017. **20**(4): p. 520-532.
49. Oyinlola, J.O., J. Campbell, and A.A. Kousoulis, Is real world evidence influencing practice? A systematic review of CPRD research in NICE guidances. *BMC Health Services Research*, 2016. **16**(1): p. 299.
50. Harpaz, R., W. DuMochel, and N.H. Shah, Big data and adverse drug reaction detection. *Clinical Pharmacology & Therapeutics*, 2016. **99**(3): p. 268-270.
51. Liu, F., A. Jagannatha, and H. Yu, Towards Drug Safety Surveillance and Pharmacovigilance: Current Progress in Detecting Medication and Adverse Drug Events from Electronic Health Records. *Drug Saf*, 2019. **42**(1): p. 95-97.
52. Vilar, S., C. Friedman, and G. Hripcsak, Detection of drug–drug interactions through data mining studies using clinical sources, scientific literature and social media. *Briefings in Bioinformatics*, 2017. **19**(5): p. 863-877.
53. World health OrganizationClinical pharmacology: scope, organization, training. Report of a WHO study group. WHO Technical Rept Ser., 446 (1970), pp. 1-21.
54. Mazhar, F., et al., Association of Hyponatraemia and Antidepressant Drugs: A Pharmacovigilance–Pharmacodynamic Assessment Through an Analysis of the US Food and Drug Administration Adverse Event Reporting System (FAERS) Database. *CNS drugs*, 2019. **33**(6): p. 581-592.
55. Carnovale, C., et al., Bullous pemphigoid induced by dipeptidyl peptidase-4 (DPP-4) inhibitors: a pharmacovigilance-pharmacodynamic/pharmacokinetic assessment through an analysis of the VigiBase®. *Expert opinion on Drug Safet*y, 2019. **18**(11): p. 1099-1108.
56. Mazhar, F., et al., Prevention of medication errors at hospital admission: a single-centre experience in elderly admitted to internal medicine. *International journal of clinical pharmacy*, 2018. **40**(6): p. 1601-1613.
57. Carnovale, C., et al., A characterization and disproportionality analysis of medication error related adverse events reported to the FAERS database. *Expert Opinion on Drug Safety*, 2018. **17**(12): p. 1161-1169.
58. Mazhar, F., et al., Changes in Anthropometric Parameters After Anti-TNFα Therapy in Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *BioDrugs*, 2020. **34**(5): p. 649-668.
59. Makady, A., et al., What is real-world data? A review of definitions based on literature and stakeholder interviews. *Value in Health*, 2017. **20**(7): p. 858-865.
60. Sherman, R.E., et al., Real-world evidence—what is it and what can it tell us. *N Engl J Med*, 2016. **375**(23): p. 2293-2297.
61. Bae, J., K.J. Rask, and E.R. Becker, The impact of electronic medical records on hospital-acquired adverse safety events: differential effects between single-source and multiple-source systems. *American Journal of Medical Quality*, 2018. **33**(1): p. 72-80.
62. Denny, J.C., Mining electronic health records in the genomics era. *PLoS Computational Biology*, 2012. **8**(12): p. e1002823.
63. Miani, C., et al., Health and healthcare: assessing the real world data policy landscape in Europe. *Rand Health Quarterly*, 2014. **4**(2).
64. Nabhan, C., A. Klink, and V. Prasad, Real-world Evidence—What Does It Really Mean? *JAMA Oncology*, 2019. **5**(6): p. 781-783.
65. Bellows, B.K., et al., Real-world evidence in pain research: a review of data sources. *Journal of Pain & Palliative Care Pharmacotherapy*, 2014. **28**(3): p. 294-304.
66. Clegg, A., et al., Development and validation of an electronic frailty index using routine primary care electronic health record data. *Age and Ageing*, 2016. **45**(3): p. 353-360.
67. Staggers, N., et al., Evaluation of a BCMA's electronic medication administration record.

Western Journal of Nursing Research, 2015. **37**(7): p. 899-921.
68. Price, J., What can big data offer the pharmacovigilance of orphan drugs? *Clinical Therapeutics*, 2016. **38**(12): p. 2533-2545.
69. Coloma, P.M., et al., Postmarketing safety surveillance. *Drug Safety*, 2013. **36**(3): p. 183-197.
70. Duggirala, H.J., et al., Use of data mining at the Food and Drug Administration. *Journal of the American Medical Informatics Association*, 2015. **23**(2): p. 428-434.
71. Bihan, K., et al., Uses of pharmacovigilance databases: An overview. Therapies, 2020. 75(6): p. 591-598.
72. European Medicine Agency. Annual Report 2018. Available from: https://www.ema.europa.eu/en/news/ema-annual-report-2018-published
73. United States Food and Drug Admionistration. FDA adverse event resporting system (FAERS) public dashboard. https://fis.fda.gov/sense/app/d10be6bb-494e-4cd2-82e4-0135608ddc13/sheet/7a47a261-d58b-4203-a8aa-6d3021737452/state/analysis. Accessed 7 Jan 2019.
74. Trifirò, G., J. Sultana, and A. Bate, From big data to smart data for pharmacovigilance: the role of healthcare databases and other emerging sources. *Drug safety*, 2018. **41**(2): p. 143-149.
75. Practical Aspects of Signal Detection in Pharmacovigilance, Council for International Organizations of Medical Sciences. Report of CIOMS Working Group VIII. CIOMS, Geneva, 2010.
76. Klein, K., et al., When more is less: an exploratory study of the precautionary reporting bias and its impact on safety signal detection. *Clinical Pharmacology & Therapeutics*, 2018. **103**(2): p. 296-303.
77. Bate, A., R.F. Reynolds, and P. Caubel, The hope, hype and reality of big data for pharmacovigilance. 2018, SAGE Publications Sage UK: London, England.
78. Borenstein, M., et al., Introduction to meta-analysis. 2011: John Wiley & Sons.
79. Berlin, J.A., et al., The Use of Metaanalysis in Pharmacoepidemiology. In Pharmacoepidemiology (eds B.L. Strom, S.E. Kimmel and S. Hennessy).
80. Strom, B.L. (2019). Choosing among the Available Data Sources for Pharmacoepidemiology Research. In Pharmacoepidemiology (eds B.L. Strom, S.E. Kimmel and S. Hennessy).
81. Evans, S., P.C. Waller, and S. Davis, Use of proportional reporting ratios (PRRs) for signal generation from spontaneous adverse drug reaction reports. *Pharmacoepidemiology and Drug Safety*, 2001. **10**(6): p. 483-486.
82. Rothman, K.J., S. Lanes, and S.T. Sacks, The reporting odds ratio and its advantages over the proportional reporting ratio. *Pharmacoepidemiology and Drug Safety*, 2004. **13**(8): p. 519-523.
83. Suzuki, Y., et al., Analysis of the interaction between clopidogrel, aspirin, and proton pump inhibitors using the FDA adverse event reporting system database. *Biological and Pharmaceutical Bulletin*, 2015. **38**(5): p. 680-686.
84. DuMouchel, W., Bayesian data mining in large frequency tables, with an application to the FDA spontaneous reporting system. *The American Statistician*, 1999. **53**(3): p. 177-190.
85. Bate, A., et al., A Bayesian neural network method for adverse drug reaction signal generation. *European Journal of Clinical Pharmacology*, 1998. **54**(4): p. 315-321.
86. DuMouchel, W. and D. Pregibon, Empirical bayes screening for multi-item associations, in Proceedings of the seventh ACM SIGKDD international conference on Knowledge discovery and data mining. 2001, Association for Computing Machinery: San Francisco, California. p. 67–76.
87. Wisniewski, A.F., et al., Good signal detection practices: evidence from IMI PROTECT. *Drug Safety*, 2016. **39**(6): p. 469-490.
88. Mannesse, C.K., et al., Hyponatraemia as an adverse drug reaction of antipsychotic drugs. *Drug Safety*, 2010. **33**(7): p. 569-578.
89. Willemen, M.J., et al., Use of dipeptidyl peptidase-4 inhibitors and the reporting of infections: a disproportionality analysis in the World Health Organization VigiBase®. *Diabetes care*, 2011. **34**(2): p. 369-374.
90. Montastruc, J.L., et al., Benefits and strengths of the disproportionality analysis for identification of adverse drug reactions in a pharmacovigilance database. *British Journal of Clinical Pharmacology*, 2011. **72**(6): p. 905-908.

91. De Bruin, M., et al., Anti-HERG activity and the risk of drug-induced arrhythmias and sudden death. *European Heart Journal*, 2005. **26**(6): p. 590-597.
92. Nguyen, T.T.H., et al., An original pharmacoepidemiological–pharmacodynamic method: application to antipsychotic-induced movement disorders. *British Journal of Clinical Pharmacology*, 2017. **83**(3): p. 612-622.
93. De Bruin, M.L., et al., Anti-HERG activity and the risk of drug-induced arrhythmias and sudden death. *European Heart Journal*, 2005. **26**(6): p. 590-597.
94. Patras de Campaigno, E., et al., Identification of cellular targets involved in cardiac failure caused by PKI in oncology: an approach combining pharmacovigilance and pharmacodynamics. *British Journal of Clinical Pharmacology*, 2017. **83**(7): p. 1544-1555.
95. Siafis, S. and G. Papazisis, Detecting a potential safety signal of antidepressants and type 2 diabetes: a pharmacovigilance-pharmacodynamic study. *British Journal of Clinical Pharmacology*, 2018. **84**(10): p. 2405-2414.
96. Cornet, L., et al., Pulmonary arterial hypertension associated with protein kinase inhibitors: a pharmacovigilance–pharmacodynamic study. *European Respiratory Journal*, 2019. **53**(5): p. 1802472.
97. Nguyen, T.T.H., et al., Role of Serotonin Transporter in Antidepressant-Induced Diabetes Mellitus: A Pharmacoepidemiological–Pharmacodynamic Study in VigiBase®. *Drug Safety*, 2018. **41**(11): p. 1087-1096.
98. Montastruc, F., et al., Role of serotonin 5-HT2C and histamine H1 receptors in antipsychotic-induced diabetes: a pharmacoepidemiological-pharmacodynamic study in VigiBase®. *European Neuropsychopharmacology*, 2015. **25**(10): p. 1556-1565.
99. Anglemyer, A., H.T. Horvath, and L. Bero, Healthcare outcomes assessed with observational study designs compared with those assessed in randomized trials. *Cochrane Database of Systematic Reviews*, 2014(4).
100. Cumpston, M., et al., Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. *Cochrane Database of Systematic Reviews*, 2019(10).
101. Sacks, H.S., et al., Meta-analyses of randomized control trials: an update of the quality and methodology. Medical Uses of Statistics. 2nd ed. Boston, Mass: NEJM Books, 1992: p. 427-442.
102. Stroup, D.F., et al., Meta-analysis of observational studies in epidemiology: a proposal for reporting. *JAMA*, 2000. **283**(15): p. 2008-2012.
103. Murad, M.H. and Z. Wang, Guidelines for reporting meta-epidemiological methodology research. *BMJ Evidence-Based Medicine*, 2017. **22**(4): p. 139-142.
104. Evidence Synthesis and Meta-analysis for Drug Safety: Report of CIOMS Working Group X. 2016: CIOMS.
105. Spigset, O. and K. Hedenmalm, Hyponatremia in relation to treatment with antidepressants: a survey of reports in the World Health Organization data base for spontaneous reporting of adverse drug reactions. *Pharmacotherapy*, 1997. **17**(2): p. 348-52.
106. Movig, K.L.L., et al., Association between antidepressant drug use and hyponatraemia: a case-control study. *British Journal of Clinical Pharmacology*, 2002. **53**(4): p. 363-369.
107. De Picker, L., et al., Antidepressants and the risk of hyponatremia: a class-by-class review of literature. *Psychosomatics*, 2014. **55**(6): p. 536-47.
108. Mulrow, C.D., et al., Efficacy of newer medications for treating depression in primary care patients. *Am J Med*, 2000. **108**(1): p. 54-64.
109. Coupland, C., et al., Antidepressant use and risk of adverse outcomes in older people: population based cohort study. *BMJ*, 2011. **343**: p. d4551.
110. Degner, D., et al., Severe adverse drug reactions of antidepressants: results of the German multicenter drug surveillance program AMSP. *Pharmacopsychiatry*, 2004. **37 Suppl 1**: p. S39-45.
111. Gandhi, S., et al., Second-Generation Antidepressants and Hyponatremia Risk: A Population-Based Cohort Study of Older Adults. *Am J Kidney Dis*, 2017. **69**(1): p. 87-96.
112. Leth-Moller, K.B., et al., Antidepressants and the risk of hyponatremia: a Danish register-based population study. *BMJ Open*, 2016. **6**(5): p. e011200.
113. Letmaier, M., et al., Hyponatraemia during psychopharmacological treatment: results of a drug surveillance programme. *Int J Neuropsychopharmacol*, 2012. **15**(6): p. 739-48.
114. Pelayo-Teran, J.M., M.M. Martinez-Perez, and Y. Zapico-Merayo, Safety in the use of

