Drug Safety in Children: Focus on hepatic concerns

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CHAPTER 1

General Introduction

1.1. THE NEED FOR PAEDIATRIC SPECIFIC DATA ON THE EFFECTS OF DRUGS

Safety issues in children have shaped pharmacovigilance

As defined by World Health Organization (WHO), pharmacovigilance is the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem (adverse drug reactions, ADRs)[1]. The aims of pharmacovigilance are to improve patient care and patient safety in relation to the use of medicines and to support public health programmes by providing reliable, balanced information on the risk-benefit profile of medicines[1].

Although pharmacovigilance is necessary for all age groups, safety issues occurring in children actually laid the foundations for pharmacovigilance. In 1937, sulphanilamide elixir caused over 100 deaths in the United States (US), of which many were children[2]. The toxic effect was induced by diethylene glycol, a solvent contained in the antimicrobial syrup, usually used in antifreeze preparations and wallpaper strippers, causing multiorgan failure with acute renal failure when ingested[3]. Despite the US Food and Drug Administration (FDA) enforced, as a consequence, regulations for pharmaceutical development, diethylene glycol continued to cause outbreaks of deaths in children treated with acetaminophen or cough syrups. The solvent accidentally or intentionally added in many other Countries till recent years[4-8]. In 1959, three newborns had died from high doses of chloramphenicol because of the "*grey baby syndrome*" (vomiting, diarrhoea, flaccidity, hypothermia, ashen-grey colour) induced by accumulation of very high concentrations of chloramphenicol in tissue due to low activity[9]. Despite these disasters, pharmacovigilance activities were boosted only after the thalidomide tragedy in 1961. Due to skeletal malformations in newborns of mothers having taken thalidomide for insomnia or nausea during pregnancy, thalidomide was withdrawn from the market[10, 11]. A few years later, however, thalidomide was reintroduced as treatment for a complication of leprosy called erythema nodosum leprosum[12]. This drama increased the awareness that safety of drugs needs to be monitored after their marketing, especially in children. In 1979, first reports appeared of valproic acid causing hepatotoxicity, particularly in infants less than 3-years[13-15]. These reports led to changes in prescribing habits, which reduced the problem. In 1980, the detection of a dose-related association between salicylate and Reye's syndrome (hepatotoxicity) led to stop the salicylates in children. In 2006 in Europe and 2007 in US, case reports on serious cardiovascular adverse events, sudden death, and psychiatric disorders led to warning from the regulatory about the use of methylphenidate in the paediatric population[16, 17]. Since August 2010,

cases of narcolepsy, especially in children and adolescents, were notified in Europe following the use of Pandemrix, a 2009 pandemic influenza A(H1N1) vaccine[18]. In August 2012, FDA launched a warning about the safety of codeine in children following the occurrence of deaths and cases of serious adverse events in children treated with this drug for post tonsillectomy and/or adenoidectomy pain[19-22]. A comprehensive safety review in FDA's Adverse Event Reporting System (FAERS) database from 1969 to 2012 identified children as more susceptible to the overdose or death from codeine[23]. One year later, the European Medicines Agency's (EMA) Pharmacovigilance Risk Assessment Committee (PRAC) addressed the same safety concerns and restricted the use of codeine for pain relief in children[24].

The above description represents a selection of adverse events occurring specifically in children to medicines that were not primarily developed and studied for use in children. These are important examples and call for the need to have paediatric specific information about the effects of drugs both pre- as well as post-licensure. Ethical and practical issues limit participation of children in clinical trials[25, 26]. For this reason, and together with the fact that paediatric patients represent only a minor proportion of the pharmaceutical market, with limited unique therapeutic indications, studies and clinical trials in children, which are often associated with little profit expectations, do not represent advantageous investments for a profit-driven pharmaceutical industry[27].

Importance to study children specifically

In the past it was quite acceptable to use adult pre-licensure data and extrapolate the results to children, but since the beginning of the millennium the call for paediatric specific research has become very loud. Children are not small adults and disease evolution as well as pharmacokinetic and pharmacodynamics processes change[28]. Body composition influences the apparent volume of distribution for drugs[29]. Infants in the first 6 months of life have markedly expanded total-body water and extracellular water, expressed as a percentage of total body weight, as compared with older infants and adults and this impacts on the pharmacokinetics of drugs[29]. The thickness, extent of perfusion, and extent of hydration of the skin and the relative size of the skin-surface area changed with age, which is important for topically applied drugs. Although skin thickness is similar in infants and adults, the extent of perfusion and hydration diminishes from infancy to adulthood[30, 31]. Age-dependent changes occur in both the structure and function of the gastrointestinal tract, which impacts absorption of orally administered drugs[32, 33]. Active tubular secretion, represented by the clearance of para-aminohippuric acid and the glomerular filtration rate, approximate adult activity only after 6 to 12 months of age, which impacts on clearance of drugs. Liver activity also varies

with age[34-36]. Most drugs are metabolized through the cytochrome P450 (CYP 450) isoenzymes. The change in maturation and activity of CYP 450 occurring with age may have a strong influence on the capacity to eliminate the same drug between newborns and adults. For instance, at birth, the CYP P450 isoenzymes are only 50% of the adult values, but their expression quickly changes during the first months and are isoform specific[37-40].

Due to potential differences in pharmacokinetics and dynamics direct extrapolation about the effects of drugs (benefits and harms) from adults to children is not always possible which underlines the need for paediatric specific data.

Problems with off-label and unlicensed drug use

Since proper studies were often not conducted for paediatrics, there is a lack of authorized medicinal products and sufficient information on medicines in children. For this reason, many old drugs are either not licensed for use in children at all (*unlicensed*) or prescribed outside the product license (*off-label*) in terms of age, dosage, therapeutic indication and route of administration[41-43]. Studies performed in several countries showed that off-label and unlicensed drug use accounted for >50% of the total prescriptions in paediatrics, with a higher prevalence of off-label than unlicensed use[41, 44-47]. Off-label and unlicensed use of drugs often represent the only evidence-based and experience-supported therapeutic options. In 2005, Pandolfini and Bonati described that the off-label/unlicensed prescriptions ranged between 16% to 62% in the paediatric hospital wards, and between 11% to 37% among outpatients[48]. This attitude concerns drugs commonly prescribed to children such as systemic anti-bacterials (i.e. amoxicillin), anti-asthmatics (i.e. salbutamol), analgesics (i.e. acetaminophen), cardiovascular agents (i.e. captopril)[49, 50]. Among different settings, higher off-label/unlicensed rates exist for neonatal versus paediatric wards and for hospital versus community setting.

Unlicensed/off-label drug use is associated with several problems. Firstly, inadequate dosage information may result into ineffective treatment through under-dosage and treatment failures and overdosing may carry a risk of adverse effects without any therapeutic benefits[51]. Moreover, unauthorized/off-label medicine use is associated with an increased risk of medication errors, especially in neonates, and of ADRs[52]. Accordingly, Cuzzolin *et al.* estimated the risk of ADRs from unlicensed/off-label use ranging between 23 and 60%[49], especially serious ones[53-55]. Actually, the most of off-label/unlicensed-related ADRs, in either oncology or non-oncology patients, are seen in paediatric wards, where the off-label/unlicensed use of medicines is frequent[56].

Guidance and regulations

Following the increase of public concerns on safer and more efficient use of medicines in children, several paediatric groups of national and international regulatory bodies emphasized the need to improve clinical and/or post-marketing research in paediatrics. Pioneering legislation addressing paediatric needs came into force in the US in 1997. Following the FDA US experience, the EMA implemented the Paediatric Regulation in 2007, by establishing a system of obligations, rewards and incentives, to ensure that drugs are systematically researched, developed and authorised to meet the therapeutic needs in children. Accordingly, the new EU Paediatric Regulation specifies the need for a Paediatric Investigation Plan (PIP) as a mandatory part of each new licence application or for the extension of an already authorised product that is still under patent protection[57]. The Paediatric Regulation also introduced the Paediatric Use Marketing Authorisation (PUMA) as an incentive to perform research into the potential paediatric use of off-patent medicinal products already authorised for adults[58]. The EU Paediatric Regulation has led to a comprehensive network of experts in paediatrics within the European countries. The Paediatric Committee at the EMA is responsible for assessing the PIPs and advising companies. The European Network for Paediatric Research at the EMA (Enpr-EMA) was established in 2009 to provide the added value of a holistic approach by linking together national and European networks, researchers and centres with specific skills in designing and conducting high quality studies in children[59].

An assessment of the impact of the new EU Paediatric Regulation after 7 years suggested a critical change of the culture in research[57]. Children have become an integral part of the overall development of a product, suggesting a good compliance of the companies to the regulation. Moreover, 600 paediatric PIPs had been approved by EMA by the end of 2012, covering a wide range of therapeutic areas, mainly endocrinology-gynaecology-fertility-metabolism, infectious diseases, and oncology. However, among those, around 80% (n= 453) still requires authorization in EU for paediatric use, while the 20% achieved new indications for patent-protected products or paediatric use marketing authorizations[57]. With respect to financial support for research in paediatrics to date, 16 projects covering at least 20 off-patent active substances have received EU funding, amounting to total support of EUR 80 million. As a result of the EU Paediatric Regulation, by the end of 2011, 72 medicines already authorized in adults received paediatric indications, and 26 new pharmaceutical forms have been authorized for paediatric use. Concerning PUMAs, so far only one authorization has been granted[57]. Between 2007 to 2011 the number of clinical trials remained stable in children, whilst they decreased overall. The EudraCT database showed an increase in the number of

paediatric study participants, comprising especially neonates and infants, from 0 to 23 months, usually not included in trials before 2008. This shows the positive impact of the regulation on efficacy data, however serious and idiosyncratic ADRs are often rare and usually not detectable, calling for larger populations and post-marketing efforts to study them. Observational studies are more efficient in evaluating the safety of medicines than clinical trials, which often are limited in size, inclusion and follow-up[60, 61]. Different types of observational data may provide different types and levels of evidence[62]. Case reports and spontaneous reporting systems, epidemiological descriptive studies and surveys allow detection of safety signals and generation of research hypotheses. Epidemiological studies may provide information on the absolute and relative risks of drugs. The strengths of observational studies are that they reflect actual clinical practice and may include patients with concomitant illnesses, large sample sizes and longer follow-up[62]. Nevertheless, due to non-random assignment of drugs and frequent use of existing data from health care databases, observational research is also hampered by methodological limitations such as confounding by indication and bias[60].

1.2. DRUG-INDUCED LIVER INJURY

Drug-induced liver injury (DILI) is defined as a liver injury caused by various medications, herbs, or other xenobiotics, leading to abnormalities in liver tests or liver dysfunction[63]. DILI is increasingly being recognised as a cause of clinically significant acute and chronic liver disease[64]. The lack of objective confirmatory diagnostic tests and the highly variable clinical presentation of DILI can often lead to a delay in recognition. DILI is a diagnosis of exclusion that relies on multiple elements[63-66]. As Fontana et al. specified, the key diagnostic tools to assess the causality in DILI include the time to onset, clinical features, the time and course of recovery, in some case, the presence of specific risk factors, exclusion of other morbidity, and previous reports on the hepatotoxicity of the implicated agent[65]. Furthermore, whenever available, the diagnosis can be improved by rechallenge and liver biopsy[66].

Epidemiology of DILI

The incidence of DILI is largely unknown because of the paucity of prospective population-based studies and the relatively low frequency of liver injury attributable to drugs. DILI represents 1.2% to 6.6% of cases of acute liver disease seen at tertiary referral centres[67-69]. The incidence of DILI in the general population has been estimated to be 1 to 2 cases per 100,000 person years[70]. However, the estimated incidence of DILI was 14 cases per 100,000 patient years in a prospective study from northern France, which is 10-fold higher than the rate reported to regulatory agencies[71].

Due to differences in prescribing patterns there is a wide geographic variability in the drugs held responsible for causing DILI[70-73]. In Western countries, antibiotics, anticonvulsants, and psychotropic agents are the medicines mostly implicated in DILI in general population, with some differences between EU and US studies[67, 69-72]. To the contrary, in Eastern countries herbal and dietary supplements are more involved in DILI than conventional drugs[74, 75].

Etiopathogenesis of DILI

Several mechanisms for and clinical presentations of DILI exist, resulting in different types of liver injury (i.e. hepatocellular, cholestatic or mixed)[76]. Briefly, hepatocellular type is characterized predominantly by a first elevation of the alanine aminotransferase levels[76], potentially associated with steatosis or necrosis[77]. Some examples of medicines causing this type of liver injury are represented by isoniazid, pyrazinamide, trovafloxacin, tetracyclines, valproic acid, selective serotonin receptor inhibitors (SSRIs), methotrexate, statins, non-steroidal anti-

inflammatory drugs, acetaminophen[76]. Clinical features of cholestatic liver injury include an initial increase of serum alkaline phosphatase (AP) levels, potentially associated with jaundice and pruritus; prolonged AP levels could result in cholangiocytes leading to progressive ductopenia or vanishing bile duct syndrome[78]. Some examples of medicines mostly implicated in cholestatic liver injury include amoxicillin/clavulanic acid and erythromycins, anabolic steroids, oral contraceptives, tricyclics antidepressants, phenothiazines[76]. DILI might sometimes be predictable in pre-marketing studies because it is often related to drug mechanism of action and dose related[63]. Typical example is represented by hepatotoxicity induced by acetaminophen[76, 79]. However, very frequently DILI is idiosyncratic, rare, and not predictable[76]. These reactions are not related to the dose, route or duration of drug administration and have different period of latency, from few days to 12 months[79]. Many drugs induce liver injury through varying patterns with specific characteristics (biochemical, clinical and histologic) and chronologic features, which, together with the low incidence of DILI, make difficult to investigate and detect this condition[79].