antidepressants: Vortioxetine-induce hyponatremia in a case report. *Rev Psiquiatr Salud Ment*, 2017. **10**(4): p. 219-220.
115. Jacobs, G.E. and A.F. Cohen, The South London and Maudsley NHS Foundation Trust and Oxleas NHS Foundation Trust Prescribing Guidelines, 9th Edition. *British Journal of Clinical Pharmacology*, 2008. **65**(4): p. 624-625.
116. Nguyen, T.T., et al., An original pharmacoepidemiological-pharmacodynamic method: application to antipsychotic-induced movement disorders. *British Journal of Clinical Pharmacology*, 2017. **83**(3): p. 612-622.
117. Nguyen, T.T.H., et al., Role of Serotonin Transporter in Antidepressant-Induced Diabetes Mellitus: A Pharmacoepidemiological-Pharmacodynamic Study in VigiBase®((R)). *Drug Safety*, 2018. **41**(11): p. 1087-1096.
118. US Food and Drug Administration/OpenFDA. Drug adverse events [/drug/event]. https://open.fda.gov/tools/downloads. Accessed 12 Mar 2018.
119. US Food and Drug Administration/OpenFDA. GitHub, Inc. 2015. https://github.com/FDA/openfda. Accessed 12 Mar 2018.
120. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Medical dictionary for regulatory activities (MedDRA®). Standardised MedDRA® Queries.
121. Madhusoodanan, S., et al., Hyponatraemia associated with psychotropic medications. A review of the literature and spontaneous reports. *Adverse Drug React Toxicol Rev*, 2002. **21**(1-2): p. 17-29.
122. Mann, J.J., The Medical Management of Depression. 2005. **353**(17): p. 1819-1834.
123. Liu, B.A., et al., Hyponatremia and the syndrome of inappropriate secretion of antidiuretic hormone associated with the use of selective serotonin reuptake inhibitors: a review of spontaneous reports. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne*, 1996. **155**(5): p. 519-527.
124. Mannesse, C.K., et al., Characteristics, prevalence, risk factors, and underlying mechanism of hyponatremia in elderly patients treated with antidepressants: a cross-sectional study. *Maturitas*, 2013. **76**(4): p. 357-63.
125. Spigset, O. and K. Hedenmalm, Hyponatraemia and the syndrome of inappropriate antidiuretic hormone secretion (SIADH) induced by psychotropic drugs. *Drug Safety*, 1995. **12**(3): p. 209-25.
126. Greenblatt, H.K. and D.J. Greenblatt, Antidepressant-Associated Hyponatremia in the Elderly. *Journal of Clinical Psychopharmacol*ogy, 2016. **36**(6): p. 545-549.
127. Spasovski, G., et al., Clinical practice guideline on diagnosis and treatment of hyponatraemia. *European Journal of Endocrinology*, 2014. **170**(3): p. G1-47.
128. Grattagliano, I., T. Mastronuzzi, and G. D'Ambrosio, Hyponatremia associated with long-term medication use in the elderly: an analysis in general practice. *Journal of Primary Health Care*, 2018. **10**(2): p. 167-173.
129. Liamis, G., H. Milionis, and M. Elisaf, A review of drug-induced hyponatremia. *American Journal of Kidney Disease*, 2008. **52**(1): p. 144-53.
130. Woodward, M., et al., Diagnosis and management of hyponatraemia in the older patient. *Internal Medicine Journal*, 2018. **48 Suppl 1**: p. 5-12.
131. The European Bioinformatics Institute ChEMBL database. Available from: (https://www.ebi.ac.uk/chembl/) (accessed Jan 2020)
132. British Pharmacological Society (BPS) and International Union of Basic Clinical Pharmacology (IUPHAR). The IUPHAR/BPS guide to pharmacology.
133. Roth, B. and J. Driscol, PDSP Ki Database. Psychoactive Drug Screening Program (PDSP). University of North Carolina at Chapel Hill and the United States National Institute of Mental Health, 2011. Available from: (https://pdsp.unc.edu/databases/kidb.php) (accessed Jan 2020)
134. Neubig, R.R., et al., International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. *Pharmacological Review*, 2003. **55**(4): p. 597-606.
135. Montastruc, J.-L., et al., Benefits and strengths of the disproportionality analysis for identification of adverse drug reactions in a pharmacovigilance database. *British Journal of Clinical Pharmacology*, 2011. **72**(6): p. 905-908.
136. Van Puijenbroek, E.P., et al., A comparison of measures of disproportionality for signal

detection in spontaneous reporting systems for adverse drug reactions. *Pharmacoepidemiology and Drug Safety*, 2002. **11**(1): p. 3-10.
137. Anderson, I.K., G.R. Martin, and A.G. Ramage, Central administration of 5-HT activates 5-HT1A receptors to cause sympathoexcitation and 5-HT2/5-HT1C receptors to release vasopressin in anaesthetized rats. *British Journal of Pharmacology*, 1992. **107**(4): p. 1020-8.
138. Keers, R. and K.J. Aitchison, Gender differences in antidepressant drug response. *International Review of Psychiatry*, 2010. **22**(5): p. 485-500.
139. Thunander Sundbom, L., et al., Are men under-treated and women over-treated with antidepressants? Findings from a cross-sectional survey in Sweden. *BJPsych Bulletin*, 2017. **41**(3): p. 145-150.
140. Rochoy, M., et al., [Antidepressive agents and hyponatremia: A literature review and a case/non-case study in the French Pharmacovigilance database]. *Therapie*, 2018. **73**(5): p. 389-398.
141. Thomas, A. and J.G. Verbalis, Hyponatraemia and the Syndrome of Inappropriate Antidiuretic Hormone Secretion Associated with Drug Therapy in Psychiatric Patients. *CNS Drugs*, 1995. **4**(5): p. 357-369.
142. Esposito, E., 1. Ennio, E., Serotonin-Dopamine Interaction as a Focus of Novel Antidepressant Drugs. *Current Drug Targets*, 2006. **7**(2): p. 177-185.
143. Alex, K.D. and E.A. Pehek, Pharmacologic mechanisms of serotonergic regulation of dopamine neurotransmission. *Pharmacological and Therapeutics*, 2007. **113**(2): p. 296-320.
144. Bhuvaneswar, C.G., et al., Adverse endocrine and metabolic effects of psychotropic drugs: selective clinical review. *CNS Drugs*, 2009. **23**(12): p. 1003-21.
145. Pozzi, M., et al., Precipitation of Carbamazepine-Controlled Seizures Due to Low-Dose Risperidone in a Child: A Conspiracy to Unbalance Blood Electrolytes. *Journal of Clinical Psychopharmacology*, 2016. **36**(6): p. 729-730.
146. Shetty, H.M., et al., Hyponatremia Secondary to Antidepressant Therapy-A Post Marketing Safety Study. *Journal of Pharmacovigilance,* 2015, 3:3.
147. Coupland, C.A., et al., A study of the safety and harms of antidepressant drugs for older people: a cohort study using a large primary care database. *Health Technology and Assessment*, 2011. **15**(28): p. 1-202, iii-iv.
148. Jung, Y.E., et al., Hyponatremia associated with selective serotonin reuptake inhibitors, mirtazapine, and venlafaxine in Korean patients with major depressive disorder. *Int. Journal of Clinical Pharmacology and Therapeutics*, 2011. **49**(7): p. 437-43.
149. Roxanas, M., E. Hibbert, and M. Field, Venlafaxine Hyponatraemia: Incidence, Mechanism and Management. *Australian & New Zealand Journal of Psychiatry*, 2007. **41**(5): p. 411-418.
150. García Fernández, E. and D.M.I. Ramos García, Syndrome of inappropriate antidiuretic hormone secretion associated with desvenlafaxine. *European Psychiatry*, 2016. **33**: p. S469.
151. Lee, G. and J. Leung, Syndrome of inappropriate secretion of antidiuretic hormone due to desvenlafaxine. *General Hospital Psychiatry*, 2013. **35**(5): p. 574.e1-3.
152. Liew, E.D. and C.P. Alderman, Syndrome of inappropriate antidiuretic hormone secretion associated with desvenlafaxine. *International Journal of Clinical Pharmacy*, 2014. **36**(2): p. 253-5.
153. D'Agostino, A., C.D. English, and J.A. Rey, Vortioxetine (brintellix): a new serotonergic antidepressant. *P & T*, 2015. **40**(1): p. 36-40.
154. Nishimura, A., et al., Randomized, double-blind, placebo-controlled 8-week trial of the efficacy, safety, and tolerability of 5, 10, and 20 mg/day vortioxetine in adults with major depressive disorder. *Psychiatry and Clinical Neurosciences*, 2018. **72**(2): p. 64-72.
155. Seshadri, M., et al., Antidepressants and Hyponatremia in a patient with Colectomy - a Case Report. *Psychiatria Danuba*, 2017. **29**(Suppl 3): p. 610-614.
156. Kapur, S. and G. Remington, Serotonin-dopamine interaction and its relevance to schizophrenia. *American Journal of Psychiatry*, 1996. 153(4): p. 466-476.
157. Bagley, S.C. and D. Yaeger, Hyponatremia Associated With Bupropion, a Case Verified by Rechallenge. *Journal of Clinical Psychopharmacology*, 2005. **25**(1): p. 98-99.
158. Kate, N., et al., Bupropion-induced hyponatremia. *General Hospital Psychiatry*, 2013. **35**(6): p. 681.e11-2.

159. Kim, C.S., et al., Hyponatremia associated with bupropion. *Electrolyte & Blood pressure*, 2011. **9**(1): p. 23-26.
160. Munjal, S. and Y. Smolin, Bupropion Induced Hyponatremia in an Elderly Patient: A Case Report and Review of the Literature. *Case Reports in Psychiatry*, 2016: p. 5103471.
161. Wiggins, A., T. Balasubramanian, and A. Ferraro, Hyponatraemia and confusion in a 70-year-old female when bupropion was added to dothiepin and escitalopram. *Australas Psychiatry*, 2015. **23**(5): p. 507-9.
162. Pariente, A., et al., Impact of safety alerts on measures of disproportionality in spontaneous reporting databases: the notoriety bias. *Drug Safety*, 2007. **30**(10): p. 891-8.
163. Kenakin, T., Principles: receptor theory in pharmacology. *Trends Pharmacological Sciences*, 2004. **25**(4): p. 186-92.
164. Verbalis, J.G., et al., Diagnosing and Treating the Syndrome of Inappropriate Antidiuretic Hormone Secretion. *American Journal of Medicine*, 2016. **129**(5): p. 537 e9-537 e23.
165. Siegel, A.J., Hyponatremia in psychiatric patients: update on evaluation and management. *Harvard Review of Psychiatry*, 2008. **16**(1): p. 13-24.
166. Hoorn, E.J. and R. Zietse, Hyponatremia and mortality: moving beyond associations. *American Journal of Kidney Disease*, 2013. **62**(1): p. 139-49.
167. Wald, R., et al., Impact of hospital-associated hyponatremia on selected outcomes. *Archives of Internal Medicine*, 2010. **170**(3): p. 294-302.
168. Ahmadi, L. and M.B. Goldman, *Primary polydipsia: Update. Best Practice & Research Clinical Endocrinology & Metabolism*, 2020: p. 101469. Doi: 10.1016/j.beem.2020.101469
169. Yasir, M. and O.J. Mechanic, Syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH), in StatPearls. 2020, StatPearls Publishing Copyright © 2020, StatPearls Publishing LLC.: Treasure Island (FL).
170. Ali, S.N. and L.A. Bazzano, Hyponatremia in Association With Second-Generation Antipsychotics: A Systematic Review of Case Reports. *Ochsner Journal*, 2018. **18**(3): p. 230-235.
171. Mannesse, C.K., et al., Hyponatraemia as an adverse drug reaction of antipsychotic drugs: a case-control study in VigiBase®. *Drug Saf*ety, 2010. **33**(7): p. 569-78.
172. Canuso, C.M. and M.B. Goldman, Does minimizing neuroleptic dosage influence hyponatremia? *Psychiatry Research*, 1996. **63**(2-3): p. 227-9.
173. Kimelman, N. and S.G. Albert, Phenothiazine-induced hyponatremia in the elderly. *Gerontology*, 1984. **30**(2): p. 132-6.
174. Spigset, O. and K. Hedenmalm, Hyponatremia during treatment with clomipramine, perphenazine, or clozapine: study of therapeutic drug monitoring samples. *J Clinical Psychopharmacology*, 1996. **16**(5): p. 412-4.
175. Meulendijks, D., et al., Antipsychotic-induced hyponatraemia: a systematic review of the published evidence. *Drug Safety*, 2010. **33**(2): p. 101-14.
176. Jorgensen, H., et al., Serotonin receptors involved in vasopressin and oxytocin secretion. *Journal of Neuroendocrinollogy*, 2003. **15**(3): p. 242-9.
177. Fabrazzo, M., et al., The unmasking of hidden severe hyponatremia after long-term combination therapy in exacerbated bipolar patients: a case series. *International journal of Clinical Psychopharmacology*, 2019. **34**(4): p. 206-210.
178. Hirayama, T., et al., Effect of chronic treatment with haloperidol on vasopressin release and behavioral changes by osmotic stimulation of the supraoptic nucleus. *Life Sci*ences, 2001. **69**(18): p. 2147-56.
179. Milella, M.S., et al., Opposite roles of dopamine and orexin in quinpirole-induced excessive drinking: a rat model of psychotic polydipsia. *Psychopharmacology* (Berl), 2010. **211**(3): p. 355-66.
180. Bun, S., M.J. Serby, and P. Friedmann, Psychotropic medication use, hyponatremia, and falls in an inpatient population: a retrospective study. *Journal of Clinical Psychopharmacology*, 2011. **31**(3): p. 395-7.
181. Falhammar, H., et al., Antipsychotics and severe hyponatremia: A Swedish population-based case-control study. *European Journal of Internal Medicine*, 2019. **60**: p. 71-77.
182. Manu, P., et al., Medical outcome of psychiatric inpatients with admission hyponatremia. *Psychiatry Research*, 2012. **198**(1): p. 24-7.
183. Yamamoto, Y., et al., Prevalence and risk factors for hyponatremia in adult epilepsy patients: Large-scale cross-sectional cohort study. *Seizure*, 2019. **73**: p. 26-30.

184. Yang, H.J. and W.J. Cheng, Antipsychotic use is a risk factor for hyponatremia in patients with schizophrenia: a 15-year follow-up study. *Psychopharmacology* (Berl), 2017. **234**(5): p. 869-876.
185. Carnovale, C., et al., Bullous pemphigoid induced by dipeptidyl peptidase-4 (DPP-4) inhibitors: a pharmacovigilance-pharmacodynamic/pharmacokinetic assessment through an analysis of the VigiBase®(R). *Expert Opinion on Drug Safety*, 2019. **18**(11): p. 1099-1108.
186. Mazhar, F., et al., Association of Hyponatraemia and Antidepressant Drugs: A Pharmacovigilance-Pharmacodynamic Assessment Through an Analysis of the US Food and Drug Administration Adverse Event Reporting System (FAERS) Database. *CNS Drugs*, 2019. **33**(6): p. 581-592.
187. Montastruc, F., et al., Role of serotonin 5-HT2C and histamine H1 receptors in antipsychotic-induced diabetes: A pharmacoepidemiological-pharmacodynamic study in VigiBase®. *European Neuropsychopharmacol*ogy, 2015. **25**(10): p. 1556-65.
188. Duggirala, H.J., et al., Use of data mining at the Food and Drug Administration. *Journal of American Medical Informatics Association*, 2016. **23**(2): p. 428-34.
189. Ham, K., OpenRefine (version 2.5). http://openrefine.org. Free, open-source tool for cleaning and transforming data. *Journal of the Medical Library Association* : JMLA, 2013. **101**(3): p. 233-234.
190. Yamada, Y., et al., Prediction and assessment of extrapyramidal side effects induced by risperidone based on dopamine D(2) receptor occupancy. *Synapse*, 2002. **46**(1): p. 32-7.
191. Matsui-Sakata, A., H. Ohtani, and Y. Sawada, Receptor occupancy-based analysis of the contributions of various receptors to antipsychotics-induced weight gain and diabetes mellitus. *Drug Metab Pharmacokinet*, 2005. **20**(5): p. 368-78.
192. Hiemke, C., et al., Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. *Pharmacopsychiatry*, 2018. **51**(1-02): p. 9-62.
193. Legehar, A., H. Xhaard, and L. Ghemtio, IDAAPM: integrated database of ADMET and adverse effects of predictive modeling based on FDA approved drug data. *J Cheminform*, 2016. **8**(1): p. 33.
194. Gilson, M.K., et al., BindingDB in 2015: A public database for medicinal chemistry, computational chemistry and systems pharmacology. *Nucleic Acids Res*, 2016. **44**(D1): p. D1045-53. Avaiable from: (https://www.bindingdb.org/bind/index.jsp) (accessed Feb 2019)
195. Liberati, A., et al., The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ*, 2009. **339**: p. b2700.
196. Wells, G.A., et al., The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2000, Oxford.
197. Hermont, A.P., et al., Tooth erosion and eating disorders: a systematic review and meta-analysis. *PLoS One*, 2014. **9**(11): p. e111123.
198. Jun, K., et al., Awareness of the use of hyponatraemia-inducing medications in older adults with hyponatraemia: a study of their prevalent use and association with recurrent symptomatic or severe hyponatraemia. *Age and Ageing*, 2020.
199. Gandhi, S., et al., Atypical antipsychotic medications and hyponatremia in older adults: a population-based cohort study. *Can J Kidney Health Dis*, 2016. **3**: p. 21.
200. Lange-Asschenfeldt, C., et al., Epidemiology, symptoms, and treatment characteristics of hyponatremic psychiatric inpatients. *J Clin Psychopharmacol*, 2013. **33**(6): p. 799-805.
201. Serrano, A., et al., Safety of long-term clozapine administration. Frequency of cardiomyopathy and hyponatraemia: two cross-sectional, naturalistic studies. *Aust N Z J Psychiatry*, 2014. **48**(2): p. 183-92.
202. Shepshelovich, D., et al., Medication-induced SIADH: distribution and characterization according to medication class. *Br J Clin Pharmacol*, 2017. **83**(8): p. 1801-1807.
203. Ilani, T., et al., A peripheral marker for schizophrenia: Increased levels of D3 dopamine receptor mRNA in blood lymphocytes. *Proc Natl Acad Sci* U S A, 2001. **98**(2): p. 625-8.
204. Le Foll, B., et al., Genetics of dopamine receptors and drug addiction: a comprehensive review. *Behav Pharmacol*, 2009. **20**(1): p. 1-17.
205. Richtand, N.M., et al., D3 dopamine receptor, behavioral sensitization, and psychosis. *Neurosci Biobehav Rev*, 2001. **25**(5): p. 427-43.
206. Vanhauwe, J.F., et al., G-protein sensitivity of ligand binding to human dopamine D(2) and D(3) receptors expressed in Escherichia coli: clues for a constrained D(3) receptor structure.

J Pharmacol Exp Ther, 2000. **295**(1): p. 274-83.
207. Girgis, R.R., et al., In vivo binding of antipsychotics to D3 and D2 receptors: a PET study in baboons with [11C]-(+)-PHNO. *Neuropsychopharmacology*, 2011. **36**(4): p. 887-95.
208. Graff-Guerrero, A., et al., The dopamine D2 receptors in high-affinity state and D3 receptors in schizophrenia: a clinical [11C]-(+)-PHNO PET study. *Neuropsychopharmacology*, 2009. **34**(4): p. 1078-86.
209. Gross, G. and K. Drescher, The role of dopamine D(3) receptors in antipsychotic activity and cognitive functions. *Handb Exp Pharmacol*, 2012(213): p. 167-210.
210. Mizrahi, R., et al., Effects of antipsychotics on D3 receptors: a clinical PET study in first episode antipsychotic naive patients with schizophrenia using [11C]-(+)-PHNO. *Schizophr Res*, 2011. **131**(1-3): p. 63-8.
211. Mugnaini, M., et al., Occupancy of brain dopamine D3 receptors and drug craving: a translational approach. *Neuropsychopharmacology*, 2013. **38**(2): p. 302-12.
212. Meltzer, H.Y. and S.M. Stahl, The dopamine hypothesis of schizophrenia: a review. *Schizophr Bull*, 1976. **2**(1): p. 19-76.
213. Kane, J.M., et al., Efficacy and Safety of Cariprazine in Acute Exacerbation of Schizophrenia: Results From an International, Phase III Clinical Trial. *J Clin Psychopharmacol*, 2015. **35**(4): p. 367-73.
214. Brownfield, M.S., et al., Neuropharmacological characterization of serotoninergic stimulation of vasopressin secretion in conscious rats. *Neuroendocrinology*, 1988. **47**(4): p. 277-83.
215. Sagi, L., et al., Autoimmune bullous diseases the spectrum of infectious agent antibodies and review of the literature. *Autoimmun Rev*, 2011. **10**(9): p. 527-35.
216. Budinger, L., et al., Identification and characterization of autoreactive T cell responses to bullous pemphigoid antigen 2 in patients and healthy controls. *J Clin Invest*, 1998. **102**(12): p. 2082-9.
217. Okazaki, A., et al., Polymorphisms of HLA-DR and -DQ genes in Japanese patients with bullous pemphigoid. *J Dermatol*, 2000. **27**(3): p. 149-56.
218. Setterfield, J., et al., Mucous membrane pemphigoid: HLA-DQB1*0301 is associated with all clinical sites of involvement and may be linked to antibasement membrane IgG production. *Br J Dermatol*, 2001. **145**(3): p. 406-14.
219. Yancey, K.B. and C.A. Egan, Pemphigoid: clinical, histologic, immunopathologic, and therapeutic considerations. *JAMA*, 2000. **284**(3): p. 350-6.
220. Tan, C.W., et al., The association between drugs and bullous pemphigoid. *Br J Dermatol*, 2017. **176**(2): p. 549-551.
221. Siegel, J., et al., Bullous disorders associated with anti-PD-1 and anti-PD-L1 therapy: A retrospective analysis evaluating the clinical and histopathologic features, frequency, and impact on cancer therapy. *J Am Acad Dermatol*, 2018. **79**(6): p. 1081-1088.
222. Stavropoulos, P.G., E. Soura, and C. Antoniou, Drug-induced pemphigoid: a review of the literature. *J Eur Acad Dermatol Venereol*, 2014. **28**(9): p. 1133-40.
223. Arai, M., et al., Bullous Pemphigoid and Dipeptidyl Peptidase 4 Inhibitors: A Disproportionality Analysis Based on the Japanese Adverse Drug Event Report Database. *Diabetes Car*e, 2018. **41**(9): p. e130-e132.
224. Gaudin, O., et al., Gliptin Accountability in Mucous Membrane Pemphigoid Induction in 24 Out of 313 Patients. *Front Immunol*, 2018. **9**: p. 1030.
225. Kawaguchi, Y., et al., Dipeptidyl peptidase-4 inhibitors-associated bullous pemphigoid: A retrospective study of 168 pemphigoid and 9,304 diabetes mellitus patients. *J Diabetes Investig*, 2019. **10**(2): p. 392-398.
226. Plaquevent, M., et al., Higher Frequency of Dipeptidyl Peptidase-4 Inhibitor Intake in Bullous Pemphigoid Patients than in the French General Population. *J Invest Dermatol*, 2019. **139**(4): p. 835-841.
227. Kridin, K. and A.D. Cohen, Dipeptidyl-peptidase IV inhibitor-associated bullous pemphigoid: A systematic review and meta-analysis*. J Am Acad Dermatol*, 2018.
228. Drucker, D.J., The biology of incretin hormones. *Cell Metab*, 2006. **3**(3): p. 153-65.
229. Drucker, D.J. and M.A. Nauck, The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*, 2006. **368**(9548): p. 1696-705.
230. Lankas, G.R., et al., Dipeptidyl peptidase IV inhibition for the treatment of type 2 diabetes: potential importance of selectivity over dipeptidyl peptidases 8 and 9. *Diabetes*, 2005.

54(10): p. 2988-94.
231. Rasmussen, H.B., et al., Crystal structure of human dipeptidyl peptidase IV/CD26 in complex with a substrate analog. *Nat Struct Biol*, 2003. **10**(1): p. 19-25.
232. Forssmann, U., et al., Inhibition of CD26/dipeptidyl peptidase IV enhances CCL11/eotaxin-mediated recruitment of eosinophils in vivo. *J Immunol*, 2008. **181**(2): p. 1120-7.
233. Ohnuma, K., et al., Dipeptidyl peptidase in autoimmune pathophysiology. *Adv Clin Chem*, 2011. **53**: p. 51-84.
234. Siafis, S. and G. Papazisis, Detecting a potential safety signal of antidepressants and type 2 diabetes: a pharmacovigilance-pharmacodynamic study. *Br J Clin Pharmacol*, 2018. **84**(10): p. 2405-2414.
235. Bate, A., M. Lindquist, and I.R. Edwards, The application of knowledge discovery in databases to post-marketing drug safety: example of the WHO database. *Fundam Clin Pharmacol*, 2008. **22**(2): p. 127-40.
236. Uppsala Monitoring Centre Uppsala Monitoring Centre. [cited 2019 Feb 3]. Available from: https://signin.who-umc.org.
237. BRENDA - The Comprehensive Enzyme Information System. The comprehensive enzyme information system. [cited 2019 Feb 10]. Available from: https://www.brenda-enzymes.org/ (Accessed Feb 2019)
238. KEGG LIGAND Database. [cited 2019 Feb 11]. Available from: https://www.genome.jp/kegg/ligand.html
239. The Metabolomics Innovation Centre. DrugBank. [cited 2019 Feb 11]. Available from: https://www.drugbank.ca
240. Garcia, M., et al., Dipeptidyl peptidase-IV inhibitors induced bullous pemphigoid: a case report and analysis of cases reported in the European pharmacovigilance database. *J Clin Pharm Ther*, 2016. **41**(3): p. 368-370.
241. Bene, J., et al., Bullous pemphigoid and dipeptidyl peptidase IV inhibitors: a case-noncase study in the French Pharmacovigilance Database. *Br J Dermatol*, 2016. **175**(2): p. 296-301.
242. Benzaquen, M., et al., Dipeptidyl peptidase IV inhibitors, a risk factor for bullous pemphigoid: Retrospective multicenter case-control study from France and Switzerland. *J Am Acad Dermatol*, 2018. **78**(6): p. 1090-1096.
243. Garcia-Diez, I., et al., Bullous pemphigoid induced by dipeptidyl peptidase-4 inhibitors. Eight cases with clinical and immunological characterization. *Int J Dermatol*, 2018. **57**(7): p. 810-816.
244. Varpuluoma, O., et al., Vildagliptin Significantly Increases the Risk of Bullous Pemphigoid: A Finnish Nationwide Registry Study. *J Invest Dermatol*, 2018. **138**(7): p. 1659-1661.
245. Faillie, J.L., Case-non-case studies: Principle, methods, bias and interpretation. *Therapie*, 2019. **74**(2): p. 225-232.
246. Dorn, J.M., et al., Sulfonamide Drug Allergy. Curr Allergy Asthma Rep, 2018. **18**(7): p. 38.
247. Heymann, W.R., Bullae for you: The increasing importance and implications of drug-induced bullous pemphigoid. *J Am Acad Dermatol*, 2018. **79**(6): p. 1026-1027.
248. Lee, S.G., et al., Association of Dipeptidyl Peptidase 4 Inhibitor Use With Risk of Bullous Pemphigoid in Patients With DiabetesBullous Pemphigoid and Dipeptidyl Peptidase-4 Inhibitors in Patients With DiabetesBullous Pemphigoid and Dipeptidyl Peptidase-4 Inhibitors in Patients With Diabetes. *JAMA Dermatology*, 2019. **155**(2): p. 172-177.
249. Lloyd-Lavery, A., et al., The associations between bullous pemphigoid and drug use: a UK case-control study. *JAMA Dermatol*, 2013. **149**(1): p. 58-62.
250. Varpuluoma, O., et al., Oral diabetes medications other than dipeptidyl peptidase 4 inhibitors are not associated with bullous pemphigoid: A Finnish nationwide case-control study. *J Am Acad Dermatol*, 2018. **79**(6): p. 1034-1038 e5.
251. Gorrell, M.D., et al. Structure and Function in Dipeptidyl Peptidase IV and Related Proteins. 2006. Boston, MA: Springer US.
252. Holst, J.J., The physiology and pharmacology of incretins in type 2 diabetes mellitus. *Diabetes Obesity & Metabolism*, 2008. **10**(s3): p. 14-21.
253. Martin, J.H., et al., Incretin-based therapies--review of the physiology, pharmacology and emerging clinical experience. *Intern Med J*, 2011. **41**(4): p. 299-307.
254. Yu, D.M., et al., The dipeptidyl peptidase IV family in cancer and cell biology. *FEBS J*, 2010. **277**(5): p. 1126-44.
255. Biftu, T., et al., Omarigliptin (MK-3102): a novel long-acting DPP-4 inhibitor for once-weekly