DILI in children

The ability of the liver to metabolize, adapt and regenerate is age dependent, which may give rise to differences in susceptibility to DILI in children compared with adults.

Causes of acute liver injury in children were attempted to be identified from a liver disease registry and showed that the aetiology of *acute liver failure* in most instances cannot be determined in almost 50% of paediatric patients, seems drug-related in around 15%, while other causes (i.e., autoimmune hepatitis or hepatopathy due to metabolic diseases or viral infections) account for less than 10% each[80].

DILI is more often reported in adults as a result of either a lower risk of toxicity in the younger patient or the increased/different exposure to drugs in adult and elderly populations[81]. For this reason, the most drugs that are known to cause hepatotoxicity in children include also analgesics (i.e. acetaminophen), and antineoplastic drugs, together with antibiotics and anticonvulsants, which are predominantly implicated in adults[82]. Some examples are given below.

Acetaminophen is one of the most common causes of DILI in children, as many children are treated with this drug for fever. Acetaminophen-induced hepatotoxicity has a well-characterized intrinsic mechanism due to the formation of a highly reactive, intermediate metabolite[83]. Young children are less susceptible to acetaminophen hepatotoxicity than older children since toxicity required metabolism which is less active in younger children[81]. Its hepatotoxicity is generally attributed to i) an acute overdose, either accidental in a toddler or intentional in

adolescents; or ii) a sub-acute form occurred in a child taking moderately large doses at regular intervals[84].

In contrast to acetaminophen-induced hepatotoxicity, children younger than 3 years are more susceptible to valproic acid-induced liver injury than older children/adolescents[85]. Hepatotoxic mechanism of valproic acid seems to be attributed to its inhibition of fatty acid transport and mitochondrial β-oxidation[86]. Clinical features include steatosis and hepatocellular damage with asymptomatic elevation of serum aminotransferases. However, a form of valproic acid-induced hepatotoxicity as a Reye-like syndrome, with marked alanine aminotransferase elevations associated with confusion, stupor and coma, has been described in children[87].

In the last decade, pemoline, used to treat paediatric attention-deficit/hyperactivity disorder (ADHD), was withdrawn from the US market because of reports of hepatotoxicity[88]. The product labelling of atomoxetine, another widely used drug for ADHD, was changed in 2004 to include a warning regarding potential hepatotoxicity[89].

The limited data may suggest that DILI is a rare cause of liver injury; however, at this point it is not clear whether absence of evidence is not just due to lack of studying the role of drugs in acute liver injury in children. A National Institutes of Health Clinical Research workshop in 2008 concluded that additional studies of paediatric DILI were needed because of age-related differences in drug metabolism, implicated drugs, and toxic doses[65].

1.3. OUTLINE AND AIMS OF THIS THESIS

This thesis aims to provide more evidence on DILI in children, by using pharmacoepidemiological approaches using different sources of routine care data.

Spontaneous Reporting Systems

Currently, Spontaneous Reporting Systems (SRS) offer the most important source for identification of safety signals both in children and in adults. The advantages include that they are large-scaled, inexpensive and easy to operate, cover all medicines during their whole life-cycle and the whole population, and can be used for hypothesis generating purposes[90].

Understanding the characteristics of these databases including their strengths and limitations represents the first step for better use of them (**Chapter 2**). Specifically, we explored the characteristics of all paediatric ADRs spontaneously reported to the Italian Pharmacovigilance Network (*Rete Nazionale di Farmacovigilanza*, RNF), established by Italian Medicines Agency (AIFA) (**Chapter 2.1**) and in the Adverse Event Reporting System maintained by US FDA (FAERS), (**Chapter 2.2**).

Second, we used a similar and larger setting, the VigiBase, ADR spontaneous reporting database of the WHO Uppsala Monitoring Centre (WHO-UMC), to identify new potential signal of hepatic toxicity among the ADRs reported in children (**Chapter 3**).

Electronic Healthcare Record databases

The increasing availability of Electronic Healthcare Record (EHR) and claims databases allows for the conduct of signal detection in these databases, which do not suffer from the same limitation as spontaneous reporting databases[91]. These systems are typically used for signal verification studies. The biggest advantages of these databases are their very large sample size, relatively inexpensive use, the presence of denominator data and flexibility for designs.

First of all, we explored the potential power of such systems in active paediatric drug safety surveillance by exploring data from the EU-ADR project in which a network of 8 databases from 4 EU countries worked together to develop signal detection methods (*Exploring and Understanding Adverse Drug Reactions by Integrative Mining of Clinical Records and Biomedical Knowledge*)[92](**Chapter 4**).

Signal detection and verification using healthcare databases

The next step in this thesis is the use of existing healthcare databases to look at the occurrence of DILI and the association between drugs and liver injury in children and adolescent outpatients (**CHAPTER 5**). The aims of the studies were:

I. To identify potentially new safety signal of drug-induced acute liver injury in a signal detection study (**CHAPTER 5.1**);
II. To investigate the characteristics and the incidence of idiopathic acute liver injury in a cohort study (**CHAPTER 5.2**);
III. To test the association between use of antibiotics and hepatotoxicity in children and adolescents (**CHAPTER 5.3**).

Network of data sharing and scientific collaboration: GRiP project

The results of this thesis show the need of very large sample sizes to study drug safety in children, especially if it concerns rare adverse events. The currently available pharmacoepidemiological databases on infants, children, and adolescents are not adequately utilized due to several reasons including a) the lack of a federation of databases for meaningful investigations; b) the lack of shared methodologies to specifically retrieve paediatric information; c) the lack of standardized methods and study designs. New methodologies and guidelines regarding the scope of pharmacoepidemiology in paediatric clinical drug and vaccine development are urgently needed.

The Global Research in Paediatrics Network of Excellence (GRiP) is an European Commission-funded network which amongst other goals aims to implement an infrastructure facilitating the development and safe use of medicines in children[93]. As part of this project, we identified databases with population based information on drugs, vaccines and potentially outcomes in children and described their characteristics (**CHAPTER 6**).

CHAPTER 2

Data on Paediatrics from Spontaneous Reporting Systems

2.1. Paediatric Drug Safety Surveillance in Italian Pharmacovigilance Network: an Overview of Adverse Drug Reactions in the years 2001-2012

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ABSTRACT

Background
Spontaneous Reporting System is an essential tool for post-marketing drug safety surveillance, especially for signal detection of less common and most serious ADRs, and in particular population, such as children.

Objective
To explore the characteristics of paediatric ADRs reported to the Italian spontaneous reporting database (*Rete Nazionale di Farmacovigilanza*, RNF) over the last decade.

Methods
Reports of suspected ADRs related to children and adolescents (<18 years) were extracted from the RNF over the period 2001-2012. Duplicates, vaccine reports and reports with missing information about age were excluded. The Medical Dictionary for Regulatory Activities (MedDRA®) and the WHO-Anatomical Therapeutic Chemical (ATC) classification were used to group ADR reports by affected System Organ Class (SOC) and suspected drug category. Main characteristics of paediatric ADRs and the most frequently implicated drug classes across MedDRA-SOC and different paediatric age-categories were investigated.

Results
Among 123,129 selected reports, 8,338 (6.8%) concerned paediatrics, with males being more involved than females up to 11 years of age (52.2% vs. 47.6%), thereafter this balance reversed. 39.4% of paediatric reports were serious and, of these, 75.2% required hospitalization, mainly in very young children. Most of the reports were issued by hospital physicians (61.9%), followed by pharmacists (10.1%), while reports from family paediatricians accounted for only 8.1%. The most frequently implicated drug categories were anti-infectives for systemic use (n= 3,743; 44.9%), drugs acting on nervous system (n= 1,304; 15.6%), and anti-inflammatory drugs (n= 849; 10.2%). As compared to the reports concerning adult population, those related to the paediatric group concerned mostly respiratory system drugs (7.8% *vs* 2.0%, respectively), and, to a lesser extent, drugs acting on blood (1.5% *vs* 10.4%) and cardiovascular system (1.0% vs. 12.0%). At single compound-level, the most frequently suspected drugs differed between children and adults. ADR reports for the same drug were likely to be more serious in adults than in children.

Conclusion

This descriptive overview of Italian SRS reflects real safety concerns for drugs used in children. Accordingly with "Guideline on conduct of pharmacovigilance for medicines" used by the paediatric population, our findings emphasize the need for stratifying analyses within specific subgroup populations to increase the sensitivity of the signal detection procedure in children.

BACKGROUND

In the past, children have been under-represented in pre-marketing clinical trials leading to market medicines not being adequately studied in the paediatric population. Consequently, the use of several medicines in children has not been authorised or has been off-label, resulting in an increased risk of ADRs[94].

The limited knowledge about benefit-risk drug profile in children has boosted in recent years initiatives to improve a safe use of medicines in paediatrics. The EMA Paediatric Committee (PDCO) came into force on 26 January 2007, obliging pharmaceutical companies to agree a PIP for all new medicines, indications and pharmaceutical forms, and to submit the results of paediatric clinical trials[95]. Despite, as a consequence, the number of pre-marketing clinical trials in children has being increased, drug safety in children remained unsatisfactorily investigated because they did not reflect the risks of a medicinal product in the 'real-life' setting. Thus, identification, quantification, and prevention of ADRs are still main concerns of paediatric pharmacovigilance. While many recent initiatives by the WHO[96] as well as the US[97] and EU[90, 91, 98, 99] policymakers are promoting the use of longitudinal EHR databases to complement traditional ADR monitoring systems, SRS, based on databases systematically collecting reports of ADRs, remains an essential post-marketing surveillance source of information to identify drug safety signal[100, 101].

Generally spontaneous reports of ADRs are nationally collected and, subsequently, transferred as individual case safety reports into international database such as Eudravigilance in Europe (that includes also reports coming from non-European countries) and VigiBase managed by the WHO-UMC, in which since the 1960s the reports are sent by the countries participating to the WHO-Drug Monitoring Programme[102]. Descriptions of the paediatric reports which document number and type of the ADR reports, their outcome and the most frequently implicated drugs have been described from several European and non-European countries as well as worldwide have been previously published[103-109].

The aim of the current study was to explore the reports of suspected ADRs concerning paediatric population from the Italian Pharmacovigilance SRS database over 11-year period and to compare them to ADR reports in adults.

METHODS

Data source

In Italy ADR reports are collected through the RNF, established by AIFA in 2001. The RNF connects each other, the national authority, the Regional/Local

Authorities and the Regional Centres of Pharmacovigilance over 200 Local Health Authorities, about 100 Hospitals, 43 Research Institutes and over 800 Pharmaceutical Companies[110].

Each suspected ADR report includes information on patient demographics (e.g. age, gender, region), report source, ADR outcome, date of onset of the suspected ADR, seriousness; MedDRA-Preferred Terms (PT) and unstructured narrative of each event; drug/vaccine information for as many medications as reported for each event (active ingredient name, trade name, Anatomical Therapeutic Classification code, ATC, therapy start and end dates, and indications of use). Duplicate reports are detected from the system by applying a dedicated automated tool. The network has been previously described as a valid system for safety surveillance[111-114].

Data setting

All the reports concerning children and adolescents (<18 years) from January 2001 till December 2012 were retrieved. Adult reports (≥18 years) were also extracted for comparison. We excluded all the reports in which age, suspected drug or event was missing. In addition, we excluded vaccine-related reports. All the reports coming from published case reports were excluded as well. The ADR reports data are loaded in the database after their codification according to MedDRA, that allow to retrieve data for analysis with different level of aggregation of the descriptive terms of SOC - divided into High-Level Group Terms (HLGT), High-Level Terms (HLT) and PT.

Data analysis

We investigated the frequency of paediatric reports according to: gender and region of origin of patients, seriousness, patients' outcome, and type of reporter. Seriousness was categorized as: death, life-threatening, hospitalization (initial or prolonged), disability, congenital anomaly, or other conditions medically significant. Main analyses were stratified by the patients' age at onset as follows: newborns and infants (<2 years), preschool children (2-≤5 years), childhood (6-≤11 years) and adolescents (12-≤17 years). Only suspected drugs were analysed (i.e. concomitant drugs were not considered). Within each age group, the most frequently involved three therapeutic classes (second-level ATC classification) were described with respect to the six most frequently reported ADRs (using MedDRA-SOC terms).

Irrespective of the type of event, we looked at the single suspected drug (by active substance) involved in more than 100 reports and the proportion of serious ADR related to these medicines, by dividing the number of serious ADRs for the specific compound with the total number of ADRs overall. In addition, we compared the frequency of the ADRs (using MedDRA-SOC) and the drugs (first-

level ATC classification) documented for children *vs* adults. Finally, the frequency statistics were calculated for reported drug-event combination.

Proportions were compared using the chi-square test; means were compared by using either students-t or Mann-Whitney U test, as appropriate; a p-value <0.05 was considered to be statistically significant.

RESULTS

During the period 2001-2012, the RNF received a total of 148,380 spontaneous ADR reports. After exclusion of the reports including vaccines (n= 24,954; 16.8%), and reports in which age was not reported (n= 297; 0.2%), a total of 123,129 re-

Table 1. Characteristics of paediatric reports of ADRs in the *Rete Nazionale di Farmacovigilanza*, distributed by age-categories over 11-years (2001-2012)

	Total n= 8,338 *(%)*	< 2years n= 1,679 *(%)*	2-5 years n= 2,250 *(%)*	6-11 years n= 2,062 *(%)*	12-17 years n= 2,347 *(%)*	P value
Mean age (± SD)	7.4 (5.5)	8.5 months (4.0)	3.3 (1.1)	8.5 (1.7)	14.7 (1.7)	<0.001
Gender						
Boys	4,356 *(52.2)*	877 *(52.2)*	1,188 *(52.8)*	1,152 *(55.9)*	1,139 *(48.5)*	<0.001
Girls	3,969 *(47.6)*	798 *(47.5)*	1,058 *(47.0)*	907 *(44.0)*	1,206 *(51.4)*	<0.001
NA	13 *(0.2)*	4 *(0.2)*	4 *(0.2)*	3 *(0.1)*	2 *(0.1)*	
Seriousness (% within age category)						
Serious	2,452 *(29.4)*	451 *(26.9)*	579 *(25.7)*	650 *(31.5)*	772 *(32.9)*	<0.001
Type of seriousness (% within seriousness)						
Hospitalization	1,843 *(75.2)*	362 *(80.3)*	435 *(75.1)*	454 *(69.8)*	592 *(76.7)*	<0.001
Other conditions medically significant	344 *(14.0)*	39 *(8.6)*	81 *(14.0)*	113 *(17.4)*	111 *(14.4)*	<0.001
Life-threatening	204 *(8.3)*	30 *(6.7)*	51 *(8.8)*	70 *(10.8)*	53 *(6.9)*	0.009
Death	26 *(1.1)*	9 *(2.0)*	7 *(1.2)*	5 *(0.8)*	5 *(0.6)*	0.287
Disability	26 *(1.1)*	3 *(0.7)*	5 *(0.9)*	8 *(1.2)*	10 *(1.3)*	0.406
Congenital anomalies	9 *(0.4)*	8 *(1.8)*	-	-	1 *(0.1)*	<0.001
Outcome (% within age category)						
Fully recovered	3,938 *(47.2)*	716 *(42.6)*	1,059 *(47.1)*	1,049 *(50.9)*	1,114 *(47.5)*	<0.001
Improved	1,893 *(22.7)*	385 *(22.9)*	528 *(23.5)*	441 *(21.4)*	539 *(23.0)*	0.403
Unknown	1,838 *(22.0)*	428 *(25.5)*	497 *(22.1)*	420 *(20.4)*	493 *(21.0)*	0.001
Not yet recovered	538 *(6.5)*	123 *(7.3)*	135 *(6.0)*	122 *(5.9)*	158 *(6.7)*	0.244
Recovered with consequences	103 *(1.2)*	17 *(1.0)*	25 *(1.1)*	24 *(1.2)*	37 *(1.6)*	0.349
Death	28 *(0.3)*	10 *(0.6)*	6 *(0.3)*	6 *(0.3)*	6 *(0.3)*	0.233

Vaccines have been excluded from this analysis. Means were compared by using either students-t or Mann-Whitney U test, as appropriate; a p-value <0.05 was considered to be statistical significant.

Abbreviations: NA= not available, SD= Standard Deviation.

ports remained, of which 8,338 (6.8%) concerned the paediatric population. The number and the proportion of ADR reports increased over the 10-year period, and most of them had been collected during the very recent years (data not shown). The mean age in these reports was 7.4 years (SD ±5.5).

Table 1 describes the characteristics of the paediatric reports distributed by age categories. The number of the reports significantly increases with age, with a small decrease in children 6-11 years old. The majority of ADRs (n= 4,356; 52.2%) were reported for boys, up to 11 years, while the majority of reports after this age were for girls. Around one third of the total reports have been considered as serious (n= 2,452; 29.4%) and, among them, most of the ADRs led to hospitalization (n= 1,843; 75.2%), and fewer ADRs have been reported as causing death or disability (n= 26, 1.1%), or determining congenital anomalies in the offspring (n= 9; 0.4%). The majority of the ADRs completely resolved (n= 3,938; 47.2%) or improved (n= 1,893; 22.7%). Among age-categories, several differences were observed in terms of seriousness and outcome of ADR. Actually, the highest proportion of serious ADR-related hospitalization was observed in children with less than one year of age (n= 362; 80.3% of serious ADRs).

Overall, ADR reports were issued by hospital physician in 61.9% of the reports (n= 5,158), followed by pharmacists (n= 841; 10.1%) and family paediatricians (FP, n= 675; 8.1%). The proportion of the reporter types was heterogeneously distributed across age categories. Actually, ADRs issued by hospital physicians (from 59.0% to 63.8%), specialists (from 4.5% to 8.8%) and general practitioners (GP, from 4.4% to 10.3%) linearly increased from reports on very young children to those on adolescents, while the opposite trend was observed for pharmacists (from

Table 2. Numbers and proportions[#] of suspected paediatric ADR reports distributed by type of reporter and age group.

Type of reporter	Total n (% on 8,338)	< 2 years n (% on 1,679)	2-5 years n (% on 2,250)	6-11 years n (% on 2,062)	12-17 years n (% on 2,347)	P value
Hospital physician	5,158 *(61.9)*	990 *(59.0)*	1,351 *(60.0)*	1,319 *(64.0)*	1,498 *(63.8)*	0.001
Pharmacist	841 *(10.1)*	222 *(13.2)*	252 *(11.2)*	162 *(7.9)*	205 *(8.7)*	<0.001
Family paediatricians	675 *(8.1)*	154 *(9.2)*	277 *(12.3)*	205 *(9.9)*	39 *(1.7)*	<0.001
Specialist	542 *(6.5)*	76 *(4.5)*	118 *(5.2)*	141 *(6.8)*	207 *(8.8)*	<0.001
General practitioner	505 *(6.1)*	74 *(4.4)*	82 *(3.6)*	107 *(5.2)*	242 *(10.3)*	<0.001
Other reporter[$]	329 *(3.9)*	87 *(5.2)*	87 *(3.9)*	67 *(3.2)*	88 *(3.7)*	0.022
Nurses	158 *(1.9)*	45 *(2.7)*	40 *(1.8)*	31 *(1.5)*	42 *(1.8)*	0.056
Poison Control Centre	66 *(0.8)*	25 *(1.5)*	22 *(1.0)*	14 *(0.7)*	5 *(0.2)*	<0.001
Patient	45 *(0.5)*	6 *(0.4)*	14 *(0.6)*	12 *(0.6)*	13 *(0.6)*	0.705
Pharmaceutical company	19 *(0.2)*	-	7 *(0.3)*	4 *(0.2)*	8 *(0.3)*	0.114

[#]Proportions are based on the number of type of reporters/the total number of suspected paediatric ADR reports within each age group.

[$]Other reporter: Other healthcare professionals.

13.2% to 8.7%), FPs (from 9.2% to 1.7%) and professional figures from the Poison Control Centres (from 1.5% to 0.2%) (**Table 2**).

The stratification by region of origin showed that the highest number of reports in children originated from Lombardy (n= 3,311). Nevertheless, compared to the total reports within each region, the highest proportion of paediatric reports was observed in Campania (n= 1,096, 18% of total reports from Campania) and Liguria (n= 323, 15%) (**Appendix 1**).

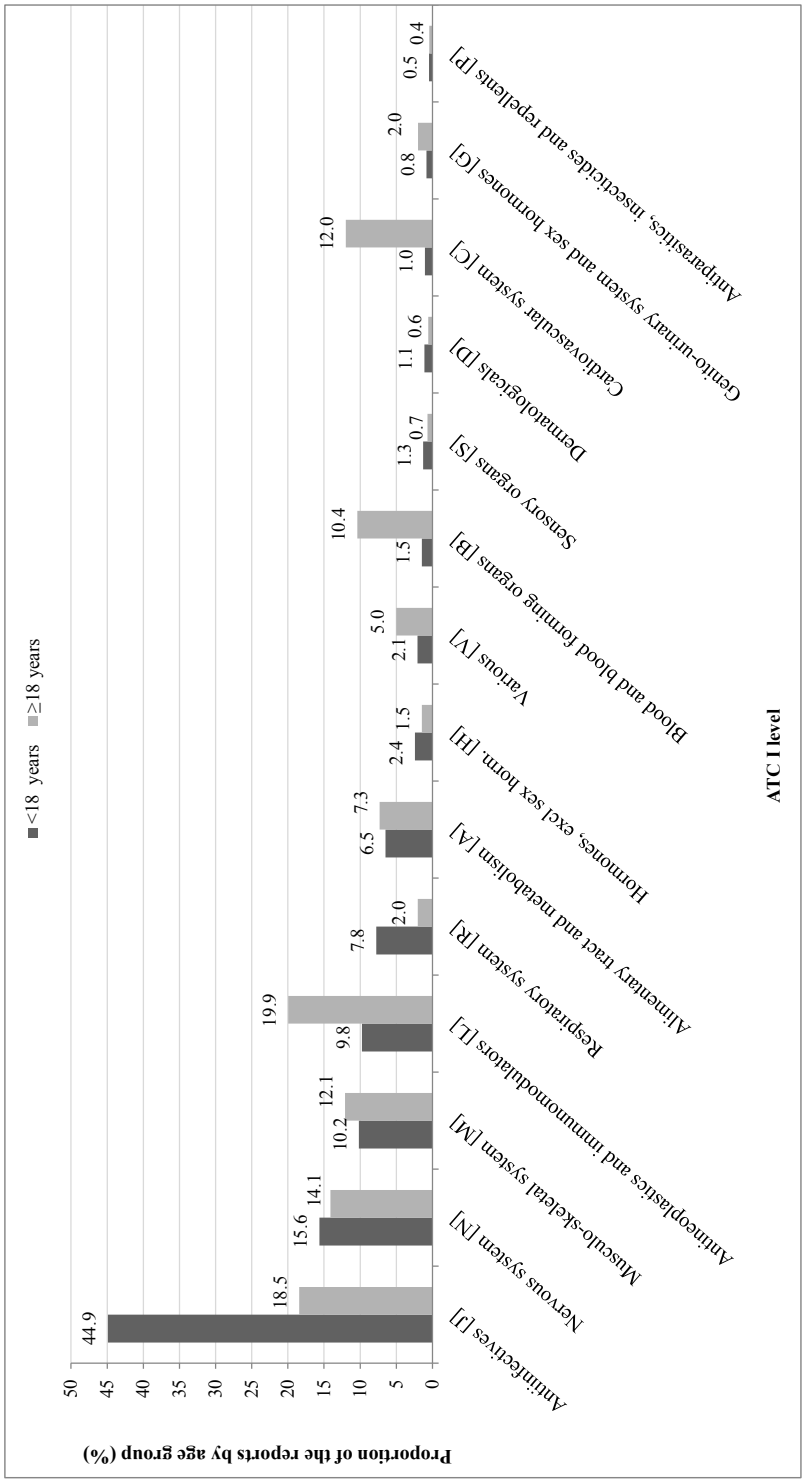
Figure 1 displays the proportions of reports by first-level ATC classification in children and in adults. "*Anti-infective agents for systemic use*" were the most frequently reported drug class among children (n= 3,743; 44.9%), followed by "*nervous system drugs*" (n= 1,304; 15.6%), *"anti-inflammatory drugs*" (n= 849; 10.2%) and "*antineoplastics and immunomodulating agents*" (n= 814; 9.8%). In the adult group, the most frequently reported drugs were the "*antineoplastics and immunomodulating agents*" (n= 22,898; 19.9%), and "*anti-infective agents*" (n= 211,181; 18.5%). In comparison to adults, the paediatric group showed a higher proportion of the reports of "*drugs belonging to the respiratory system*" (7.8% *vs*. 2.0%), while the opposite was found for "*agents acting to the blood and blood-forming organs*" (1.5% *vs*. 10.4%) and for "*drugs for cardiovascular system*" (1.0% *vs*. 12.0%).

Proportion is based on the number of the reports with the specific ATC within each age group (n=8,338, in children, n= 114,791 in adults) within the ATC category (1st level). One report can describe one or more drug/event combination, thus one report can be counted in more than one ATC category. The figure is sorted out by descending order of the total number of paediatric reports per ATC.