treatment of type 2 diabetes. *J Med Chem*, 2014. **57**(8): p. 3205-12.
256. Grimshaw, C.E., et al., Trelagliptin (SYR-472, Zafatek), Novel Once-Weekly Treatment for Type 2 Diabetes, Inhibits Dipeptidyl Peptidase-4 (DPP-4) via a Non-Covalent Mechanism. *PLoS One*, 2016. **11**(6): p. e0157509.
257. Boulton, D.W., Clinical Pharmacokinetics and Pharmacodynamics of Saxagliptin, a Dipeptidyl Peptidase-4 Inhibitor. *Clin Pharmacokinet*, 2017. **56**(1): p. 11-24.
258. Brandt, I., et al., Inhibition of dipeptidyl-peptidase IV catalyzed peptide truncation by Vildagliptin ((2S)-{[(3-hydroxyadamantan-1-yl)amino]acetyl}-pyrrolidine-2-carbonitrile). *Biochem Pharmacol*, 2005. **70**(1): p. 134-43.
259. Hughes, T.E., et al., NVP-LAF237, a highly selective and long-acting dipeptidyl peptidase IV inhibitor. *Diabetes*, 2002. **51**: p. A67-A67.
260. Villhauer, E.B., et al., 1-[[(3-hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(S)-pyrrolidine: a potent, selective, and orally bioavailable dipeptidyl peptidase IV inhibitor with antihyperglycemic properties. *J Med Chem*, 2003. **46**(13): p. 2774-89.
261. Matteucci, E. and O. Giampietro, Dipeptidyl peptidase-4 (CD26): knowing the function before inhibiting the enzyme. *Curr Med Chem*, 2009. **16**(23): p. 2943-51.
262. Coburn, M.C., D.C. Hixson, and J.S. Reichner, In vitro immune responsiveness of rats lacking active dipeptidylpeptidase IV. *Cell Immunol*, 1994. **158**(2): p. 269-80.
263. Steeg, C., U. Hartwig, and B. Fleischer, Unchanged signaling capacity of mutant CD26/dipeptidylpeptidase IV molecules devoid of enzymatic activity. *Cell Immunol*, 1995. **164**(2): p. 311-5.
264. Tanaka, S., et al., Suppression of arthritis by the inhibitors of dipeptidyl peptidase IV. *Int J Immunopharmacol*, 1997. **19**(1): p. 15-24.
265. Vora, K.A., et al., Genetic ablation or pharmacological blockade of dipeptidyl peptidase IV does not impact T cell-dependent immune responses. *BMC Immunol*, 2009. **10**: p. 19.
266. Kodera, R., et al., [Clinical characteristics of bullous pemphigoid in elderly patients with type 2 diabetes mellitus: The association with the use of dipeptidyl peptidase-4 inhibitors]. *Nihon Ronen Igakkai Zasshi*, 2019. **56**(1): p. 43-50.
267. Ujiie, H., et al., HLA-DQB1*03:01 as a Biomarker for Genetic Susceptibility to Bullous Pemphigoid Induced by DPP-4 Inhibitors. *J Invest Dermatol*, 2018. **138**(5): p. 1201-1204.
268. Kushwaha, R.N., Haq, W. and Katti, S.B. (2014) Discovery of 17 Gliptins in 17-Years of Research for the Treatment of Type 2 Diabetes: A Synthetic Overview. *Chemistry & Biology Interface*, 4, 137-162.
269. Chen, X.W., et al., Clinical pharmacology of dipeptidyl peptidase 4 inhibitors indicated for the treatment of type 2 diabetes mellitus. *Clinical and Experimental Pharmacology and Physiology*, 2015. **42**(10): p. 999-1024.
270. Hauben, M. and J.K. Aronson, Gold standards in pharmacovigilance: the use of definitive anecdotal reports of adverse drug reactions as pure gold and high-grade ore. *Drug Safety*, 2007. **30**(8): p. 645-55.
271. Kirby, M., et al., Inhibitor selectivity in the clinical application of dipeptidyl peptidase-4 inhibition. *Clinical science*, 2010. **118**(1): p. 31-41.
272. Kalliokoski, T., et al., Comparability of mixed IC(5)(0) data - a statistical analysis. *PLoS One*, 2013. **8**(4): p. e61007.
273. Gisondi, P., et al., Anti–tumour necrosis factor-α therapy increases body weight in patients with chronic plaque psoriasis: a retrospective cohort study. *Journal of the European Academy of Dermatology and Venereology*, 2008. **22**(3): p. 341-344.
274. Sfriso, P., et al., Impact of 24 months of anti-TNF therapy versus methotrexate on body weight in patients with rheumatoid arthritis: a prospective observational study. *Clinical Rheumatology*, 2016. **35**(6): p. 1615-1618.
275. Beutler, B., et al., Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature*, 1985. **316**(6028): p. 552-554.
276. Plata-Salamán, C.R., Cytokines and Feeding. *Physiology*, 1998. **13**(6): p. 298-304.
277. Chen, X., et al., TNF-α, a potent lipid metabolism regulator. *Cell Biochemistry and Function*, 2009. **27**(7): p. 407-416.
278. Florin, V., et al., Body weight increment in patients treated with infliximab for plaque psoriasis. *Journal of the European Academy of Dermatology and Venereology*, 2012. **27**(2): p. e186-e190.
279. Eder, P., et al., The Role of Adipose Tissue in the Pathogenesis and Therapeutic Outcomes

of Inflammatory Bowel Disease. *Cells*, 2019. **8**(6): p. 628.
280. Harper, J.W. and T.L. Zisman, Interaction of obesity and inflammatory bowel disease. *World journal of gastroenterology*, 2016. **22**(35): p. 7868-7881.
281. Long, M.D., et al., Prevalence and epidemiology of overweight and obesity in children with inflammatory bowel disease. *Inflammatory bowel diseases*, 2011. **17**(10): p. 2162-2168.
282. Moran, G.W., et al., The Increasing Weight of Crohn's Disease Subjects in Clinical Trials. *Inflammatory Bowel Diseases*, 2013. **19**(13): p. 2949-2956.
283. Nic Suibhne, T., et al., High prevalence of overweight and obesity in adults with Crohn's disease: Associations with disease and lifestyle factors. *Journal of Crohn's and Colitis*, 2013. **7**(7): p. e241-e248.
284. Singh, S., et al., Obesity in IBD: epidemiology, pathogenesis, disease course and treatment outcomes. *Nature reviews. Gastroenterology & hepatology*, 2017. **14**(2): p. 110-121.
285. Haas, L., et al., Biologic Agents Are Associated with Excessive Weight Gain in Children with Inflammatory Bowel Disease. *Digestive Diseases and Sciences*, 2017. **62**(11): p. 3110-3116.
286. Mostafa, N.M., et al., Impact of immunogenicity on pharmacokinetics, efficacy and safety of adalimumab in adult patients with moderate to severe chronic plaque psoriasis. *Journal of the European Academy of Dermatology and Venereology*, 2016. **31**(3): p. 490-497.
287. Passot, C., et al., The underlying inflammatory chronic disease influences infliximab pharmacokinetics. *mAbs*, 2016. **8**(7): p. 1407-1416.
288. Wade, J.R., et al., Population pharmacokinetic analysis of certolizumab pegol in patients with Crohn's disease. *Journal of clinical pharmacology*, 2015. **55**(8): p. 866-874.
289. Moher, D., Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *Annals of Internal Medicine*, 2009. **151**(4): p. 264.
290. Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa: Ottawa Hospital Research Institute; 2011. Avaiable from: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
291. Rosenthal, R., Meta-Analytic Procedures for Social Science Research. *Educational Researcher*, 1986. **15**(8): p. 18-20.
292. Cohen, J., A power primer. *Psychological Bulletin*, 1992. **112**(1): p. 155-159.
293. Adams, D.W., et al., Sarcopenia Is Common in Overweight Patients with Inflammatory Bowel Disease and May Predict Need for Surgery. *Inflammatory Bowel Diseases*, 2017. **23**(7): p. 1182-1186.
294. Amato, L., et al., P.1.221: serum lipid alterations in patients with inflammatory bowel disease treated with tnf alpha antagonists. *Digestive and Liver Disease*, 2011. **43**: p. S221.
295. Assa, A., et al., Long-term outcome of tumor necrosis factor alpha antagonist's treatment in pediatric Crohn's disease. *Journal of Crohn's and Colitis*, 2013. **7**(5): p. 369-376.
296. Borrelli, O., et al., Infliximab heals intestinal inflammatory lesions and restores growth in children with Crohn's disease. Digestive and Liver Disease, 2004. **36**(5): p. 342-347.
297. Branquinho DPF, Mendes S, Ferreira M, Portela F, Sofia C, et al. Body mass index variation in INFLIXIMAB-treated IBD patients: United European Gastroenterology Week Vienna, Austria, October 18-22. *United Eur Gastroenterol J*. 2014;2(1S):A132–605.
298. Csontos Á, A., et al., The Effect of anti-TNFα Induction Therapy on the Nutritional Status and Dietary Intake in Inflammatory Bowel Disease. *J Gastrointestin Liver Dis*, 2016. **25**(1): p. 49-56.
299. DeBoer, M.D., et al., Increases in Sex Hormones during Anti-Tumor Necrosis Factor α Therapy in Adolescents with Crohn's Disease. *The Journal of pediatrics*, 2016. **171**: p. 146-52.e522.
300. DeBoer, M.D., et al., Increases in IGF-1 After Anti-TNF-α Therapy Are Associated With Bone and Muscle Accrual in Pediatric Crohn Disease. *Journal of Clinical Endocrinology and Metabolism*, 2018. **103**(3): p. 936-945.
301. Santos, J.C.d., et al., Impact of biological therapy on body composition of patients with Chron's disease. *Revista da Associação Médica Brasileira*, 2017. **63**(5): p. 407-413.
302. Emerenziani, S., et al., Nutritional status and bioelectrical phase angle assessment in adult Crohn disease patients receiving anti-TNFα therapy. *Digestive and Liver Disease*, 2017. **49**(5): p. 495-499.
303. Franchimont, D., et al., Impact of Infliximab on Serum Leptin Levels in Patients with Crohn's

Disease. *Journal of Clinical Endocrinology & Metabolism*, 2005. **90**(6): p. 3510-3516.
304. Gouldthorpe, O., et al., Loss of response to long-term infliximab therapy in children with Crohn's disease. *Pharmaceuticals* (Basel, Switzerland), 2013. **6**(10): p. 1322-1334.
305. Griffin, L.M., et al., Improvements in Bone Density and Structure during Anti-TNF-α Therapy in Pediatric Crohn's Disease. *Journal of clinical Endocrinology and Metabolism*, 2015. **100**(7): p. 2630-2639.
306. Kierkus, J., et al., Maintenance therapy with infliximab for paediatric Crohn's disease: impact on clinical remission and mucosal healing in Polish paediatric patients with severe Crohn's disease. *Gastroenterology Review*, 2012. **1**: p. 26-30.
307. Kierkus, J., et al., The impact of infliximab induction therapy on mucosal healing and clinical remission in Polish pediatric patients with moderate-to-severe Crohn's disease. *European Journal of Gastroenterology & Hepatology*, 2012. **24**(5): p. 495-500.
308. Koutroubakis, I.E., et al., Effects of tumor necrosis factor alpha inhibition with infliximab on lipid levels and insulin resistance in patients with inflammatory bowel disease. *European Journal of Gastroenterology & Hepatology*, 2009. **21**(3): p. 283-288.
309. Malik, S., et al., The effects of anti-TNF-α treatment with adalimumab on growth in children with Crohn's disease (CD). *Journal of Crohn's and Colitis*, 2012. **6**(3): p. 337-344.
310. Miranda-Bautista, J., et al., Lipid Profile in Inflammatory Bowel Disease Patients on Anti-TNFα Therapy. *Digestive Diseases and Sciences*, 2015. **60**(7): p. 2130-2135.
311. Parmentier-Decrucq, E., et al., Effects of infliximab therapy on abdominal fat and metabolic profile in patients with Crohn's disease. *Inflammatory Bowel Diseases*, 2009. **15**(10): p. 1476-1484.
312. Vadan, R., et al., The prevalence of malnutrition and the evolution of nutritional status in patients with moderate to severe forms of Crohn's disease treated with Infliximab. *Clinical Nutrition*, 2011. **30**(1): p. 86-91.
313. van Hoeve, K., et al., Efficacy, Pharmacokinetics, and Immunogenicity is Not Affected by Switching From Infliximab Originator to a Biosimilar in Pediatric Patients With Inflammatory Bowel Disease. *Therapeutic Drug Monitoring*, 2019. **41**(3): p. 317-324.
314. Wiese, D., B. Lashner, and D. Seidner, Measurement of nutrition status in Crohn's disease patients receiving infliximab therapy. *Nutrition in clinical practice*, 2008. **23**(5): p. 551-556.
315. Engvall, I.-L., et al., Infliximab therapy increases body fat mass in early rheumatoid arthritis independently of changes in disease activity and levels of leptin and adiponectin: a randomised study over 21 months. *Arthritis Research & Therapy*, 2010. **12**(5): p. R197-R197.
316. Renzo, L.D., et al., Prospective assessment of body weight and body composition changes in patients with psoriasis receiving anti-TNF-α treatment. *Dermatologic Therapy*, 2011. **24**(4): p. 446-451.
317. Serelis, J., et al., Effect of anti-TNF treatment on body composition and serum adiponectin levels of women with rheumatoid arthritis. *Clinical Rheumatology*, 2008. **27**(6): p. 795-797.
318. Nikolaus, S., et al., Mechanisms in failure of infliximab for Crohn's disease. *The Lancet*, 2000. **356**(9240): p. 1475-1479.
319. Juge-Aubry, C.E., E. Henrichot, and C.A. Meier, Adipose tissue: a regulator of inflammation. *Best Practice & Research Clinical Endocrinology & Metabolism*, 2005. **19**(4): p. 547-566.
320. Pratesi, A., F. Tarantini, and M. Di Bari, Skeletal muscle: an endocrine organ. *Clinical cases in mineral and bone metabolism* :, 2013. **10**(1): p. 11-14.
321. Motil, K.J., et al., Growth failure in children with inflammatory bowel disease: A prospective study. *Gastroenterology*, 1993. **105**(3): p. 681-691.
322. Sawczenko, A. and B.K. Sandhu, Presenting features of inflammatory bowel disease in Great Britain and Ireland. *Archives of disease in childhood*, 2003. **88**(11): p. 995-1000.
323. MacRae, V.E., C. Farquharson, and S.F. Ahmed, The restricted potential for recovery of growth plate chondrogenesis and longitudinal bone growth following exposure to pro-inflammatory cytokines. *Journal of Endocrinology*, 2006. **189**(2): p. 319-328.
324. Pfefferkorn, M., et al., Growth Abnormalities Persist in Newly Diagnosed Children With Crohn Disease Despite Current Treatment Paradigms. *Journal of Pediatric Gastroenterology and Nutrition*, 2009. **48**(2): p. 168-174.
325. Wong, S.C., et al., The Growth Hormone -Insulin Like Growth Factor 1 Axis In Children & Adolescents With Inflammatory Bowel Disease & Growth Retardation. *Clinical Endocrinology*, 2010.