The distribution of the reports by MedDRA-SOCs in children and in adults is displayed in **Figure 2**. "*Skin and Subcutaneous Tissue Disorders*" were most commonly reported ADRs in children (n= 4,352, 52.2%), followed by "*Gastrointestinal Disorders*" (n= 1,439; 17.3%), "*Nervous System Disorders*" (n= 1,024; 12.3%), "*General Disorders"* (n= 1,008; 12.1%) and "*Respiratory, Thoracic & Mediastinal Disorders"* (n= 538; 6.5%). Despite the top five SOCs were consistently reported in both children and adults in terms of absolute number of reports, different percentages were identified within each age-group (<18 years and ≥ 18 years). Actually, the proportion of the reports including skin reactions in children exceeded those recorded in adults by 19.8% and, reversely, the proportion of the reports on respiratory disorders in adults exceeded those recorded in children by 5.1%.

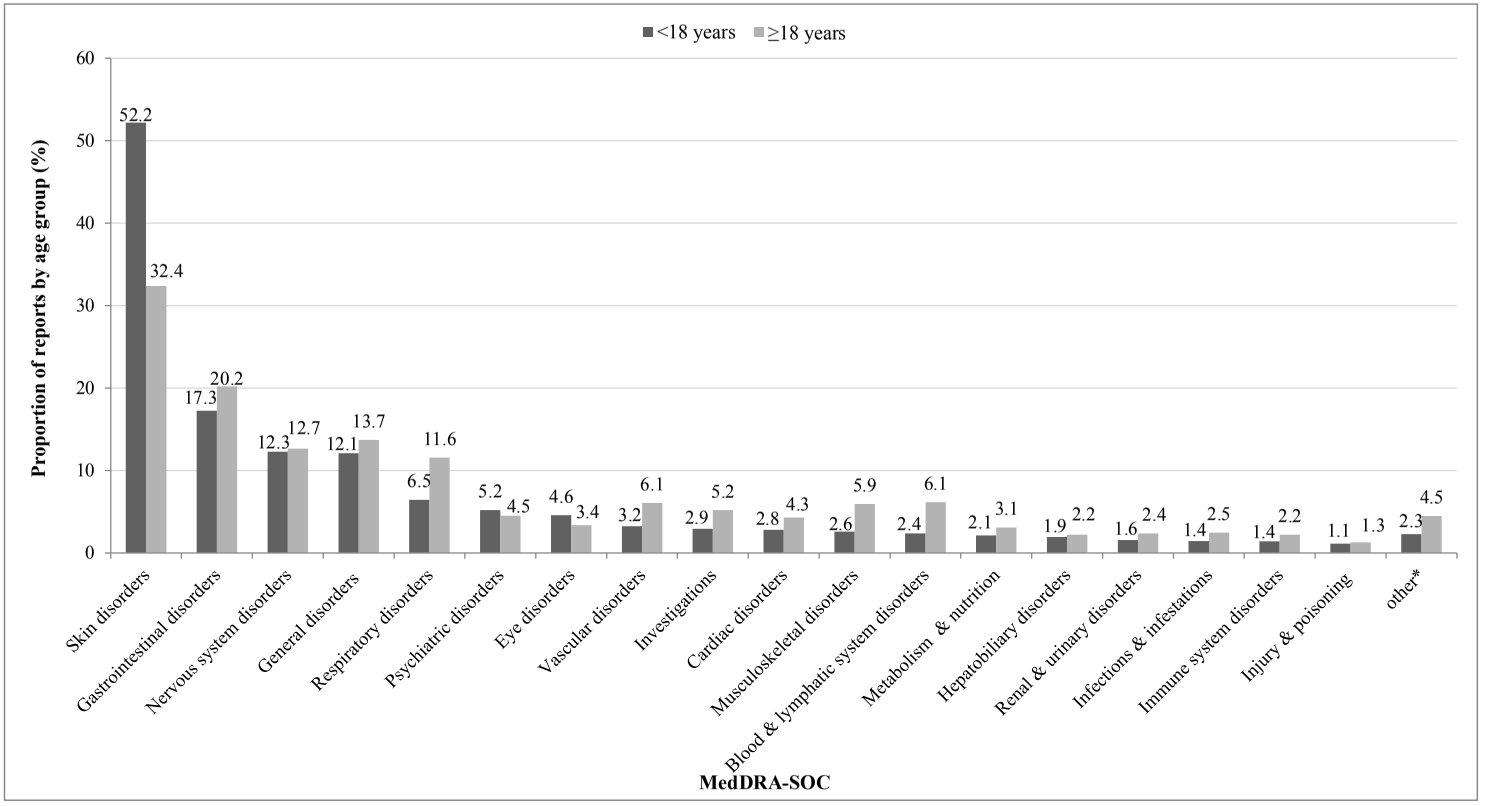
The three most frequently drug therapeutic classes (by second-level ATC) in relation to each ADR in terms of MedDRA-SOCs are stratified by the four paediatric age-categories (**Table 3**). The distribution of drug classes was comparable when we looked at skin reactions, gastrointestinal and general disorders, for which events "*antibiotics"* (ATC: J01), "*analgesics*" (N02, mainly acetaminophen),

Figure 1. Proportions of reports within each age group by drug class (first-level ATC)



Proportion is based on the number of the reports with the specific ATC within each age group (n=8338, in children, n= 114,791 in adults) within the ATC category (1st level). One report can describe one or more drug/event combination, thus one report can be counted in more than one ATC category. The figure is sorted out by descending order of the total number of paediatric reports per ATC.

Figure 2. Proportions of reports within each age group by System Organ Class (SOC).



Proportions on the number of the reports with the specific Medical Dictionary for Regulatory Activities MedDRA-SOC/total number of reports in the specific age group. (n=8338 in children; n= 114,791 in adults). One report can describe one or more drug/event combination, thus one report can be counted in more than one SOC. The figure is sorted in descending order of the total number of paediatric reports per SOC.

*Other includes the MedDRA-SOCs reported in less than 1% of paediatric reports (Ear & labyrinth disorders, Reproductive system & breast disorders, Endocrine disorders, Congenital, familial & genetic disorders, Neoplasm benign, malignant & unspecified, Surgical & medical procedures, Pregnancy, puerperium & perinatal conditions, and Social circumstances)

Table 3. Distribution of the most frequently reported drug groups stratified by SOC and age group

SOC (top 6 in each age class)	N. of ADRs *(% within each age category)**	Three most frequently reported therapeutic classes ATC 2nd level *(% within SOC in each age category)*
< 2 years (n= 1,679)		
Skin and subcutaneous tissue disorders	1,237 *(74)*	J01 *(70)*; N02 *(8)*; M01 *(5)*
Gastrointestinal disorders	324 *(19)*	J01 *(54)*; M01 *(9)*; L01 *(5)*
Nervous system disorders	216 *(13)*	A03 *(19)*; R06 *(12)*; J01 *(9)*
General disorders & administration site conditions	179 *(11)*	J01 *(37)*; L01 *(8)*; M01 *(7)*
Respiratory, thoracic & mediastinal disorders	121 *(7)*	R05 and J01 *(17)*; M01 *(8)*
Psychiatric disorders	100 *(6)*	R03 *(18)*; R06 (15); J01 (11)
2-5 years (n= 2,250)		
Skin and subcutaneous tissue disorders	1,756 (78)	J01 (*64*); M01 (9); N02 (6)
Gastrointestinal disorders	570 (25)	J01 (35); L01 (*22*); M01 (13)
General disorders & administration site conditions	339 (15)	J01 (*31*); L01 (*20*); N02 (*7*)
Nervous system disorders	253 (11)	R06 (15); J01 (*14*); A03 (13)
Psychiatric disorders	178 (8)	R03 (21); J01 (17); R06 (*14*)
Respiratory, thoracic & mediastinal disorders	148 (7)	J01 (26), M01 (12); L01 (11)
6-11 years (n= 2,062)		
Skin and subcutaneous tissue disorders	1,301 (63)	J01 (54); M01 (11); N02 (6)
Gastrointestinal disorders	633 (31)	J01 (30); L01 (22); M01 (14)
Nervous system disorders	383 (19)	A03 (*16*); J01 (14); L01 (10)
General disorders & administration site conditions	320 (16)	J01 (21); L01 (15); N03 (10)
Respiratory, thoracic & mediastinal disorders	191 (9)	J01 (31); L01(14); M01 (*9*)
Psychiatric disorders	189 (9)	N06 (14); R03 (13); N05 (11)
12-17 years (n= 2,347)		
Skin and subcutaneous tissue disorders	1,538 (66)	J01 (*51*); M01 (*13*); N02 (*9*)
Gastrointestinal disorders	689 (29)	J01 (*23*); L01 (20); M01 (*13*)
Nervous system disorders	469 (20)	A03 (*14*); N05 (*13*); J01 (11)
General disorders & administration site conditions	402 (17)	J01 (23); L01 (13); M01 (9)
Respiratory, thoracic & mediastinal disorders	210 (9)	J01 (*24*); M01 (11); L01 (10)
Psychiatric disorders	157 (7)	N05 (*25*); N06 (22); N03 (9)

Within each age group, the SOC are ranked according to the absolute number of reports with the specific SOC (TOP 6). For each SOC, the three most frequently involved therapeutic classes [ATC 2 level] are described.

"*anti-inflammatory drugs for systemic use*" (M01) and "*antineoplastic agents*" (L01) were uniformly the most frequently implicated agents. Small drug class variation occurred when exploring the ADRs related *to nervous and respiratory systems and psychiatric disorders*: "*drug for respiratory system use"* (R06, R05, R03) recorded in young children were replaced by *anti-convulsant* (N03) and *psycholeptic drugs* (N05) notified in older children and adolescents.

The single drug entities (fifth-level ATC) involved in 100 reports and over were notified in **Table 4**. Except for amoxicillin, with or without clavulanic acid, cef-

Table 4. Drugs* identified as suspected cause of paediatric ADRs

Drug	Paediatrics			Adults		
	N. of ADRs	N. of serious ADRs (%)#	Rank	N. of ADRs	N. of serious ADRs (%)#	Rank
Amoxicillin/Clavulanic Ac.	1,107	185 (17)	**1**	4,069	1,433 (35)	**1**
Amoxicillin	751	113 (15)	**2**	2,495	910 (36)	**4**
Ibuprofen	385	105 (27)	**3**	1,096	378 (34)	**21**
Acetaminophen	350	115 (33)	**4**	1,204	466 (39)	**17**
Clarithromycin	284	67 (24)	**5**	1,076	302 (28)	**22**
Ceftriaxone	264	100 (38)	**6**	1,646	885 (54)	**9**
Cefaclor	215	65 (30)	**7**	34	15 (44)	**508**
Ketoprofen	191	76 (40)	**8**	2,242	789 (35)	**6**
Cytarabine	173	54 (31)	**9**	154	123 (80)	**180**
Vincristine	173	61 (35)	**9**	169	105 (62)	**160**
Azithromycin	152	23 (15)	**10**	415	119 (29)	**70**
Cefixime	124	28 (23)	**11**	203	73 (36)	**134**
Methotrexate	111	34 (31)	**12**	525	268 (51)	**56**

*only drugs involved in ≥ 100 ADRs have been included in the tables. The total numbers of ADRs is higher than the total numbers of reports because each report may describe more than one drug/event combination.

#Proportion of the serious ADR is given by dividing the number of serious ADRs for the specific compound with the total number of ADRs for each compound.

triaxone and ketoprofen, the top twelve most frequently suspected drugs differed between children and adults. Concerning the seriousness, ADRs related to the same drug were likely to be more serious in adults than in children.

The twenty most frequently reported drug- event combinations in paediatric (active substance-MedDRA-SOC) are displayed in **Table 5**.

Based on these ADR reports, the AIFA, issued several regulatory actions to reduce the potential risk of ADR for such medicines as detected from the SRS during the last decade. The most important interventions, including restriction, caution, or contraindications for use in children are displayed on **Table 6**.

DISCUSSION

This is an exploratory study identifying the characteristics of the reports of ADRs in the Italian paediatric population during the years 2001-2012. We found that the number of the reports increased with age, reports in males were more frequent than females up to 11 years of age after which it reversed. Only one third of the total reports have been judged as serious with the majority of them requiring an hospitalization. However, two thirds of the reactions fully recovered or improved.

Table 5. Top twenty of most frequent paediatric drug-event combination (active substance-MedDRA-SOC)

Active substance	Reaction (MedDRA-SOC)	N. of combinations	% of all paediatric reports
Amoxicillin/clavulanic ac.	Skin and subcutaneous tissue disorders	765	6.6%
	Gastrointestinal system disorders	147	1.3%
	Body as a whole-general disorders	85	0.7%
Amoxicillin	Skin and subcutaneous tissue disorders	569	4.9%
	Gastrointestinal system disorders	61	0.5%
Acetaminophen	Skin and subcutaneous tissue disorders	208	1.8%
Ibuprofen	Skin and subcutaneous tissue disorders	198	1.7%
	Gastrointestinal system disorders	79	0.7%
	Body as a whole-general disorders	61	0.5%
Cefaclor	Skin and subcutaneous tissue disorders	195	1.7%
Ceftriaxone	Skin and subcutaneous tissue disorders	192	1.6%
Clarithromycin	Skin and subcutaneous tissue disorders	177	1.5%
Metoclopramide	Central & peripheral nervous system disorders	110	0.9%
Ketoprofen	Skin and subcutaneous tissue disorders	105	0.9%
Azithromycin	Skin and subcutaneous tissue disorders	81	0.7%
Cefixime	Skin and subcutaneous tissue disorders	80	0.7%
Domperidone	Central & peripheral nervous system disorders	58	0.5%
Vincristine	Gastrointestinal system disorders	56	0.5%
Montelukast	Psychiatric disorders	56	0.5%
Nimesulide	Skin and subcutaneous tissue disorders	54	0.5%

The types of reporter were heterogeneously distributed across age categories, with the highest proportion of the reports issued by hospital physicians while paediatricians accounted only for a smaller proportion (8%). Reports concerning anti-infective agents and drugs for respiratory system as suspected drugs and skin reactions as adverse event were proportionally higher in children compared with those in adults. At drug-event combination level, antibiotics, acetaminophen, anti-inflammatory drugs and antineoplastic agents were the mostly implicated drugs in skin and gastrointestinal adverse reactions consistently across all four paediatric age-categories.

Lombardy, Campania, Tuscany, Sicily and Veneto, which contributed the most of the reports concerning children to RNF, were the first regions that institutionalized a Regional Centre of Pharmacovigilance. These Centres allocated at regional level cooperate to the proper functioning of the National Pharmacovigilance System and provide for dissemination of knowledge about pharmacovigilance and training programmes for operators in that field[116, 117]. Then, within each Regions, reports were proportionally higher for Campania and Liguria compared

Table 6. Main regulatory actions issued by AIFA in children in the last decade.

Year	Suspected Drug (or active entity)	Reported Adverse Event (SOC or PT)	Action	Issued by
2004	Metoclopramide	NEUROLOGIC SIDE EFFECT (incl. extrapyramidal disorders)	Revision of SPCs/PIL: - contraindications for patients less than 16	*Nota Informativa Importante AIFA* (2004, March and 2014 Jan*)
	Niflumic acid	Mucocutaneous events	Revision of SPC/PIL: - Warning about the risk of serious mucocutaneous reactions (OR 2.3; 95% CI 1.2-3.7) in children less than 12 months; contraindicated in pregnancy	
2007	Nasal decongestant incl. sympathomymetics agents: - NARLISIM *rino drops* (inc, lysozyme-thonzylamine-phenylephrine) - RINOVIT PAIDO*cream* (inc. ephedrine)	SKIN DISORDERS (erythema), NERVOUS SYSTEM DISORDERS (unintentional muscle contractions, hypotonic-hyporesponsive episode, ataxia, drowsiness, lethargy, excitability), CARDIOVASCULAR SYSTEM (bradycardia), RESPIRATORY SYSTEM DISORDERS (dyspnea, pharyngeal oedema),	Reclassification and revision of the dispensing mode: - switch from over the counter pharmacy medicines to Medicines by GP prescription only; Revision of SPC/PIL: - contraindications for children less than 12 years;	*Nota Informativa Importante AIFA* (05/14/2009)
	Cefaclor	IMMUNE SYSTEM DISORDERS (inc. skin-allergic reactions)	Revision of SmPCs/PIL: - update on the Sections 4.4 "Special recommendations and caution" and 4.8 "Side effects"	
	Domperidone	Neurologic side effects	Recommendations about the risk of neurological effects.	AIFA Peadiatric Working Group
2008	Ceftriaxone	Convulsions/Anaphylactic shock	Recommendations from http://www.agenziafarmaco.gov.it/sites/defa and http://www.agenziafarmaco.gov.it/sites/default/files/ceftriaxone_e_shock_anafilattico_ok.pdf	BIF XVN.4 2008
	VISUMIDIATRIC*eye drops* (inc tropicamide/ phenilephrina)	Hypertensive crisis and acute pulmonary oedema in children	Revision of SmPCs/PIL: - Contraindications for children less than 12 years	AIFA *Nota Informativa Importante*, 2008, July
2009	TINSET (incl. Oxatomide)	SERIOUS CARDIAC SIDE EFFECTS in children due to dose medication error	Revision of SPC/PIL: - Removal of indication of use of pills in children; - Black-box Warning about the risk of overdose in children	AIFA *Nota Informativa Importante* (02/02/2009; 08/03/2010; 15/12/2010):
2011	NSAIDS AND Acetaminophen	Increased risk of overall ADR reporting in children from 0.7 (in 2005) to 1.7 (in 2010)/100,000, particularly for SKIN AND GASTROINTESTINAL DISORDERS	Recommendations about the correct use in children	AIFA Peadiatric Working Group
2012	Lamotrigine/Valproate	SKIN DISORDERS: Steven-Johnson And Lyell-Syndrome, Toxic Epidermal Necrolysis	Recommendations to avoid the overdose in paediatrics	

*EMA CHMP: 443003/2013 26 July 2013Jan 2014 publication of other actions on metoclopramide: EMA CHMP: 443003/2013 26 July 2013; EMA CHMP: 13239/201420 December 2013[1].recommendations to reduce dosage and duration (up to 5 days) of treatment to avoid serious neurological side effects.

with the overall reporting ADR. The presence of two major paediatric Italian hospital located in these Regions, which play a crucial geographical role for clinically relevance in children from the Southern- and Northern-Italy, respectively, is probably the most plausible explanation for the higher number of reports from these regions. However, since several local drug safety monitoring programmes started in the recent years, the reasons of the jeopardized reporting need to be investigated.

Our results are consistent with several previous studies exploring the spontaneous reports in children in other nationwide and worldwide networks[103-105, 108, 109, 118]. In our study, exactly in line with the results from VigiBase, we found that 52% of all reports in children were recorded for boys, mainly younger than 11 years[108]. However, this proportion reversed from 12 years onward, where girls showed the higher number of reports. Several factors may explain these findings. First of all, the incidence and prevalence of some childhood diseases, i.e. asthma[119], certain infections[120], ADHD[121], are greater among younger boys than girls; afterwards, during the adolescence the incidence changes, mainly for asthma and urinary tract infections, with greater occurrence among girls than boys. Alternatively, among children, younger boys and older girls could be physiologically vulnerable to ADR occurrence. Irrespective of the gender, we found an overall increase of the absolute number of reports with age increasing that could be attributed to a greater exposure to the medications in older children[122].

There is an expected variation between age-categories in the type of reporter. With increasing age, the decrease of the proportion of the ADRs notified by FPs and professional figures from the Poison Control Centres is counterbalanced by the increase of those recorded by hospital physicians, specialists and GPs. This is not surprising because Italian children receive routinely medical care by FPs up to the age of 14 years, and thereafter by GPs and are being inspected and monitored systematically during growth by the paediatrician[99]. On the other hand, we would have expected a greater number of reports issued by paediatricians, because of their crucial role in child's healthcare. In order to increase their awareness of drug safety monitoring, specific learning programs or software should be adopted to facilitate the ADR reporting. Moreover, childhood, especially younger than 5 years of age, is at particular risk of drug poisoning, either accidental or as resulting from therapeutic error[123, 124], uniformly with the greater proportion of ADRs recorded by Poison Control Centres in children less than 5 years of age.

The distribution of the reports in terms of drugs and events is not surprising and in line with the other SRSs. The highest proportion of reports including anti-infective agents and respiratory system drugs compared to the adults is likely to reflect the fact that these are the most frequently prescribed drugs, excluded

vaccines, in overall paediatric age-categories in Italy as well as in other European countries[122]. In fact, compared with the adulthood disease, infections and asthma are more prevalent childhood diseases than cardiovascular disorders. Diseases' pattern changes with increasing age, resulting in different drug prescribing and, consecutively, different type of ADRs. Consistently, we found a replacement of reports concerning drugs for respiratory tract with reports including drugs for nervous system from babies to adolescents.

As previously described[103, 104, 106-109], adverse skin reactions are more frequently reported in children than in adults, which could be attributed to underlying causes. Indeed, skin physiology is different in children compared to adults[125], potentially causing to a greater susceptibility to skin reactions. On the other hand, as confirmed by our analysis, allergic reactions are well-known to be induced by antibiotics use[126]. These findings need to be interpreted considering that special characteristics related to the different stage of growth and development could explain the differences in terms of drugs and adverse events observed across age.

Consistently, at single entity-level, the mostly reported medications are not similar among children and adults. Moreover, when referring to same suspected drug, ADRs are more serious in adults than in children. Thus, these findings emphasize the need of analyses stratified within specific subgroup populations to increase the sensitivity of the signal detection procedure in children. Overall, the characteristics of paediatric reports from Italian SRS overlap those from the other nationwide and worldwide SRSs, showing a very similar and collaborative approach on pharmacovigilance rules across countries. However, small differences across countries could reflect differences in drug use or country-specific active surveillance initiatives. Definitely, while a worldwide SRS could provide for a larger and heterogeneous data sources enough powerful to detect new signals of rare event and low exposure drug, nationwide systems could offer a smaller but better qualified data sources reflecting local healthcare policy, such as drug prescription trends and specific initiatives to stimulate the ADR surveillance.

This present overview of the ADRs in children cumulatively reported to RNF, therefore, increases knowledge about the structure and the scope of this database and its respective strengths and limitations, that is essential for its correct use and interpretation, as first step for evaluating new signals. The system allows routine analysis of the safety of drugs in children including an in-depth analysis of new reports received on a bi-yearly basis in a signal detection activities at the AIFA with the Regional Centres of Pharmacovigilance. As part of this activity, whenever needed, AIFA lunches regulatory actions, addressed to healthcare professionals "*Dear Doctor Letters*", including restrictions regarding the distribution and reimbursement of drugs, revision of the Summary of Product Characteristics/Patient

Information Leaflet (SmPCs/PIL) adding warning or caution of use, or, rarely, withdrawal from the market of incriminated medicines. Noteworthy, over the last decade several issued regulatory agency measures in children related some of the 20 most frequently reported drug-event combinations, such as the restriction of combined use of NSAIDs (ibuprofen and ketoprofen) and acetaminophen, or the warning about the neurologic effects induced by metoclopramide and domperidone. Of interest in Italy, already in 2004, AIFA contraindicated the use of metoclopramide in children less than the age of 16, according to the evidence of an increased risk to develop neurological events, with extrapyramidal disorders, in this age-group population[127], in line with the increased trend of number of ADRs in RNF. In February 2007, the Dutch Medicines Evaluation Board also restricted paediatric indications of metoclopramide because of the risk of developing extra-pyramidal disorders. More recently, in October 2013, after a formal evaluation of metoclopramide, as requested by French Medicines Regulatory Agency, following safety concerns over side effects and efficacy, EMA Committee on Medicinal Products for Human Use (CHMP) recommended that metoclopramide should not be used in children <1 year of age and that in children > 1 year of age should only be used as second-choice prevent treatment of delayed nausea and vomiting after chemotherapy and as treatment for post-operative nausea and vomiting[115].

Limitations

Post-marketing surveillance through SRS is sensitive and capable to quickly identify side effects after market-launch, particularly rare, unpredictable or serious reactions[128]. The system reflects both real-life events and real-life drug use, included drug use patterns that cannot be studied in clinical trials for ethical reasons and cannot be recorded in electronic healthcare registries because of their limited secondary care use. These data has also limitations:

- ✓ the number of reports can vary in time and space due to several reasons that include a different attitude to the reporting activities;
- ✓ set up of regional or local projects of active Pharmacovigilance;
- ✓ the presence or the lack of hospitals for children in the area;
- ✓ the frequency (volume) of the reports for any individual drug might be influenced by the length of time on the market;
- ✓ no information on the severity of underlying illness;
- ✓ the public attention to specific safety issues (i.e. post-marketing surveillance activities);
- ✓ if the adverse event was already known for the drug label;
- ✓ the lack of the knowledge of the real exposure.

Furthermore in the comparison of the ADRs between children and adults many other aspects need to be taken into account; the percentage of serious adverse reactions could be explained by the attitude to report also less severe reactions in children, by the concomitant use of more drug in adults (especially in elderly), to possible organ impairment in adults suffering from concomitant diseases.

For all these reasons, when using passive systems for signal detection or hypothesis testing, the investigators need to apply several approaches and different tools in order to address specific bias and confounding factors, nevertheless the spontaneous reporting system has own value in the monitoring especially for those population not enough studies (i.e. paediatric and elderly patients)[129-131].

CONCLUSION

This study provides an overview of reported ADRs in paediatric population and a frame of reference for additional research on specific drugs in relation to their prescribing patterns. The characteristics of paediatric reports on Italian spontaneous reporting database are consistent with those from the other nationwide and worldwide spontaneous reporting systems.