326. Walters, T.D., A.R. Gilman, and A.M. Griffiths, Linear Growth Improves during Infliximab Therapy in Children with Chronically Active Severe Crohn's Disease. *Inflammatory Bowel Diseases*, 2007. **13**(4): p. 424-430.
327. Barbosa-Silva, M.C.G., et al., Bioelectrical impedance analysis: population reference values for phase angle by age and sex. The *American Journal of Clinical Nutrition*, 2005. **82**(1): p. 49-52.
328. Kyle, U.G., et al., Bioelectrical impedance analysis—part II: utilization in clinical practice. *Clinical Nutrition*, 2004. **23**(6): p. 1430-1453.
329. Nagano, M., S. Suita, and T. Yamanouchi, The validity of bioelectrical impedance phase angle for nutritional assessment in children. *Journal of Pediatric Surgery*, 2000. **35**(7): p. 1035-1039.
330. Werkstetter, K.J., et al., Lean body mass, physical activity and quality of life in paediatric patients with inflammatory bowel disease and in healthy controls. *Journal of Crohn's and Colitis*, 2012. **6**(6): p. 665-673.
331. Tan, E., C. Baker, and P. Foley, Weight gain and tumour necrosis factor-alpha inhibitors in patients with psoriasis. *Australas J Dermatol*, 2013. **54**(4): p. 259-63.
332. Issa, M., et al. Infliximab significantly increases proportion of overweight/obese Crohn's disease patients receiving Longterm maintenance therapy. in Gastroenterology. 2006.
333. Bray, G.A. and T. Bellanger, Epidemiology, trends, and morbidities of obesity and the metabolic syndrome. *Endocrine*, 2006. **29**(1): p. 109-117.
334. Grundy, S.M., et al., Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation*, 2004. **109**(3): p. 433-8.
335. Liu, Y., et al., Impact of Obesity on Remission and Disease Activity in Rheumatoid Arthritis: A Systematic Review and Meta-Analysis. *Arthritis Care Res* (Hoboken), 2017. **69**(2): p. 157-165.
336. Dai, Z.-h., X.-t. Xu, and Z.-h. Ran, Associations Between Obesity and the Effectiveness of Anti–Tumor Necrosis Factor-α Agents in Inflammatory Bowel Disease Patients: A Literature Review and Meta-analysis. *Annals of Pharmacotherapy*, 2020. **54**(8): p. 729-741.
337. Singh, S., et al., Obesity and Response to Infliximab in Patients with Inflammatory Bowel Diseases: Pooled Analysis of Individual Participant Data from Clinical Trials. *Am J Gastroenterol*, 2018. **113**(6): p. 883-889.
338. Control, C.f.D. and Prevention. A SAS program for the WHO growth charts (ages 0 to< 2 years). 2016 30 July 2020]; Available from: https://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas-who.htm
339. Kuczmarski RJ. CDC growth charts: United States. US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 2000.
340. Guo, S.S., et al., Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. *Am J Clin Nutr*, 2002. **76**(3): p. 653-8.
341. Vande Casteele, N., et al., Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology*, 2015. **148**(7): p. 1320-9.e3.
342. Pozzi, M., et al., Adverse Drug Reactions Related to Mood and Emotion in Pediatric Patients Treated for Attention Deficit/Hyperactivity Disorder: A Comparative Analysis of the US Food and Drug Administration Adverse Event Reporting System Database. *J Clin Psychopharmacol*, 2019. **39**(4): p. 386-392.
343. Carnovale, C., et al., Interaction between paracetamol and lamotrigine: new insights from the FDA Adverse Event Reporting System (FAERS) database. *Eur J Clin Pharmacol*, 2019. **75**(9): p. 1323-1325.
344. Nagashima, T., et al., Prevention of antipsychotic-induced hyperglycaemia by vitamin D: a data mining prediction followed by experimental exploration of the molecular mechanism. *Sci Rep*, 2016. **6**: p. 26375.
345. Administration, U.S.F.a.D. FDA Adverse Event Reporting System (FAERS). 30/07/2020]; Available from: https://www.fda.gov/drugs/drug-approvals-and-databases/fda-adverse-event-reporting-system-faers.
346. Fescharek, R., et al., Medical dictionary for regulatory activities (MedDRA). *International journal of pharmaceutical medicine*, 2004. **18**(5): p. 259-269.
347. Cornelius, V.R., O. Sauzet, and S.J. Evans, A signal detection method to detect adverse drug

reactions using a parametric time-to-event model in simulated cohort data. *Drug Saf*, 2012. **35**(7): p. 599-610.
348. Yamada, M. and J. Handa, 2. Comparison of the Onset Time Profile among the Interferon Formulations in Adverse Drug Reaction of Suicide-or Diabetes-Related. *Japanese Journal of Pharmacoepidemiology*, 2014. **19**(1): p. 23-30.
349. Sauzet, O., et al., Illustration of the weibull shape parameter signal detection tool using electronic healthcare record data. *Drug Saf*, 2013. **36**(10): p. 995-1006.
350. Team, R.C., R: A language and environment for statistical computing [Computer software, version 3.6. 2]. 2019, Vienna, Austria: R Foundation for Statistical Computing.
351. El-Matary, W., et al., Trends of Utilization of Tumor Necrosis Factor Antagonists in Children With Inflammatory Bowel Disease: A Canadian Population-Based Study. *Inflammatory Bowel Diseases*, 2019. **26**(1): p. 134-138.
352. Mazhar, F., et al., Changes in Anthropometric Parameters After Anti-TNFα Therapy in Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. BioDrugs, 2020. **34**(5): p. 649-668.
353. Haas, L., et al., Biologic Agents Are Associated with Excessive Weight Gain in Children with Inflammatory Bowel Disease. *Digestive Diseases and Sciences*, 2017. 62(11): p. 3110-3116.
354. Christian, K.E., et al., Gender Differences and Other Factors Associated with Weight Gain Following Initiation of Infliximab: A Post Hoc Analysis of Clinical Trials. *Dig Dis Sci*, 2020. **26**(1): p. 125-131.
355. Lepp, J., et al., P345. Rapid weight increase in Infliximab treated Crohn's disease patients is sustained over time. *Journal of Crohn's and Colitis*, 2015. **9**(suppl_1): p. S251-S251.
356. Wu, M.-Y., et al., Change in body weight and body mass index in psoriasis patients receiving biologics: A systematic review and network meta-analysis. *Journal of the American Academy of Dermatology*, 2020. **82**(1): p. 101-109.
357. Peluso, I. and M. Palmery, The relationship between body weight and inflammation: Lesson from anti-TNF-α antibody therapy. *Human Immunology*, 2016. **77**(1): p. 47-53.
358. Koutroubakis, I.E., et al., Effects of tumor necrosis factor alpha inhibition with infliximab on lipid levels and insulin resistance in patients with inflammatory bowel disease. *Eur J Gastroenterol Hepatol*, 2009. **21**(3): p. 283-8.
359. Kiortsis, D.N., et al., Effects of infliximab treatment on insulin resistance in patients with rheumatoid arthritis and ankylosing spondylitis. *Ann Rheum Dis*, 2005. **64**(5): p. 765-6.
360. Toussirot, É., et al., TNFα blockade for inflammatory rheumatic diseases is associated with a significant gain in android fat mass and has varying effects on adipokines: a 2-year prospective study. *Eur J Nutr*, 2014. **53**(3): p. 951-61.
361. Florin, V., et al., Body weight increment in patients treated with infliximab for plaque psoriasis. *J Eur Acad Dermatol Venereol*, 2013. **27**(2): p. e186-90.
362. Daïen, C.I., et al., Effect of TNF inhibitors on lipid profile in rheumatoid arthritis: a systematic review with meta-analysis. *Annals of the Rheumatic Diseases*, 2012. **71**(6): p. 862.
363. Charabaty, A., Are We Ready for Proactive Therapeutic Drug Monitoring of Anti-TNF to Optimize Care of Patients With Inflammatory Bowel Disease? *Crohn's & Colitis* 360, 2020. **2**(1).
364. Sobrado, C.W., N.S.F. Queiroz, and C.A. Perez, Inhibitors of Tumoral Necrosis Factor Alpha in Inflammatory Bowel Disease, in Biological Therapy for Inflammatory Bowel Disease. 2020, IntechOpen.
365. Kennedy, N.A., et al., Predictors of anti-TNF treatment failure in anti-TNF-naive patients with active luminal Crohn's disease: a prospective, multicentre, cohort study. *Lancet Gastroenterol Hepatol*, 2019. **4**(5): p. 341-353.
366. Nordin, F., B. Shadbolt, and K. Subramaniam, P526 Body mass index and response to TNF-α inhibitors in inflammatory bowel disease. *Journal of Crohn's and Colitis*, 2020. **14**(Supplement_1): p. S453-S453.
367. Singh, S., et al., Obesity and response to anti-tumor necrosis factor-α agents in patients with select immune-mediated inflammatory diseases: A systematic review and meta-analysis. *PLoS One*, 2018. **13**(5): p. e0195123.
368. Wiggins, A., T. Balasubramanian, and A. Ferraro, Hyponatraemia and confusion in a 70-year-old female when bupropion was added to dothiepin and escitalopram. *Australasian Psychiatry*, 2015. **23**(5): p. 507-509.

DOCTORAL ACTIVITY REPORT

This report summarizes research activities during the tenure of my PhD candidacy conducted at the Clinical Pharmacology Unit, Fatebenefratelli-Sacco Hospital, Department of Biomedical and Clinical Sciences "L. Sacco, I have gain experiences and acquired skills related to:

- Bibliographic search, review of scientific literature (through PubMed, EMBASE and Web of Science databases) and interpretation of epidemiological data. Systematic reviewing, meta-analysis, and meta-synthesis (including network meta-analysis).
- Organization and management of large pharmacovigilance databases.
- Design, conduction and analysis of pharmacoepidemiology and studies, mainly through pharmacovigilance databases.
- Adverse drug events knowledge discovery in FDA Adverse Event Reporting System (FAERS), VigiBase®, and AIFA pharmacovigilance databases with R.
- Cleaned and merged adverse event datasets and stored them in inhouse database.
- Frequentist pharmacovigilance signal detection with R and STATA.
- Integrate pharmacodynamic data with pharmacoepidemiologic datasets.
- Leveraging regional administrative medical records to make a report for administrative purpose.
- Assessment of drug prescribing quality and rational drug use by determining specific indicators (drug-drug interactions, therapeutic duplicates, off-label use, irrational prescribing in the elderly) through the analysis of in-patient medical records.
- Methods for detecting medication errors and associated adverse drug-related events through pharmacovigilance databases and in-patient health record.

- Prevention of medication errors through pharmacist-led medication reconciliation process.
- Evaluation of the risk/benefit profile of drugs in the context of real-world clinical practice, through the estimation of the association between their use and the reduction of the incidence of events (effectiveness) or the development of adverse events (safety), through both the use of databases (administrative, clinical or pharmacovigilance) and the application of meta-analysis methodologies based on the published results of clinical and/or experimental studies.
- Mentoring activity for the students and research fellows.
- I also acquired skills in using advanced statistical analysis software such as R-Studio, Stata and SAS JMP.

Most of my research activity was dedicated to the validation of proposed research work, that is the object of the present thesis. The protocol of this framework is still ongoing and expanding to include Pharmacoeconomics and use of administrative health-care databases. We also planned several projects based on the same research framework. Some of the manuscripts concerning the analyses presented in my thesis are still under peer-review.

Besides this project, during my PhD course, I have also collaborated with the research group of Marco Pozzi, at the Scientific Institute, IRCCS E. Medea, Bosisio Parini. In this context, I gained experience in systematic reviewing and meta-analysis. In particular, we conducted antidepressant effects of glucagon-like peptide 1 (GLP-1) functional agonists that have been published in the *Journal of Affective Disorders*. We also performed a pharmacovigilance retrospective observational study exploring the adverse drug reactions related to mood and emotion in paediatric patients treated for attention-deficit/hyperactivity disorder that has been published in the *Journal of Clinical Psychopharmacology*.

Furthermore, I have also assisted the Trauma & Burns research group

of Cook County HHS, Chicago, Illinois in design and statistical analyses. In this context, I have also enhanced my knowledge on propensity score-based approaches and multivariate predictive approaches for treatment effect heterogeneity and individualized predictions of different surgical interventions using American College of Surgeon-NSQIP Database.

During my PhD years, I served as an external research tutor of two master's thesis. In this context, I have also enhanced my knowledge and experienced the conduction of studies based on the principles of prediction of early treatment response and mixed methods (qualitative and quantitative).

Muhammad Saqlain, Department of Pharmacy, Quaid-I-Azam University, Islamabad, Pakistan	MPhil thesis 'Potentially Inappropriate Medications Use and Its Association with Health-Related Quality of Life Among Elderly Cardiac Patients'
Subesh V Vikram, Department of Pharmacy Practice, MS Ramaiah University of Applied sciences, India	MPharm thesis: 'Finding Early Improvement Threshold to Predict Response after 8-Weeks of Treatment Using Risperidone in First-Episode Psychosis'

I am serving as an Extended member of the International Society for Pharmacoepidemiology (ISPE) Real-World Evidence Taskforce Statistical Methods that promote global knowledge exchange of scientific methods and standards, education, and policies related to the development, implementation, and interpretation of Real World-Evidence generated from Real-World Data to study the utilization, effectiveness, and safety of treatment interventions in population health.

LIST OF PUBLICATIONS DURING PHD CANDIDATURE:

- **Mazhar F**, Battini V, Carnovale C. (2021) Antiemetic Drugs During Pregnancy: What Can We Learn from Spontaneous Reporting System Database Analyses? *JAMA Pediatr*. doi:

10.1001/jamapediatrics.2020.5173

- Subeesh V, Maheswari E, Singh H, Neha R, **Mazhar F** (2021) Finding Early Improvement Threshold to Predict Response After 8 Weeks of Treatment Using Risperidone in First-Episode Psychosis. *J Clin Psychopharmacol* 41(1):58-61
- Saadat GH, Toor R, **Mazhar F**, Bajani F, Tatebe L, Schlanser V, Kaminsky M, Messer T, Starr F, Dennis A, Poulakidas S, Bokhari F. (2021) Severe burn injury: Body Mass Index and the Baux score. *Burns* 47(1):72-77.
- **Mazhar F**, Hadi MA, Kow CS, Marran AM, Merchant HA, Hassan SS. Use of hydroxychloroquine and chloroquine in COVID-19: How good is the quality of randomized controlled trials? (2020) *Int J Infect Dis*. 101:107-120
- **Mazhar F**, Battini, V., Pozzi, M. et al. Changes in Anthropometric Parameters After Anti-TNFα Therapy in Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. *BioDrugs* 34, 649–668 (2020). doi:10.1007/s40259-020-00444-9
- Hasan SS, Capstick T, Ahmed R, Kow S, **Mazhar F**, …. Zaidi (2020) Mortality in COVID-19 patients with acute respiratory distress syndrome and corticosteroids use: a systematic review and meta-analysis, *Expert Rev. Respir. Med.* DOI: 10.1080/17476348.2020.1804365
- Saqlain M, Ali H, Kamran S, Munir MU, Jahan S, **Mazhar F**. (2020) Potentially Inappropriate Medications Use and Its Association with Health-Related Quality of Life Among Elderly Cardiac Patients. *Qual Life Res* 29(10):2715-2724.
- **Mazhar F**, Carnovale C, Haider N, Ahmed R, Taha M. (2020) Paliperidone associated Hyponatremia: Report of a Fatal Case with Analysis of Cases Reported in the Literature and to The US Food and Drug Administration Adverse Event Reporting System (FAERS). *J Clin Psychopharmacol* 40(2):202-205
- Carnovale, C., Battini, V., **Mazhar, F**. et al. (2020). Are dizziness-related symptoms signals for suboptimal treatment of hypothyroidism? New insights from the FDA adverse event reporting system (FAERS) database. *Eur J Clin Pharmacol* 76(5):733-734.

- Carnovale C, Maffioli A, Zaffaroni G, **Mazhar F**, Battini V, Mosini G, Pozzi M, Radice S, Clementi E, Danelli P. (2020) Efficacy of Tumour Necrosis Factor-alpha therapy in paediatric Crohn's disease patients with perianal lesions: a systematic review. *Expert Opin. Biol. Ther.* 20:3, 239- 251.
- Carnovale C*, **Mazhar F***, Arzenton E, Moretti U, Pozzi M, Mosini G, Leoni O, Scatigna M, Clementi E, Radice S. (2019). Bullous pemphigoid induced by dipeptidyl peptidase-4 (DPP-4) inhibitors: A Pharmacovigilance-Pharmacodynamic/Pharmacokinetic assessment through an analysis of the VigiBase®. *Expert Opinion on Drug Safety*, 18 (11), 1099-1108. **[*co-first author].**
- Pozzi M, Carnovale C, **Mazhar F**, Peeters GGAM, Gentili M, Nobile M, Radice S, Clementi E. (2019) Adverse Drug Reactions Related to Mood and Emotion in Pediatric Patients Treated for Attention-Deficit/Hyperactivity Disorder: A Comparative Analysis of the US Food and Drug Administration Adverse Event Reporting System Database. *J Clin Psychopharmacol 39*(4):386-392.
- Carnovale C, Mosini G, Gringeri M, Battini V, **Mazhar F**, Pozzi M, Clementi E, Radice S (2019) Interaction between paracetamol and lamotrigine: new insights from the FDA Adverse Event Reporting System (FAERS) database. *Eur J Clin Pharmacol* 75(9):1323-1325.
- Carnovale C, Dassano A, Mosini G, **Mazhar F**, D'Addio F, Pozzi M, Radice S, Fiorina P, Clementi E. (2020). The β-cell effect of verapamil-based treatment in patients with type 2 diabetes: a systematic review. *Acta Diabetologica* 57, 117–131.
- Pozzi M, **Mazhar F**, Peeters GGAM, Vantaggiato C, Nobile M, Clementi E, Radice S, Carnovale C. (2019) A systematic review of the antidepressant effects of glucagon-like peptide 1 (GLP-1) functional agonists: Further link between metabolism and psychopathology. *J. Affect. Disord*. 1;257: S0165-0327(19)30593-2.
- **Mazhar F**, Pozzi M, Gentili M, Scatigna M, Clementi E, Radice S, Carnovale C. (2019) Association of Hyponatraemia and Antidepressant Drugs: A Pharmacovigilance-Pharmacodynamic Assessment Through an Analysis of the US Food and Drug Administration Adverse Event Reporting System (FAERS) Database. *CNS Drugs*. 33(6):581-592.
- Carnovale, C., Gentili, M., Magni, C., **Mazhar, F**., Mosini, G., Clementi,

E., & Radice, S. (2019). The impact of a successful treatment of HCV on glyco-metabolic control in diabetic patients. *Antiviral Therapy.* 24(2):147-149.

- Carnovale, C.*, **Mazhar, F***., Pozzi, M., Gentili, M., Clementi, E., & Radice, S. (2018) A characterization and disproportionality analysis of medication error related adverse events reported to the FAERS database. *Expert Opinion on Drug Safety* 17(12):1161-1169. **[*co-first author].**
- Carnovale, C., **Mahzar, F**., Scibelli, S., Gentili, M., Arzenton, E., Moretti, U., & Medaglia, M. (2019). Central nervous system-active drug abused and overdose in children: a worldwide exploratory study using the WHO pharmacovigilance database. *European Journal of Pediatrics* 178(2);161-172.
- Carnovale, C., Pozzi, M., **Mazhar, F**., Mosini, G., Gentili, M., Peeters, G.G. Clementi, E. and Radice, S. (2019) Interactions Between Antiepileptic and Antibiotic Drugs: A Systematic Review and Meta-Analysis with Dosing Implications. *Clinical Pharmacokinetics* 58(7):875-886.
- **Mazhar, F**., Haider, N., Al-Osaimi, Y. A., Ahmed, R., Akram, S., & Carnovale, C. (2018). Prevention of medication errors at hospital admission: a single-centre experience in elderly admitted to internal medicine. *International Journal of Clinical Pharmacy* 40(6):1601-1613.

Under review after minor revisions:

- **Mazhar F**, Battini V, … Carnovale C. (2020) Hyponatraemia following antipsychotic treatment: In-silico pharmacodynamics analysis of spontaneous reports from the US Food and Drug Administration Adverse Event Reporting System Database and an Updated Systematic Review. [under review] *Int Journal Neuropsychopharmacology.*

Finally, during my PhD programme, I attended several congresses (outlined below), at the national and international level, in the belief that sharing experiences with other research groups working on the same topic of interest is a valuable key point to broaden knowledge and develop and optimize research practices.

PARTICIPATION IN SCIENTIFIC CONFERENCES DURING PHD CANDIDATURE:

- Role of dopamine D3 and serotonin 5-HT2A receptors in antipsychotic associated hyponatraemia: A Pharmacovigilance-pharmacodynamic assessment through an analysis of the US food and drug administration adverse event reporting system (FAERS) database. 36th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Virtual, September 16–17, 2020. **Oral contribution.**
- Efficacy of Tumour Necrosis Factor-alpha therapy in paediatric Crohn's disease patients with perianal lesions: a systematic review. 2020 ISPE Mid-Year Meeting April 18-21, 2020 Orlando, Florida, USA (Conference cancelled). **poster presentation**
- Bullous pemphigoid induced by dipeptidyl peptidase-4 (DPP-4) inhibitors: A Pharmacovigilance-Pharmacodynamic/Pharmacokinetic assessment through an analysis of the VigiBase®., 2019 ACPE October 11-13, 2019 Kyoto, Japan. **Symposium session**.
- Antidepressant-induced hyponatremia: a pharmacoepidemiological–pharmacodynamic analysis of suspected adverse drug reactions in the US-FAERS database. 14th Congress of the European Association for Clinical Pharmacology and Therapeutics (EACPT2019), "Clinical pharmacology meets tomorrow´s health care challenges" in Stockholm, Sweden, from the 29th June to 2nd July 2019. **Oral contribution.**
- Characterization and Disproportionality Analysis of Spontaneously Reported Cases of Medication Errors to the U SF DA Adverse Event Reporting System. 18th Annual Meeting of the International Society of Pharmacovigilance (ISoP 2018), "Pharmacovigilance without borders", in Geneva, Switzerland 11th to 14th November 2018. **Oral contribution.**
- Prevention of medication errors at hospital admission: a single-centre experience in elderly admitted to internal medicine. International Society for Pharmacoepidemiology (ISPE) "11th Asian Conference on Pharmacoepidemiology" in Xi'an, China, 27-29 October 2018. **Oral contribution.**

APPENDICES

APPENDICES

APPENDIX I

Drugs associated with hyponatremia

Category	Associated pharmacological classes/drugs
CNS	Benzodiazepines, antidepressants, valproate, carbamazepine, oxcarbazepine, lamotrigine, phenytoin, NSAIDs, opioids, MDMA (ecstasy), levamisole
Cardiovascular	Diuretics, beta-blockers, ACE inhibitors, nitrates, calcium channel antagonists, amiodarone, Clofibrate.
Endocrine	Chlorpropamide, sulphonylureas, biguanides, thiazolidinediones, vasopressin, desmopressin, bromocriptine, oxytocin, terlipressin
Cytotoxic drugs	Vinca alkaloids, platinum compounds, ifosfamide, melphalan, cyclophosphamide, methotrexate, pentostatin
Miscellaneous	Interferon, interlukin-2, nicotine, proton pump inhibitors, monoclonal antibodies, antimicrobials (trimethoprim-sulfamethoxazole, ciprofloxacin, cefoperazone/sulbactam, rifabutin)

References:

- Movig KLL, Leufkens HGM, Lenderink AW, van den Akker VGA, Hodiamont PPG, Goldschmidt HMJ, et al. Association between antidepressant drug use and hyponatraemia: a case–control study. Br J Clin Pharmacol. 2002;53(4):363–9.
- Liu BA, Mittmann N, Knowles SR, Shear NH. Hyponatraemia and the syndrome of inappropriate secretion of antidiuretic hormone associated with the use of selective serotonin reuptake inhibitors: a review of spontaneous reports. CMAJ. 1996;155(5):519–27.
- Spigset O, Hedenmalm K. Hyponatraemia and the syndrome of inappropriate antidiuretic hormone secretion (SIADH) induced by psychotropic drugs. Drug Saf. 1995;12(3):209–25.
- Mannesse CK, Jansen PA, Van Marum RJ, Sival RC, Kok RM, Haffmans PM, et al. Characteristics, prevalence, risk factors, and underlying mechanism of hyponatraemia in elderly patients treated with antidepressants: a cross-sectional study. Maturitas. 2013;76(4):357–63.

APPENDIX II

Receptor occupancy for the 19 antipsychotics of interest.

Drug	C′ T* (ng ml−1)	fu (%)	Receptor systems																					
			D1		D2		D3		D4		H1		M1		M2		Alpha1A		Alpha2A		5-HT1A		5-HT2A		5-HT2C	
			Ki (nM)	%	Ki (nM)	%	Ki (nM)	%	Ki (nM)	%	Ki (nM)	%	Ki (nM)	%	Ki (nM)	%	Ki (nM)	%	Ki (nM)	%	Ki (nM)	%	Ki (nM)	%	Ki (nM)	%
Olanzapine	80	7%	52.24	25.54	20	46.74	43.83	29.02	27	39.89	2	89.96	4.7	79.22	87.09	17.07	111.96	13.8	314.05	5.4	2063	0.86	3.12	85.19	10.61	62.81
Risperidone	60	11%	523	2.99	2	87.98	5.9	73.18	7	69.7	18.62	46.38	8128.31	0.2	7852.36	0.2	5	76.31	58.89	21.47	371.54	4.15	0.29	98.23	26	38.25
Quetiapine	500	17%	1282.61	14.75	180	55.21	329.85	40.21	2200	9.16	8.7	96.22	127.28	63.54	630.96	26.01	22	90.98	3630	5.76	304.48	42.15	209.18	51.47	1400	13.68
Clozapine	600	5%	196	31.9	130	41.39	245.47	27.22	28.9	76.06	1.8	98.08	3.1	96.73	63.19	59.23	1.6	98.29	142	39.26	140	39.6	6.51	93.38	15.14	85.84
Aripiprazole	500	1%	870.93	1.26	2	86.3	3.1	78.25	716.94	1.53	29	27.77	6776	0.16	3510	0.32	25.7	30.26	74	13.09	4.34	71.96	8.7	56.17	15	42.64
Haloperidol	10	8%	83	2.5	2	52.19	4.18	33.71	3	41.5	1427.69	0.15	3090.3	0.07	10000	0.02	12	15.06	1130	0.19	1949.84	0.11	80.15	2.59	4700	0.05
Paliperidone	60	26%	10000	0.37	8	81.51	1	97.34	1.58	95.86	3.98	90.2	10000	0.37	10000	0.37	4.07	90	30.2	54.83	10000	0.37	5.25	87.48	10000	0.37
Ziprasidone	200	0%	130	0.39	4	12.54	7.3	6.53	32.36	1.55	44.85	1.12	5100	0.01	3000	0.02	18	2.76	160	0.32	4	11.31	0.39	56.67	0.72	41.31
Amisulpride	320	84%	10000	6.79	2	99.73	2.4	99.67	2369	23.52	10000	6.79	10000	6.79	10000	6.79	10000	6.79	1114	39.54	10000	6.79	8304	8.06	10000	6.79
Fluphenazine	10	5%	19.05	5.67	1	67.06	1.78	39.17	40.74	2.73	32.36	3.42	2187.76	0.05	4677.35	0.02	6.61	14.77	316.23	0.36	831.76	0.14	33.11	3.34	803.53	0.14
Asenapine	5	5%	10	8.05	2	35.57	0.43	67.23	>10000	0	1	46.67	8128.31	0.01	8128.31	0.01	1.2	42.12	1.2	42.12	5.62	13.46	0.1	89.74	0.03	96.19
Flupentixol	5	5%	3.46	14.24	1	45.31	1.1	34.33	14.48	3.82	0.86	40.07	>10000	0	>10000	0	>10000	0	>10000	0	8028	0.01	87.5	0.65	125.89	0.45
Lurasidone	40	1%	263.03	0.31	2	31.85	100	0.81	100	0.81	10000	0.01	10000	0.01	10000	0.01	48.98	1.63	1.62	33.36	6.75	10.74	2.03	28.57	416.87	0.19
Chlorpromazine	300	4%	83.71	31.04	3	93.48	3	92.63	12.21	75.52	6	86.26	34.28	52.36	215	14.91	0.28	99.26	184	17	3000	1.24	2.8	93.08	12.35	75.32
Prochlorperazine	40	5%	>10000	0.01	1	89	2.95	64.44	5.5	49.32	19.05	21.92	199.53	2.61	>10000	0	23.99	18.23	1698.24	0.31	10000	0.05	15.14	26.11	123.03	4.17
Pipamperone	400	25%	2454.71	9.78	32	89.37	251.19	51.43	>10000	0.27	2454.71	9.78	>10000	0.27	>10000	0.27	66.07	80.1	870.96	23.4	2818.38	8.62	3.16	98.83	199.53	57.14
Pimozide	20	1%	10000	0	1	36.86	0.39	52.67	1.8	19.43	131.53	0.33	812.83	0.05	812.83	0.05	197.7	0.22	1593	0.03	502.87	0.09	26.57	1.61	874	0.05
Loxapine	30	3%	54	4.84	12	18.62	21.49	11.32	8.38	24.68	4.95	35.67	112.2	2.39	524.81	0.52	31	8.13	150.9	1.79	2571.07	0.11	2.5	52.34	12.71	17.76
Sulpiride	1000	60%	10000	14.95	10	99.45	8.2	99.54	54	97.02	10000	14.95	>10000	14.95	10000	14.95	10000	14.95	1584.89	52.59	10000	14.95	4786.3	26.86	10000	14.95

C′T*, total plasma concentration of a drug; fu, free fraction of a drug in the plasma; Ki, equilibrium dissociation constant

APPENDIX III

The Newcastle Ottawa scale for the quality assessment of studies included in the study 2