Monitoring of ADRs for signal detection analysis is generally performed considering reports related to the entire population. Accordingly to the Guideline on conduct of pharmacovigilance for medicines used by the paediatric population[132], our results stressed the need for using specific age-group setting when performing signal detection or validation analyses on children, in light of observed differences in the frequency and the type of reports between children and adults.

Appendix 1. Distribution of suspected paediatric ADR reports by Italian Regions.

Region	Total reports n= 123,426	N. of Paediatrics reports (% on total paediatric ADR reports)	(% of total reports within each region)*
Lombardy	44,749	3,311 (39.7)	7.4%
Campania	6,015	1,096 (13.1)	18.2%
Tuscany	15,002	803 (9.6)	5.4%
Sicily	5,020	507 (6.1)	10.1%
Veneto	12,327	488 (5.9)	4.0%
Emilia Romagna	8,834	330 (4.0)	3.7%
Liguria	2,206	323 (3.9)	14.6%
Puglia	4,043	285 (3.4)	7.0%
Lazio	5,052	224 (2.7)	4.4%
Sardinia	2,419	198 (2.4)	8.2%
Piedmont	6,233	197 (2.4)	3.2%
Trento PA	1,283	139 (1.7)	10.8%
Friuli Venetia Giulia	2,165	93 (1.1)	4.3%
Marche	2,019	81 (1.0)	4.0%
Abruzzo	1,516	67 (0.8)	4.4%
Calabria	1,191	57 (0.7)	4.8%
Basilicata	1,107	50 (0.6)	4.5%
Umbria	1,056	34 (0.4)	3.2%
Molise	318	13 (0.2)	4.1%
Bolzano PA	451	12 (0.1)	2.7%
Valle d'Aosta	118	2 (0.0)	1.7%
NA	302	28 (0.3)	9.3%

2.2. Paediatric Drug Safety Surveillance in FDA-AERS, a description of Adverse Events: a GRiP Study

Submitted for publication

ABSTRACT

Objective

Individual case safety reports (ICSRs) are a cornerstone in drug safety surveillance. The knowledge on using these data specifically for children is limited. Therefore we studied characteristics of paediatric ICSRs reported to the US FDA Adverse Event Reporting System (FAERS).

Methods

Public available ICSRs reported in children (0-18 years) to FAERS were downloaded from the FDA-website for the period Jan 2004-Dec 2011. Characteristics of these ICSRs, including the reported drugs and events, were described and stratified by age-groups.

Results

We included 106,122 paediatric ICSRs (55% boys and 58% from US) with a median of 1 drug [range 0-157] and 3 events [1-94] per ICSR. Mean age was 9.1 years. 90% was submitted through expedited (15-days, 65%) or periodic reporting (25%) and 10% by non-manufacturers. The proportion and type of paediatric ICSRs reported were relatively stable over time. Most commonly reported drug classes by decreasing frequency were '*neurological*' (58%), '*antineoplastic*' (32%) and '*anti-infectives*' (25%). Most commonly reported SOCs were '*general*' (13%), '*nervous system*' (12%) and '*psychiatric*' (11%) disorders. Duration of use could be calculated for 19.7% of the reported drugs, of which 14.5% concerned drugs being used long-term (>6 months).

Conclusion

Knowledge on the distribution of the drug classes and events within FAERS is a key first step in developing paediatric specific methods for drug safety surveillance. Analysis of the reported drugs indicate disproportionate safety reporting of neurological/psychiatric and antineoplastic agents. Studying multiple databases is useful because of worldwide differences in drug utilization and type of reports.

BACKGROUND

The limited knowledge about the effects of drugs in children has boosted initiatives by the WHO and triggered new legislation in recent years[95, 133]. The Global Research in Paediatrics Network of Excellence (GRiP) is an EU-funded consortium, which aims to implement an infrastructure facilitating the development and safe use of medicines in children. This entails the development of a comprehensive educational programme and integrated use of existing research capacity, whilst reducing the fragmentation and duplication of efforts[134, 135].

Post-marketing drug safety surveillance using spontaneous reporting systems is essential in studying drug safety[100]. An important part of the GRiP project is evaluating current and developing new methodology for post-marketing drug safety studies specifically for the paediatric population. Typical large spontaneous reporting systems include VigiBase of the WHO-UMC, the FAERS, maintained by US FDA, the Vaccine Adverse Effect Reporting System (VAERS), maintained by FDA and CDC (Centres for Disease Control and Prevention), and EudraVigilance of the EMA[136-138].

While these spontaneous reporting databases were predominantly used by regulatory authorities to monitor drug safety and to perform safety signal detection, these data are increasingly available for research purposes. The FDA databases offer publicly downloadable datasets[137, 138], while the EMA published their access policy for EudraVigilance in 2011[139], and WHO-UMC is preparing summary VigiBase data to be made accessible via their website[140]. Understanding the structure and scope of these datasets and their respective strengths and limitations is essential for their correct use and interpretation and a first and important step for evaluating current and developing new methodology. In 2011 an overview of paediatric ICSRs reported to WHO-UMC was published[108]. Published descriptions on the paediatric reports within FAERS include the number of reports, their outcome, and the most frequently reported drugs[141-143]. However, studies rarely investigated the reported AEs within FAERS.

In the current study we aimed to describe the paediatric ICSRs as reported within FAERS. Specific attention was given to describing AEs occurring after long-term drug use or with delayed onset after cessation of treatment.

METHODS

Data source

FAERS is a database that contains information on AE and medication error reports submitted to FDA. It is a passive surveillance system that relies on voluntary reporting by healthcare professionals and consumers, as well as required reporting by pharmaceutical manufacturers. FAERS includes spontaneous reports from US sources; serious and unlabelled spontaneous reports from non-US sources; and serious, unlabelled, and attributable post-marketing clinical trial reports from all sources[25].

FAERS data is publicly available and files containing the raw data of ICSRs as contained within the database are available[25]. The information include: patient demographic and administrative information; drug/biologic information; preferred terms-MedDRA® of the events; patient outcomes for the event; report sources for the event; therapy start dates and end dates; and indications of use (diagnosis) for the reported drugs.

Data preparation

We extracted all ICSRs for the period January 2004 till December 2011 and included all ICSRs on children, aged <18 years. We excluded the following reports from the analyses; adults reports (≥18 years); reports in which the age was missing; reports in which the reported drug or event was missing; duplicate reports (e.g. in a follow-up report, were only included once). All reported events are coded in PT-MedDRA. To facilitate high level descriptive we recoded the reported terms to a single SOCs of MedDRA. The reported drugs are described either as a valid trade name or as unstructured narrative. As far as possible the reported drug names were recoded to ATC drug classes using drug dictionaries[144-147]. This recoding was possible for >90% of the reported drugs reported in the selected ICSRs. The entries for which recoding was not possible included reports without a specified drug name, spelling errors. For the analyses on type of reported drugs, only those records with a known ATC-code were included.

Data analysis

Each of the included ICSRs was classified by the age at time of the event, sex, number of drugs and number of events. Results were stratified by age-categories in which age at onset was stratified based on the ICH (International Conference On Harmonisation) age-groups: neonates (≤27 days), infants (28 days-≤23 months), children (2-≤11 years), and adolescents (12-≤17 years)[148].

The role of the reported drugs, being either primary suspect, secondary suspect, concomitant or interacting was provided. The most frequently reported drugs and events were described. In addition, the reported events were stratified with respect to the outcome of the event. The outcome was registered in terms of the seriousness criteria: death, life-threatening, hospitalization (initial or prolonged), disability, congenital anomaly, requiring intervention to prevent permanent impairment or damage or other. Using the primary suspect and secondary suspect drugs only, the most frequent reported drug-event combinations were described.

For those drugs for which the starting date of the drug and the date of the event were known, the time to event was calculated. An event occurred after *long-term use* if it occurred at least 6 months after starting of therapy[149]. We also studied *delayed events*. For those records with a known stopping date of therapy and date of the event, the type of reported events occurring >3 months after drug cessation were compared with the type of events occurring during drug use.

Characteristics of the ICSRs were compared using chi-square to compare proportions and either students-t test, if variable was normally distributed or Mann-Whitney tests if the variable was not normally distributed to compare means. A *p*-value <0.05 was considered to be statistical significant.

RESULTS

The overall publicly available dataset of FAERS included 3,691,417 ICSRs; 106,122 (2.9%) ICSRs occurred in children 0-<18 years and were included in the analyses. The mean age of the children in these reports was 9.1 years. 10.5% of the ICSRs were on children up to one year of age, after which this decreased to 3.6% at 4 to 5 years of age and gradually increased again to 8.9% at 17-18 years of age (**Figure 1**).

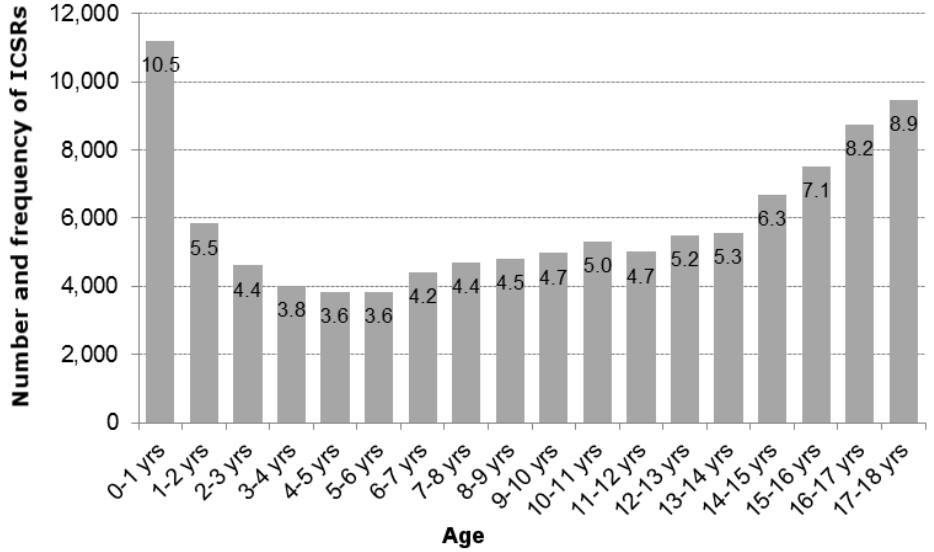
The majority of the ICSRs (54.5%) were reported for boys, reports for boys exceeded those for girls up to the age of 11 years (54.1-59.9%) and this reversed from the age of 12 years onwards (47.7%), the mean age in the female reports was higher than for male reports (*p*=0.000) (**Table 1**).

The number of ICSRs reported by calendar year is increasing, with a small dip in 2010 (**Figure 2**).

The majority of the reports originated from the US (58%), followed by Japan (7.0%) and the United Kingdom (6.6%).

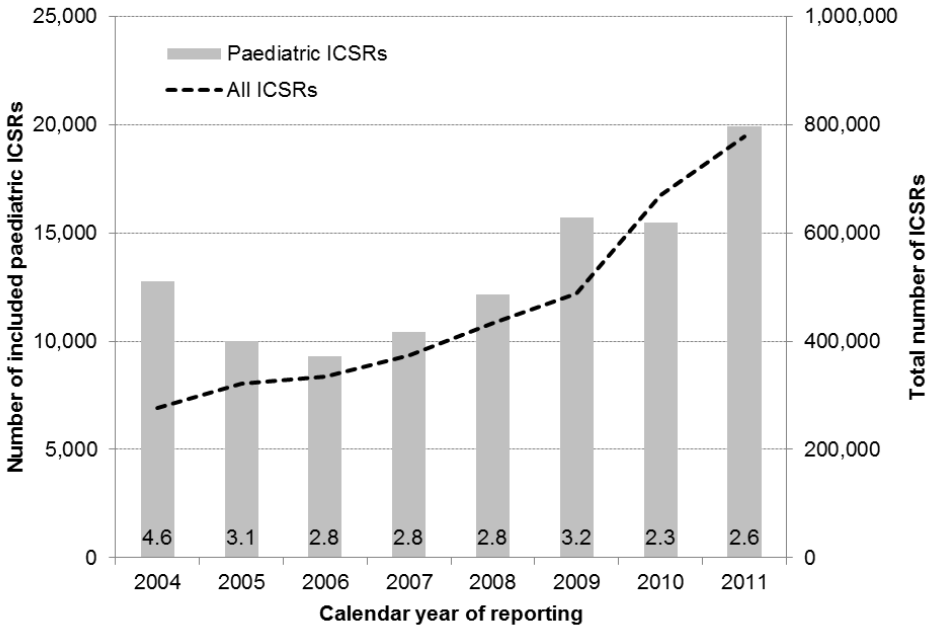
Of the included ICSRs, 10.0% (N=10,576) concerned "direct" reporting, defined as ICSRs being voluntary submitted by "non-manufacturers". The majority of the ICSRs were submitted by manufacturers; 64.9% (n= 68,886) were expedited reports and 25.1% (n= 26,660) were periodic reports (**Table 1**). The reporter was a

Figure 1. Number of reported ICSRs by age.