Cross sectional studies ^

List of studies	Selection (max. 5 points)				Comparability (max. 2 points)	Assessment of the outcome (max. 3 points)		Total Score	Quality rating
	Representativeness of the sample	*Sample size*	*Non-respondents*	*Ascertainment of the risk-factors*	*The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled.*	*Ascertainment of the outcome*	*Statistical test*		
Shepshelovich et al., 2017	★	0	0	★	0	★★	0	4	Low
Serrano et al., 2014	0	0	0	★	0	0	★	2	Low
Letmaier et al., 2012	★	★	★	★	★	★	★	7	High

^The NOS for cohort studies was adapted for use with cross sectional studies in a similar manner to previous research

Cohort studies

List of studies	Selection (score)				Comparability (score)			Outcome (score)			Total Score	Quality rating
	Representative of exposed cohort	*Selection of non-exposed cohort*	*Assessment of exposure*	*Absence of outcome at start of study*	*Control for age or obesity or smoking or exercise*	*Control for other variables (second important variables)*	*Cohorts are not comparable on the basis of the design or analysis controlled for confounders*	*Assessment of outcome*	*Follow-up period*	*Adequacy of follow-up*		

Gandhi et al., 2016	0	★	★	★	★	★	0	★	★	★	8	High
Lange-Asschenfeldt et al., 2013	0	0	★	★	0	0	0	★	0	0	3	Low

Case-control studies

List of studies	**Selection** (score)				**Comparability** (score)	**Exposure** (score)			**Total Score**	**Quality rating**
	Is the case definition adequate?	*Representativeness of the cases:*	*Selection of controls:*	*Definition of controls:*	*Comparability of cases and controls on the basis of the design or analysis controlled for confounders*	*Assessment of outcome*	*Same method of ascertainment for cases and controls:*	*Non-response rate*		
Jun et al., 2020	★	★	★	0	★★	★	★	★	8	High
Falhammar et al., 2019	★	★	★	★	★★	★	★	★	9	High
Yamamoto et al., 2019	★	★	0	★	0	0	★	★	5	Moderate
Yang and Cheng, 2017	★	★	★	★	★	★	★	★	8	High
Manu et al., 2012	★	★	0	★	0	0	★	★	5	Moderate
Bun et al., 2011	★	★	0	★	★	0	★	★	6	Moderate

Overall quality (mean ± SD) = 5.7 ± 2.31 (moderate)

A score of '0' was awarded when criteria was not satisfied, a star (★, score of '1') was awarded when criteria was satisfied, and in some cases a score of '2' was awarded when criteria was satisfied using a validated method or an established model. The sum of scores for all subscale items were used to categorise overall study quality as either high (7-9), moderate (5-6), or low (0-4).

Legend: Refer to http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, for a description of Newcastle-Ottawa Quality Assessment Scale for cohort studies and case control studies. For cross-sectional studies refer to main text for relevant citation.

APPENDIX IV

List of drugs reported to induce bullous pemphigoid

Antibiotics
Actinomycin
Penicillins
Cephalexin
Ciprofloxacin
Chloroquine
Dactinomycin
Levofloxacin
Rifampin
Sulfamethoxazole; Trimethoprim
NSAID
Azapropazone
Celecoxib
Diclofenac (topical)
Ibuprofen
Mefenamic acid
Phenacetin
Diuretics
Furosemide
Spironolactone
Anti TNF-α
Adalimumab
Efalizumab
Etanercept
Antiarrythmics-antihypertensives
Amlodipine
Nifedipine
Captopril
Enalapril
Lisinopril
Ramipril
Nadolol
Practolol
Losartan
Valsartan
Salicylates
Aspirin
Sulphasalazine
Salicylazosulfapyridine
Antirheumatics
D-penicillamine
Tiobutarit
Other
Arsenic
Azathioprine
Clonidine
Denosumab
Erlotinib
Fluoxetine
Flupenthixol
Gabapentin
Galantamine hydrobromide
Ipilimumab
Gold thiosulfate
Interleukin-2
Levetiracetam
Mepolizumab
Methyldopa
Natalizumab
Terbinafine
Omeprazole
Potassium iodide
Risperidone
Nivolumab
Pembrolizumab
Omalizumab
Rituximab
Secukinumab
Ustekinumab
Topical
Anthralin
Benzyl benzoate
Coal tar
5-fluorouracil
Iodophor in adhesive bandage
Epinephrine
Idoxuridine
Pilocarpine
Timolol

Information from:

- Stavropoulos PG, Soura E, Antoniou C (2014) Drug-induced pemphigoid: a review of the literature. *J Eur Acad Dermatol Venereol.* 28: 1133-1140. https://doi.org/10.1111/jdv.12366
- Lee JJ, Downham TF. (2006). Furosemide-induced bullous pemphigoid: case report and review of literature. *J Drugs Dermatol.* 5(6):562–4
- Vassileva S. (1998) Drug-induced pemphigoid: Bullous and cicatricial. *Clin. Dermatol.* 1998; 16:379. https://doi.org/10.1016/S0738-081X(98)00008-X
- Ahronowitz I, Fox L. (2014). Severe drug-induced dermatoses. *Semin Cutan Med Surg*. 33:49–58.

APPENDIX V

Selectivity profile of different DPP-4 inhibitors for inhibition of DPP-4 enzyme over DPP-8/DPP-9 along with pharmacokinetic data

DPP-4 inhibitor	DPP-4 selectivity vs DPP-8		DPP-4 selectivity vs DPP-9		Haf life (hrs)	Volume of Distribution (L)	Ref.
	fold	**log2 fold**	**fold**	**log2 fold**			
Alogliptin	14,285	13.80	14,285	13.80	21	417	[1, 2]
Anagliptin	84,700	16.37	56,100	15.78	4.37	112	[3, 4]
Linagliptin	10,000	13.29	10,000	13.29	160	1,110	[5, 6]
Omarigliptin	10,000	13.29	10,000	13.29	22	77	[7-9]
Saxagliptin	72	6.17	31	4.95	3.8	151	[10, 11]
Sitagliptin	2,667	11.38	5,550	12.44	12.4	198	[12]
Teneligliptin	11,248	13.46	11,248	13.46	24.2	107	[13, 14]
Trelagliptin	100,000	16.61	100,000	16.61	55.56	1,194	[15, 16]
Vildagliptin	270	8.08	32	5.00	12.4	198	[17-19]

References:

[1] Scott, L. J. (2010). Alogliptin. *Drugs*. 70(15), 2051-2072.

[2] Capuano A, Sportiello L, Maiorino MI, et al. (2013) Dipeptidyl peptidase-4 inhibitors in type 2 diabetes therapy–focus on alogliptin. *Drug Des Devel Ther*. 17; 7:989-1001.

[3] Watanabe YS, Yasuda Y, Kojima Y, et al. (2015) Anagliptin, a potent dipeptidyl peptidase IV inhibitor: its single-crystal structure and enzyme interactions. *J Enzyme Inhib Med Chem.* 30(6):981-8.

[4] Retlich S, Duval V, Graefe-Mody U, et al. (2015). Population Pharmacokinetics and Pharmacodynamics of Linagliptin in Patients with Type 2 Diabetes Mellitus. *Clin Pharmacokinet.* 54(7): 737-750.

[5] Sortino MA, Sinagra T, Canonico PL (2013). Linagliptin: A thorough Characterization beyond Its Clinical Efficacy. *Front Endocrinol (Lausanne).* 4: 16-16

[6] Graefe-Mody U, Retlich S, Friedrich C (2012) Clinical pharmacokinetics and pharmacodynamics of linagliptin. *Clin. Pharmacokinet.* 51: 411-427

[7] Wang X, Li X, Qie S, Zheng Y, Liu Y, Liu G (2018) The efficacy and safety of once-weekly DPP-4 inhibitor omarigliptin in patients with type 2 diabetes mellitus: A systemic review and meta-analysis. *Medicine (Baltimore).* 97: e11946-e11946.

[8] Biftu T, Sinha-Roy R, Chen P, et al. (2014) Omarigliptin (MK-3102): a novel long-acting DPP-4 inhibitor for once-weekly treatment of type 2 diabetes. *J Med Chem.* 57: 3205-3212
[9] Krishna R, Addy C, Tatosian D, et al. (2016). Pharmacokinetics and Pharmacodynamics of Omarigliptin, a Once-Weekly Dipeptidyl Peptidase-4 (DPP-4) Inhibitor, After Single and Multiple Doses in Healthy Subjects. *J Clin Pharmacol*. 56: 1528-1537.
[10] Wang A, Dorso C, Kopcho L, et al. (2012) Potency, selectivity and prolonged binding of saxagliptin to DPP4: maintenance of DPP4 inhibition by saxagliptin in vitro and ex vivo when compared to a rapidly-dissociating DPP4 inhibitor. *BMC Pharmacol*. 12: 2-2.
[11] ONGLYZA: package insert. Bristol-Myers Squibb (2009). https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/022350s011lbl.pdf
[12] Thornberry NA, Weber AE (2007) Discovery of JANUVIA (Sitagliptin), a selective dipeptidyl peptidase IV inhibitor for the treatment of type 2 diabetes. *Curr Top Med Chem*. 7: 557-568
[13] Singh AK (2017) Efficacy and safety of teneligliptin. *Indian J Endocrinol Metab.* 21: 11-17
[14] Kishimoto M (2013) Teneligliptin: a DPP-4 inhibitor for the treatment of type 2 diabetes. *Diabetes Metab Syndr Obes.* 6: 187-195
[15] Grimshaw CE, Jennings A, Kamran R, et al. (2016) Trelagliptin (SYR-472, Zafatek), Novel Once-Weekly Treatment for Type 2 Diabetes, Inhibits Dipeptidyl Peptidase-4 (DPP-4) via a Non-Covalent Mechanism. *PloS One* 11: e0157509
[16] Zafatek®(trelagliptin succinate) tablet prescribing information. https://www.pmda.go.jp/files/000213963.pdf
[17] Tatosian DA, Guo Y, Schaeffer AK, et al. (2013) Dipeptidyl peptidase-4 inhibition in patients with type 2 diabetes treated with saxagliptin, sitagliptin, or vildagliptin. *Diabetes Ther.* 4: 431-442
[18] Berger JP, SinhaRoy R, Pocai A, et al. (2018) A comparative study of the binding properties, dipeptidyl peptidase-4 (DPP-4) inhibitory activity and glucose-lowering efficacy of the DPP-4 inhibitors alogliptin, linagliptin, saxagliptin, sitagliptin and vildagliptin in mice. *Endocrinol Diab Metab.*1:e2. https://doi.org/10.1002/edm2.2
[19] He YL (2012) Clinical pharmacokinetics and pharmacodynamics of vildagliptin. *Clin. Pharmacokinet.* 51: 147-162

APPENDIX VI

The Newcastle Ottawa scale for the quality assessment of studies included in study IV.

STUDY DETAILS		SELECTION					COMPARABILITY			OUTCOME				
Study.	**Outcomes**	**Repre-sentativeness of the exposed cohort (A, B stars)**	**Selection of the non-exposed cohort (A star)**	**Ascertain-ment of exposure (A, B stars)**	**Demon-stration that outcome of interest was not present at start of study (A star)**	**Total**	**The study controls for age, sex (star)**	**Study controls for other factors (cortico-steroids) (star)**	**Total**	**Assessment of outcome (A, B star)**	**Was follow-up long enough for outcomes to occur (A star)**	**Adequacy of follow-up of cohorts (A, B star)**	**Total**	**QUALITY**
Adams (2017)	Weight, BMI, CRP, ESR, HBI	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**
Amato (2011)	BMI, CDAI, Mayo score	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**
Assa (2013)	BMI	*	0	*	*	3	0	0	0	*	*	0	2	**5/9**
Borrelli (2004)	Weight, height, CRP, ESR, PCDAI	*	0	*	*	3	0	0	0	*	*	0	2	**5/9**

Branquinho, (2014)	BMI	*	0	*	*	3	0	0	0	*	*	0	2	**5/9**
Csontos (2016)	Weight, BMI, fat mass, lean mass, CRP, CDAI, partial Mayo score	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**
DeBoer (2016)	Weight, BMI, height, CRP, ESR, PCDAI	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**
DeBoer (2018)	BMI, height, lean mass, CRP, ESR, PCDAI	*	0	*	*	3	0	0	0	*	*	0	2	**5/9**
Dos Santos (2017)	Weight, BMI, waist cir., fat mass, lean mass, phase angle, HBI	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**
Emerenziani (2017)	Lean mass, phase angle, CRP	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**

Franchimont (2005)	Weight, fat mass, CRP, CDAI	*	*	*	*	4	0	0	0	*	0	*	2	**6/9**
Gouldthorpe (2013)	Weight, height, PCDAI	*	0	*	*	3	0	0	0	*	*	0	2	**5/9**
Griffin (2015)	BMI, height, lean mass, CRP, ESR, PCDAI	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**
Haas (2017)	Weight, BMI	*	0	*	*	3	0	0	0	*	*	0	2	**5/9**
Kierkus [1] (2012)	Weight, BMI, height, CRP, PCDAI	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**
Kierkus [2] (2012)	BMI, CRP, ESR, PCDAI	*	0	*	*	3	0	0	0	*	0	*	2	**5/9**
Koutroubakis (2009)	BMI, CDAI, SCCAI	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**
Malik (2011)	Height vel., PCDAI	*	0	*	*	3	0	0	0	*	*	0	2	**5/9**
Miranda-Bautista (2015)	BMI	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**
Parmentier (2009)	BMI, fat mass	*	0	*	*	3	0	0	0	*	0	*	2	**5/9**

Vadan (2011)	Weight, BMI	*	0	*	*	3	0	0	0	*	*	0	2	**5/9**
Van Hoeve (2019)	Weight, BMI, height, CRP, ESR	*	0	*	*	3	0	0	0	*	*	0	2	**5/9**
Wiese (2008).	BMI, fat mass, lean mass, REE, RQ, CRP, HBI	*	0	*	*	3	0	0	0	*	*	*	3	**6/9**

Overall quality: (mean ± SD) = 5.5 ± 0.51 (moderate)

A score of '0' was awarded when criteria was not satisfied, a star (★, score of '1') was awarded when criteria was satisfied, and in some cases a score of '2' was awarded when criteria was satisfied using a validated method or an established model. The sum of scores for all subscale items were used to categorise overall study quality as either high (7-9), moderate (5-6), or low (0-4).

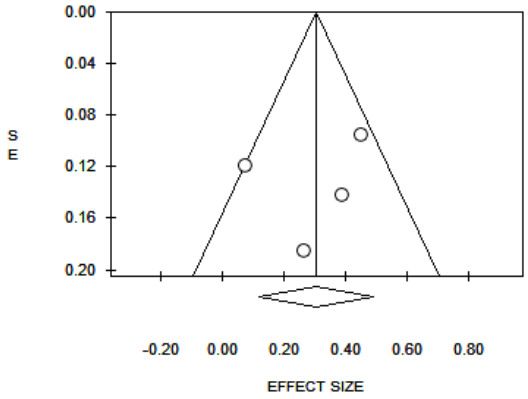
Legend: Refer to http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp, for a description of Newcastle-Ottawa Quality Assessment Scale for cohort studies

BFM =Body Fat Mass; BIA=Bioelectrical impedance analysis; CDAI= Crohn Disease Activity Index; DXA=Dual-energy X-ray absorptiometry; FFM=Fat Free Mass; FFMI =Fat Free Mass Index; FMI=Fat mass Index; HBI=Harvey Bradshaw Index; LM=Lean Mass; LMI=Lean mass Index; LegLM =Leg Lean Mass; PCDAI=Paediatric Crohn Disease Activity Index; SCCAI= Simple Clinical Colitis Activity Index; SMI=Skeletal Muscle Index; TAF=Total Abdominal Fat; VFA=Visceral Fat Area; WC=Waist Circu

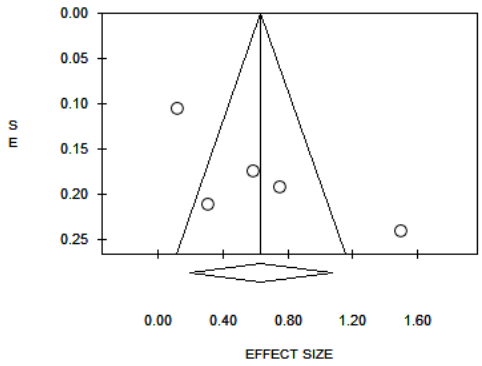
APPENDIX VII

Funnel plot assessment of evidence for publication bias in study IV

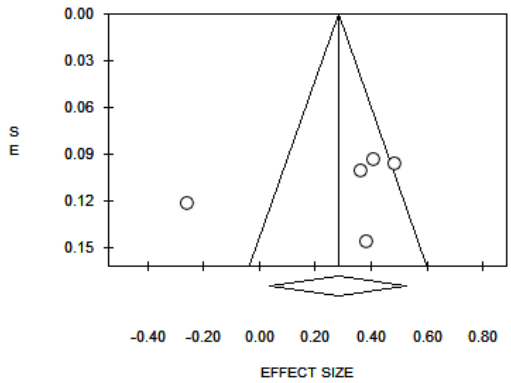
a) Weight



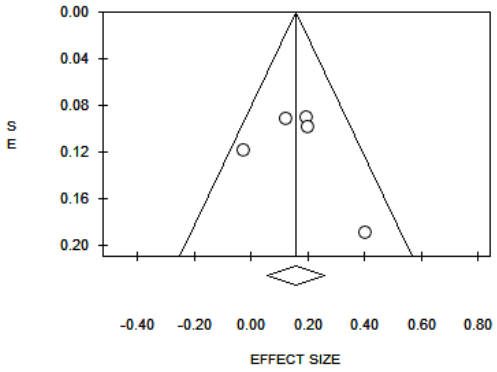
b) BMI change in adults



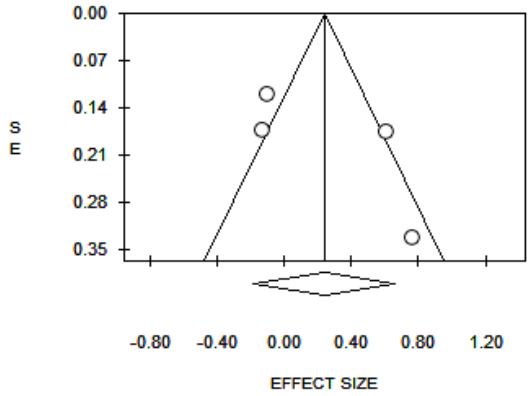
c) BMI change in paediatrics



d) Height change in children



e) Fat mass change in adults



APPENDIX VIII

Overview of secondary outcomes and their relationship with primary outcomes (Study IV)

ID	CRP (mg/dL)				ESR (mm/h)				ACTIVITY INDEX					BODY COMPOSITION	
	Baseline (Mean±SD)	**Endpoint (Mean±SD)**	**Change (Mean±SD)**	**P**	**Baseline (Mean ±SD)**	**Endpoint (Mean ±SD)**	**Change (Mean± SD)**	**P**	**Scale**	**Baseline (Mean± SD)**	**Endpoint (Mean± SD)**	**Change (Mean ±SD)**	**P**	**Change (↑ ,↔, ↓)**	**P**
Adams 2017 [22]	NA	NA	-26.05 mg/L	**0.006**	NA	NA	-9.51	**<0.001**	HBI	6 (2-9) [&]	NA	-1.2	0.13	Weight: ↑	0.06
Amato 2011 [23]	NA	NA	NA	**-**	NA	NA	NA	**-**	CDAI	117.9±55.7	75.2±37.1	NA	**<0.001**	BMI: ↑	**0.016**
									Mayo score	7.3±2.5	3.1±1.3		**<0.001**		
Assa 2013 [32]	NA	NA	*Induction:* Responders: ↓ non-responders: ↓ *Maintenance:* Responders: ↓ non-responders: ↑	**<0.001 0.04 <0.001 0.009**	NA	NA	*Induction*: Responders: ↓ non-responders: ↓ *Maintenance*: Responders: ↓ non-responders: ↑	**<0.001 <0.001 <0.001 0.03**	NA	NA	NA	NA	-	BMI: ↑	**0.04**
Borrelli 2004 [33]	7.11±3.8	1.35±0.45	NA	NA	35±13.1	12.75±2.37	NA	NA	PCDAI	34.11±5.15	15.11±5.45	NA	**<0.01**	Weight: ↑ Height: ↑	**<0.01 <0.01**

Csontos 2016 [24]	23.9 g/L	13.4 g/L	NA	**0.01**	NA	NA	NA	NA	CDAI	270.5±98.7	96.7±80.9	NA	NA	Weight: ↑ BMI: ↑ FMI (kg/m2) : ↑ BFM (%): ↓ VFA (cm^2): ↓ FFMI (Kg/m^2): ↑ SMI(Kg/m^2): ↑	**<0.001** **<0.001** 0.120 0.532 0.730 **<0.001** **0.003**
									Mayo score	7.3±0.8	2.3±2.4				
DeBoer 2016 [25]	1.2 (0.5-2.5) * mg/L	0.5 (0.3-0.8) * mg/L	NA	**< 0.0001**	21 (12-39) *	11 (5-17) *	NA	**< 0.0001**	PCDAI	25 (15-37.5) *	10 (2.5-15)	NA	**< 0.0001**	Weight: ↑ BMI: ↑ Height: ↑	**< 0.0001** **< 0.0001** **< 0.0001**
DeBoer 2018 [35]	2.1±2.9	0.8±0.8	NA	**< 0.0001**	30±22	15±14	NA	**< 0.0001**	NA	NA	NA	NA	NA	BMI: ↑ Height: ↑ LegLM (z-score): ↑	**<0.001** **<0.001** **<0.001**
Dos Santos 2017 [42]	NA	NA	NA	NA	NA	NA	NA	NA	HBI	7	2	NA	**<0.001**	Weight: ↑ BMI: ↑ BFM (%): ↑ FMI: ↑ LM (%): ↓ LMI: ↑ WC (cm): ↑	**0.006** **0.005** **0.008** **0.000** **0.008** **0.000** **0.002**
Emerenziani 2017 [26]	10.6±7.3	3.4±2.4	NA	**<0.05**	NA	NA	NA	NA	NA	NA	NA	NA	NA	FFM (Kg): ↑	>0.05
Franchimont 2005 [21]	5.3 (1.2) §	1.68 (0.6) §	NA	**<0.01**	NA	NA	NA	NA	CDAI	268 (26) §	135 (17) §	NA	**0.003**	Weight: ↑ BFM (Kg): ↑ BFM	**0.013** 0.38 0.58

														(%): ↑	
Griffin 2015 [27]	1.3(0.3–19.7) * mg/L	0.5 (0.3–5.2) * mg/L	NA	NA	26(0 – 115) *	13(0 – 64) *	NA	NA	NA	NA	NA	NA	NA	BMI: ↑ Height: ↑ Muscle area(mm^2): ↑	**< 0.001 < 0.001 <0.001**
Kierkus 2012a [28]	0.3 (0.15-2.9) &	0.8 (0.2-3.0) &	NA	**<0.05**	NA	NA	NA	NA	PCDAI	12.5 (6.2-15.0) &	5 (0.0;12.5) &	NA	**<0.05**	Weight: ↑ BMI: ↑ Height: ↑	**<0.05 <0.05** NA
Kierkus 2012b [41]	1.6 (0.3-3.5) * mg/L	0.3 (0.2-2.1) * mg/L	NA	NA	53±11.2	NA	NA	NA	PCDAI	52.5 (45.0-57.5) *	15 (10.0-30.0) *	NA	**<0.05**	BMI: ↑	NA
Koutroubakis 2009 [29]	3.9 (1)	1.2 (0.5)	NA	0.02	NA	NA	NA	NA	NA	261.6± 20.6	151.2±9 .1	NA	**< 0.0001**	BMI: ↑	**0.006**
									SCCAI	8.7±0.6	5.7±0.5	NA	0.19		
Malik 2011 [37]	NA	NA	NA	NA	NA	NA	NA	NA	PCDAI	25 (7.5-65) £	5 (0-37.5)	NA		Height vel.: ↑	0.11
Van Hoeve 2019 [40]	0.6 (0.3-1.2) * mg/L	0.3 (0.3-1.2) * mg/L	NA	0.367	9.5 (2.0-16.5) *	8 (2.5-19.0) *	NA	0.549	NA	NA	NA	NA	NA	Weight: ↑ BMI: ↑ Height: ↔	0.502 0.757 0.117
Wiese 2008 [31]	1.79	0.68	NA	**0.03**	NA	NA	NA	NA	HBI	12.93	8	NA	0.22	BMI: ↑ BFM-BIA (%): ↑ BFM-DXA (%): ↑ LM (Kg): ↑	**0.03** **0.09** 0.10 0.44

* Median (IQR); § Mean (SEM); & Median (range); # Median; £ Median (10th-90th centiles)
NA: not available in the primary study; BFM =Body Fat Mass; BIA=Bioelectrical impedance analysis; CDAI= Crohn Disease Activity Index; DXA=Dual-energy X-ray absorptiometry; FFM=Fat Free Mass; FFMI =Fat Free Mass Index; FMI=Fat mass Index; HBI=Harvey Bradshaw Index; LM=Lean Mass; LMI=Lean mass Index; LegLM =Leg Lean Mass; PCDAI=Paediatric Crohn Disease Activity Index; SCCAI= Simple Clinical Colitis Activity Index; SMI=Skeletal Muscle Index; TAF=Total Abdominal Fat; VFA=Visceral Fat Area; WC=Waist Circumference
Please refer main text for phase angle

APPENDIX IX

List of Medical Dictionary for Regulatory Activities (MedDRA) Lower-level term to create custom 'Body-changes' query (STUDY VI).

Must include:
WEIGHT ABNORMAL
WEIGHT INCREASE
WEIGHT GAIN
HIGH WEIGHT
WEIGHT INCREASED
WEIGHT ABOVE NORMAL
ABNORMAL WEIGHT GAIN
WAIST CIRCUMFERENCE INCREASED
BODY MASS INDEX HIGH
BODY MASS INDEX ABNORMAL
BODY MASS INDEX INCREASED
OVERWEIGHT
OBESITY
GROSS OBESITY
CENTRAL OBESITY
TRUNCAL OBESITY
MORBID OBESITY
ABDOMINAL OBESITY
At the same time, cases MUST NOT include:
WEIGHT DECREASED
WEIGHT DECREASE
WEIGHT GAIN POOR
WEIGHT NORMAL
ABNORMAL LOSS OF WEIGHT
UNDERWEIGHT

APPENDIX X

List of weight drugs associated with significant weight gain.

CATEGORY	SUBCATEGORY	DRUG
Corticosteroid	Mineralocorticoid	Aldosterone Desoxycortone Fludrocortisone
	Glucocorticoid	Betamethasone Cloprednol Cortisone Cortivazol Deflazacort Dexamethasone Fluocortolone Hydrocortisone Meprednisone Methylprednisolone Paramethasone Prednisolone Prednisone Prednylidene Rimexolone Triamcinolone
Antidiabetic agent	Insulin	Insulin
	Sulfonylurea	Acetohexamide Carbutamide Chlorpropamide Glibenclamide Glibornuride Gliclazide Glimepiride Glipizide Gliquidone Glisoxepide Metahexamide Tolazamide Tolbutamide
	Thiazolidinedione	Pioglitazone Rosiglitazone Troglitazone
Antihypertensive treatment	Beta-blocker	Atenolol

		Metoprolol Propranolol
	Thiazide and thiazide-like diuretic	Chlorothiazide
		Chlortalidone
		Diazoxide
		Hydrochlorothiazide
		Indapamide Methylclothiazide
		Metolazone
	Lithium	Lithium
Psychotropic medication	Second generation antipsychotic	Amisulpride
		Aripiprazole
		Clozapine
		Iloperidone
		Olanzapine Paliperidone
		Quetiapine
		Risperidone
		Ziprasidone
	Antiepileptic drug	Carbamazepine Gabapentin
		Pregabalin
		Valproic acid
Antibiotic	Quinolone	Gatifloxacin
		Levofloxacin
Calcineurin inhibitor		Ciclosporin Sirolimus
		Tacrolimus
Protease inhibitor		Atazanavir
		Darunavir
		Fosamprenavir
		Indinavir
		Nelfinavir
		Ritonavir
		Saquinavir
		Tipranavir

APPENDIX XI

Quartile and parameters of Weibull distribution and failure pattern for all anti-TNF-α and IBD indication.

	Cases	**Median**	**Lower quartile**	**Upper quartile**	**Minimum**	**Maximum**	**Scale parameter**		**Shape parameter**		**Failure type**
	(n)	**(months)**	**(months)**	**(months)**	**(months)**	**(months)**	**α**	**95 % CI**	**β**	**95 % CI**	
Overall	46	8	2.33	12.25	1	60	9.25	6.30-13.39	0.83	0.65-0.1.03	random failure type profile
IBD	27	8.5	3.25	12	0.6	50	9.13	5.88-13.93	0.97	0.69-1.28	wear-out failure-type profile