Number of reported ICSRs by age. Within the bars the proportion of ICSRs within this age-stratum of the total reported paediatric ICSRs is given.

Figure 2. Number of reported ICSRs over time.



Number of reported ICSRs over time. The number of included paediatric ICSRs are plotted on the left y-axis. The total number of ICSRs within the database are plotted on the right y-axis. Within the bars the proportion of paediatric ICSRs of the total reported ICSRs is given.

Table 1. General characteristics of paediatric ICSRs (n= 106,122) within FAERS

Number of included paediatric ICSRs	**n (%)**	**Males**
0 to 27 days	4,717 (4.4%)	2,114 (54.1%)
28 days to 23 months	16,096 (15.2%)	7,921 (55.3%)
2 to 11 years	47,248 (44.5%)	27,075 (59.9%)
12-17 years	38,061 (35.9%)	17,658 (47.7%)
Total	106,122 (100%)	54,768 (54.5%)
Mean age [mean(95%CI)]		
Male	8.9 (95%CI 8.8-8.9)	
Female	9.7 (95% CI 9.6-9.8)	
Total	9.1 (95%CI 9.0-9.1)	
Number of reported drugs	**Total no. of drugs n (%)**	**No. of drugs/ICSR Median(range)**
0 to 27 days	12,180 (5.2%)	1 (0-36)
28 days to 23 months	34,575 (14.6%)	1 (0-55)
2 to 11 years	103,988 (44.0%)	1 (0-75)
12-17 years	85,748 (36.3%)	1 (0-61)
Total	236,491 (100%)	1 (0-157)
Number of reported events	**Total no. of events n (%)**	**No. of events/ICSR Median(range)**
0 to 27 days	21,265 (5.4%)	1 (1-15)
28 days to 23 months	59,306 (14.9%)	1 (1-15)
2 to 11 years	173,395 (43.7%)	1 (1-19)
12-17 years	143,254 (36.1%)	1 (1-30)
Total	397,220 (100%)	3 (1-94)
Type of report	**n (%)**	
Direct reporting	10,576 (10.0%)	
Expedited reports (“15 day reports”)	68,886 (64.9%)	
Periodic reports	26,660 (25.1%)	
Reporter	**n (%)**	
Physician	33,990 (32.0)	
Consumer/non-health professional	26,378 (24.9)	
Other health professional	21,193 (20.0)	
Pharmacist	6,159 (5.8)	
Lawyer	1,301 (1.2)	
Unspecified	17,101 (16.1)	
Initial source	**n (%)**	
Foreign	10,290 (9.7%)	
Study	164 (0.2%)	
Literature	882 (0.8%)	
Consumer	10,123 (9.5%)	
Health Professional	11,196 (10.6%)	
User Facility	14 (0.0%)	
Company representative	3,964 (3.7%)	
Distributor	229 (0.2%)	
Other	554 (0.5%)	
Unknown	68,706 (64.7%)	
Country	**n (%)**	
United States	50,625 (47.7%)	
Japan	6,119 (5.8%)	
United Kingdom	5,722 (5.4%)	
France	4,656 (4.4%)	
Germany	2,758 (2.6%)	
Unknown	18,827 (17.7%)	

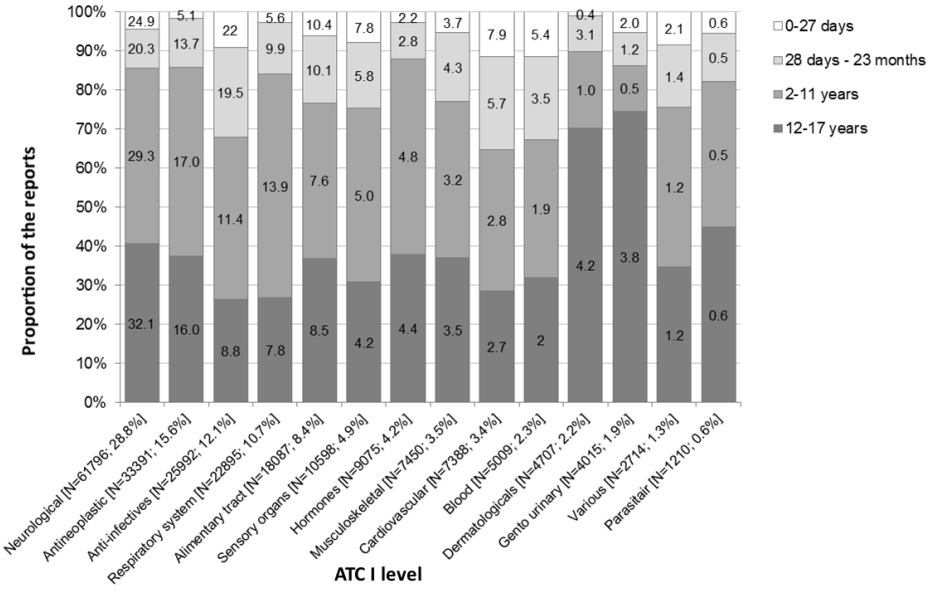
physician in 32.0% of the ICSRs, a consumer in 24.9% and another health professional in 20.0%.

The ICSRs comprised a total of 236,491 drug records (median 1 drug/ICSR) (**Table 1**). Of these, 35% were indicated as the primary suspected drug and 21%as secondary suspected drug. The other drugs were either indicated as concomitant (45%) or interacting (0.3%).

"*Nervous system drugs*" were the most frequently reported drug class in all age-categories (**Figure 3**). "*Anti-infective agents*" were an important group of the reported drugs for the youngest children, covering 22% of the reported drugs in children ≤27 days of age (e.g. "*antiretroviral drugs*" and "*antibiotics*") and 19.5% of the drugs in children aged 28 days to 23 months (e.g. "*specific immunoglobulins*" and "*antibiotics*"). In the older children the "*anti-infective agents*" covered a smaller proportion of the drugs (11.4% in children aged 2-11 years and 8.8% in adolescents aged 12-17 years) and "*antineoplastic drugs*" became of more importance (17% in children aged 2-11 years and 16% in adolescents aged 12-17 years).

The ICSRs comprised a total 397,220 event records (median 3 events/ICSR). (**Table 1**). The outcome in terms of seriousness criteria was: 33% hospitalization (initial or prolonged); 12% death; 3% life-threatening; 3% disability; 2% congenital

Figure 3. Number of reported drugs by anatomical main group



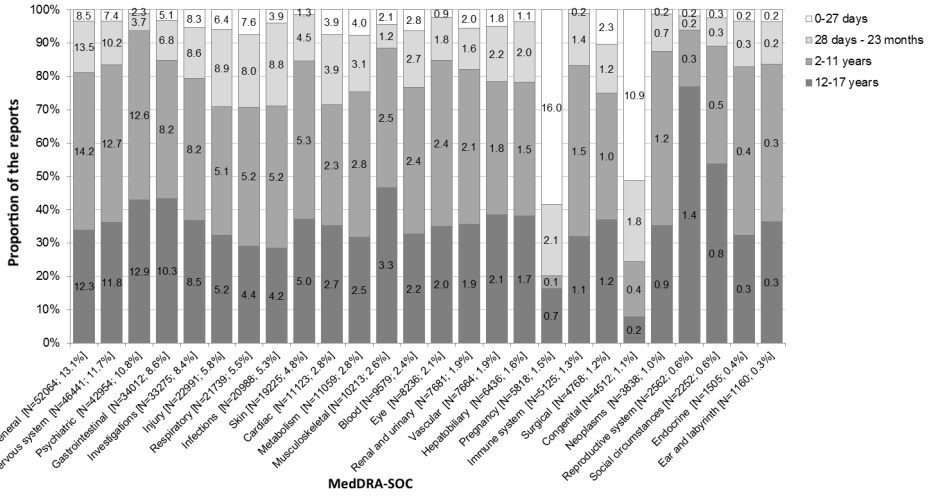
Distribution of the number of reported drugs over anatomical main group (1st level ATC), stratified by age-categories. The reported ATC classes are presented at the X-axis including the number of reports and the percentage of total. On the y-axis the distribution of the age-categories within each ATC class is presented, counting up to 100%. Within the bars the proportion of this ATC class within the total number of reported drugs within the specified age-category is presented. Only those drugs with a recoded ATC code are included (N=214,327).

anomaly; 1% required intervention to prevent permanent impairment or damage; 31% other and was missing in 15%. In **Figure 4** the reported events are stratified by their SOCs and by age-categories and the ten most frequently reported events are presented in **Table 2**. The reported events were most frequently situated in the SOCs "*General disorders and administration site conditions*" (13%) (e.g. vomiting and pyrexia), "*Nervous system disorders*" (12%) (e.g. convulsion and headache), and "*Psychiatric disorders*" (11%) (e.g. abnormal behaviour and aggression). In the youngest group of children "*Pregnancy, puerperium and perinatal conditions*" (16%) and "*Congenital, familial and genetic disorders*" (11%) covered a large part of the reported events (drug exposure during pregnancy, premature baby and maternal drugs affecting foetus). The proportion of "*Psychiatric disorders*" increased with age from 5% at ≤27 days of age to 13% at 12-17 years of age. Also reporting of "*Nervous system disorders*" increased with age, incrementing from 7.4% at ≤27 days of age to 11.8-12.7% at 2-17 years of age.

The number of reported drug-event pairs, calculated using the primary and secondary suspected drugs only, are displayed in **Table 3**. The number of unique drug-event combinations was 180,100 and within the age groups: 2,606 (≤27 days); 14,800 (28 days-23 months); 62,788 (2-11 years); 59,101 (12-17 years).

Duration of drug use could be calculated for 63,311 drug records (26.8%). The median duration of use was 10 days (range 0-6,209). The starting and event date

Figure 4. Number of reported event by system organ classes



Distribution of the number of reported events over system organ classes (SOCs), stratified by age-categories. The reported SOCs are presented at the X-axis including the number of reports and the percentage of total. On the y-axis the distribution of the age-categories within each SOC is presented, counting up to 100%. Within the bars the proportion of this SOC within the total number of reported events within the specified age-category is presented.

Table 2. Most frequently reported events

≤27 days (n= 21,265)		28 days - 23 months (n= 59,306)		2-11 years (n= 173,395)		12-17 years (n= 143,254)		Total (n= 397,220)	
AE	**n (%)**	**AE**	**n (%)**	**AE**	**n (%)**	**AE**	**n (%)**	**AE**	**n (%)**
Drug Exposure During Pregnancy	1,350 (6.3%)	Pyrexia	1,068 (1.8%)	Vomiting	2,818 (1.6%)	Vomiting	1,878 (1.3%)	Vomiting	5,827 (1.5%)
Premature Baby	562 (2.6%)	Vomiting	1,046 (1.8%)	Pyrexia	2,425 (1.4%)	Headache	1,747 (1.2%)	Pyrexia	4,880 (1.2%)
Maternal Drugs Affecting Foetus	484 (2.3%)	Convulsion	895 (1.5%)	Drug Ineffective	2,394 (1.4%)	Nausea	1,641 (1.1%)	Convulsion	4,720 (1.2%)
Neonatal Disorder	381 (1.8%)	Accidental Drug Intake By Child	789 (1.3%)	Convulsion	2,334 (1.3%)	Depression	1,581 (1.1%)	Drug Ineffective	4,392 (1.1%)
Drug Withdrawal Syndrome Neon.	333 (1.6%)	Diarrhoea	645 (1.1%)	Abnormal Behaviour	2,261 (1.3%)	Convulsion	1,399 (1.0%)	Headache	3,531 (0.9%)
Caesarean Section	328 (1.5%)	Accidental Exposure	622 (1.0%)	Aggression	1,755 (1.0%)	Drug Ineffective	1,365 (1.0%)	Abnormal Behaviour	3,264 (0.8%)
Maternal Exp. During Pregnancy	158 (0.7%)	Drug Ineffective	548 (0.9%)	Headache	1,733 (1.0%)	Pyrexia	1,322 (0.9%)	Nausea	3,093 (0.8%)
Neonatal Resp. Distress Syndr.	140 (0.7%)	Somnolence	544 (0.9%)	Somnolence	1,392 (0.8%)	Suicidal Ideation	1,140 (0.8%)	Somnolence	2,903 (0.7%)
Drug Exposure Via Breast Milk	134 (0.6%)	Product Quality Issue	541 (0.9%)	Nausea	1,330 (0.8%)	Overdose	1,118 (0.8%)	Overdose	2,713 (0.7%)
Patent Ductus Arteriosus	122 (0.6%)	Overdose	540 (0.9%)	Product Quality Issue	1,238 (0.7%)	Suicide Attempt	1,113 (0.8%)	Aggression	2,710 (0.7%)

Table 3. Most frequently reported drug-ADR combinations (Primary and Secondary suspected drugs only)

0-27 days (n= 3,274)		28 days - 23 months (n= 21,356)		2-11 years (total n= 146,094)		12-17 years (n =129,699)		Total (n= 548,640)	
Combination	**n (%)**	**Combination**	**n (%)**	**Combination**	**n (%)**	**Combination**	**n (%)**	**Combination**	**n (%)**
Buprenorphine – Drug withdrawal syndrome neonatal	32 (1.0%)	Valproate - Drug exposure during pregnancy	84 (0.4%)	Atomoxetine –Prescribed overdose	473 (0.3%)	Isotretinoin - Depression	472 (0.4%)	Isotretinoin - Depression	669 (0.1%)
Heparin - Maternal drugs affecting foetus	29 (0.9%)	Fluoxetine- Drug exposure during pregnancy	49 (0.2%)	Atomoxetine – Drug Ineffective	462 (0.3%)	Isotretinoin -Inflammatory bowel disease	337 (0.3%)	Atomoxetine – Drug Ineffective	664 (0.1%)
Heparin - Premature baby	21 (0.6%)	Valproate – Foetal anticonvulsant syndrome	40 (0.2%)	Atomoxetine –Abnormal behaviour	396 (0.3%)	Isotretinoin -Colitis Ulcerosa	257 (0.2%)	Atomoxetine –Prescribed overdose	654 (0.1%)
Levetiracetam - Maternal drugs affecting foetus	17 (0.5%)	Olanzapine - Drug exposure during pregnancy	39 (0.2%)	Methylphenidate –Product quality issue	393 (0.3%)	Isotretinoin -Crohn's disease	234 (0.2%)	Atomoxetine –Abnormal behaviour	579 (0.1%)
Heparin – Caesarean section	14 (0.4%)	Fentanyl – Accidental drug intake by child	38 (0.2%)	Atomoxetine – Somnolence	356 (0.2%)	Isotretinoin -Suicidal ideation	225 (0.2%)	Methylphenidate –Product quality issue	573 (0.1%)

Presented proportion are based on the total number of "primary suspect" and "secondary suspect" reported drugs. The number of unique drug-event combinations was 180,100 and within the age groups: 2,606 (0-27 days); 14,800 (28 days-23 months); 62,788 (2-11 years); 59,101 (12-17 years).

were equal in 28.4% of the records, 19.1% were reported after 1-7 days since starting. Time to event was 8-30 days in 14.4% of the records, 31-182 days in 17.8% and >182 days (defined as long-term use) in 20.2% of the records. The proportion of drugs being used long-term increased with age: 0.0% (≤27 days); 7.8% (28 days-23 months); 22.0% (2-11 years) and 24.1% (12-17 years). The drugs used long-term use more often concerned drugs within the drug groups '*Systemical hormonal preparations*' (10.1% *vs*. 2.9%; *P* <0.01), '*Alimentary drugs*' (10.8% *vs.* 5.8%; *P* <0.01) and '*Antineoplastic and immunomodulating agents*' (16.5% *vs*. 13.9%; *P* <0.01). The most frequently reported drugs after long-term use were '*Somatropin*' (n= 955; 7.5%), '*Atomoxetine*' (n= 507; 4.0%), and '*Methylphenidate*' (n= 462; 3.6%). Also the type of reported events differed; events within the SOCs '*Neoplasms benign and malignant*' (2.1% *vs*. 0.5%; *P* <0.01), '*Infections and infestations*' (7.0% vs. 4.8%; *P* <0.01) and '*Musculoskeletal, connective tissue and bone disorders*' (3.8% vs. 2.3%; *P* <0.01) were more often reported after long-term use. The most frequently reported events after long-term use were '*Vomiting*' (n= 415; 1.2%), '*Convulsion*' (n= 412; 1.2%), and '*Pyrexia*' (n= 389; 1.2%) (data not shown).

For 47,301 drug records (20.0%) the time between ceasing of therapy and occurrence of the event was known. The event occurred prior to stopping of the drug in 42.1% of the records, on the day of stopping of the drug in 31.5% and after stopping of therapy in 26.4%. Of the drugs occurring after stopping therapy, 45.2% occurred within 1-7 days, 27.0% occurred within 8-30 days, 11.3% within 31-90 days, 5.4% within 91-182 days, 5.1% within 183-365 days and 6.0% after 365 days. When comparing the drug classes reported >90 days of ceasing of therapy with those drug classes reported during drug use, the largest differences, with higher proportion for delayed effects, were present for '*Antineoplastic and immunomodulating agents*' (26.6% *vs.* 9.9%; *P* <0.01), '*Dermatologicals*' (10.8% *vs.* 5.1%; *P* <0.01), and '*Hormones*' (6.1% *vs.* 3.7%; *P* <0.01). The most frequently reported drugs after delayed use (>3 months) were '*Isotretinoin*' (n= 184; 8.8%), '*Palivizumab*' (n= 95; 4.5%), and '*Infliximab*' (n= 75; 3.6%). '*Neoplasms benign and malignant*' (2.9% *vs.* 0.6%; *P* <0.01), '*Gastrointestinal disorders*' (14.8% *vs.* 9.8%; *P* <0.01), and *'Infections and infestations*' (7.5% *vs*. 4.6%; *P* <0.01) were more frequently SOCs reported 3 months after stopping. The most frequently reported delayed events were '*Crohn's disease*' (n= 106; 1.7%), '*Inflammatory bowel disease*' (n= 98; 1.6%), and '*Depression*' (n= 89; 1.4%) (data not shown).

DISCUSSION

Signal detection within spontaneous reporting databases is the first step in the detection of a safety signal, which may be followed by signal prioritization and evaluation[150]. The GRiP network aims to create an infrastructure that supports this workflow and active safety surveillance in children. Knowledge about the available data in systems as FAERS is a key first step in the development of paediatric specific methodology for post-marketing drug safety studies. Signal detection is influenced by the type of ICSRs that are reported, in a previous study we demonstrated the influence of the proportion of vaccines within VigiBase on the sensitivity of data mining algorithms[151]. Also the distribution of other factors including the type of reporter and year of reporting can influence the results. Knowledge on the difference in the distribution of reported drugs and events within the different databases, like FAERS and VigiBase, gives insight on which factors might be of influence on the results but also helps choosing the right database for a specific research hypothesis.

The paediatric ICSRs reported within VigiBase were studied by Star *et al*[108]. Although US reports make up the largest proportion of the ICSRs both within FAERS (58%) and within VigiBase (39%), striking differences between the datasets are present. First, the type of reporters differed. While more than half of the ICSRs of VigiBase were reported by physicians, only a third of the FAERS ICSRs were reported by physicians. The most notable difference was for consumer reports; 24.9% of the FAERS reports versus 4.3% within VigiBase. This difference might be due to different time-periods; consumer reporting is increasing in latest years[152]. Second, only a small proportion of the FAERS ICSRs concerned reporting by non-manufacturers. The majority was either reported as part of expedited reporting (65%) or as part of periodic reporting. Earlier it was shown that the US reports within VigiBase are mainly reported by manufacturers, while these form only a small proportion of the reports from the other continents[153]. Third, the reported drug groups and events differed. VigiBase reports more often concerned anti-infective and dermatological drugs, while within FAERS neurological drugs and antineoplastic drugs were most frequent reported. This also reflects utilization differences between the US and EU countries, with high rates of prescriptions of methylphenidate in US adolescents in recent years[154]. Choosing an appropriate time-period to study these kind of drugs is essential since the utilization of neurological drugs and especially for the treatment of ADHD has changed tremendously since the start of VigiBase in 1968[154, 155].

Describing of ICSRs reported after long-term drug use was a topic of special interest. Long-term drug use and long-term adverse events are of importance dur-

ing childhood because of possible effects on growth and development However, they are difficult to study. Studies often lack sufficient time of follow-up and adverse events occurring long after initiating therapy are not easily recognised. Especially for drugs being used chronically, studies investigating long-term safety should be performed. The reported types of drugs before and after 6 months of use differed significantly. Systemical hormonal preparations, alimentary drugs and antineoplastic/immunomodulating agents were prominently reported after long-term treatment, while anti-infective drugs, musculoskeletal system drugs and sensory organ drugs were reported mostly with short term use. These findings are in line with drugs known to be used short-term or are known to be used for long periods of time[122]. New onset neoplasms are an important concern and were more often reported after long-term drug use. It is not possible to infer any causal association based on spontaneous reporting data. However, the distribution of the drug classes and events reported after long-term drug use are in line with what is expected and therefore a complete dataset of paediatric ICSRs might be a suitable additional source to generate signals on delayed events and new onset chronic events.

The use of spontaneous reporting data has many well-known limitations[150]. Since the publically available datasets often do not include all variables there are analytic limitations and since case-narratives are lacking it is difficult to draw inferences on causality. For example, the non-availability of case-narratives implies a loss of potentially important information not otherwise coded in the ICSR. Another well-known, limitation is the volume of duplicates[156]. Duplicate reports are present in all spontaneous reporting databases[157]. The identification and elimination of duplicates from analyses is advantageous for using the data and is important for a correct interpretation of the data, however, so far, few easy to use duplicate-detection methods are currently available and enhanced methods of duplicate detection are being developed[156]. For this study, we dealt with the issue of duplicate reports by only including unique ICSRs. However it is inevitable that duplicate reports are still present within the used database.

CONCLUSION

Knowledge on the distribution of the drug classes and events within FAERS is a key first step in developing paediatric specific methods for drug safety surveillance. Studying multiple databases is useful because global differences in drug utilization and type of reports.

CHAPTER 3

Signal Detection on Spontaneous Reporting Systems

3.1. Drug-Induced Hepatic Injury in Children: a Case/Non-Case Study of Suspected Adverse Drug Reactions in VigiBase

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ABSTRACT

Objective

To identify which drugs are associated with reports of suspected hepatic injury in children and adolescents.

Methods

Using a worldwide pharmacovigilance database, VigiBase, we conducted a case/non-case study on suspected ADRs occurring in the population <18 years old. Cases were all the records with hepatic ADRs; non-cases were all the other ADR records. Records regarding topically administered drugs were excluded from both groups. The association between drug and suspected hepatic ADRs was calculated using the reporting odds ratio (ROR) as a measure of disproportionality while adjusting for gender, country, reporter and calendar year. Sub-analyses were performed within therapeutic class and by excluding vaccination-related reports to reduce confounding.

Results

Overall, 6,595 (1%) out of 624,673 ADR records in children and adolescents concerned hepatic injury. Most of the reported hepatic injuries concerned children 12-17 years of age. Drugs that were most frequently reported as suspected cause and were associated with hepatic injury comprised acetaminophen, valproic acid, carbamazepine, methotrexate, minocyclin, zidovudine, pemoline, ceftriaxone, bosentan, ciclosporin, atomoxetine, olanzapine, basiliximab, erythromycin, and voriconazole. The association between hepatotoxicity and all these drugs, except for basiliximab, is already known.

Conclusion

Drug-induced hepatic injury is infrequently reported (only 1% of total) as suspected ADR in children and adolescents. The drugs associated with reported hepatotoxicity (acetaminophen, antiepileptic and anti-tuberculosis agents) are known to be hepatotoxic in adults as well, but age related changes in associations were observed. VigiBase is useful as a start to plan further drug safety studies in children.

BACKGROUND

Drug-induced hepatic injury is one of the most important reasons for drug withdrawal[158], but very little is known about drug-induced hepatic injury in the paediatric population; most of the evidence comes from small case series[159].

Although pharmacovigilance activities were boosted after the thalidomide disaster in children, pharmacovigilance and pharmacoepidemiology studies in children are still infrequent. There is no enough systematic monitoring of drug safety (i.e. signal generation) in children and adolescents separately. On the contrary, signal generation is generally performed considering the entire population. Children are not just small adults and the pharmacologic effects (both therapeutic and adverse ones) of drugs in these patients cannot be extrapolated from the observed effects in adults. Susceptibility to drug toxicity changes by age and can differ largely between newborns, toddlers, adolescents, and adults, because of age-dependent maturation of pharmacokinetic processes. This is particularly so for the liver which is the main organism for drug metabolism[34-36]. Most drugs are metabolized through the CYP P450 isoenzymes. The change in maturation and activity of CYP P450 occurring with age may have a strong influence on the capacity to eliminate the drugs between newborns and adults. For instance, at birth, the CYP P450 isoenzymes are only 50% of the adult values, but their expression quickly changes during the first months[37].

Considering the lack of comprehensive information about drug-induced hepatic injury in children and adolescents, aim of this study was to assess which drugs are associated with hepatic injury in paediatric population, in a worldwide spontaneous reporting database.

METHODS

Data source and selection of cases and non-cases

For this study we analysed the reports of suspected ADRs in VigiBase, the global ICSR database that was established in 1968 and is maintained by the WHO-UMC[160]. VigiBase is the largest database worldwide with > 4 million ICSRs covering more than 40 years. The suspected ADRs are sent to UMC from the national centres participating in the WHO Programme for International Drug Monitoring. Currently, 95 countries submit ICSRs to VigiBase. The origin of reports is heterogeneous as some of these countries have voluntary reporting and others more mandatory systems. Healthcare professionals, consumers and marketing authorization holders may fill the reports. A significant proportion of

the WHO-UMC database comprises data from the US FAERS[161]. Due to the multiple entry modes and duplicate reporting of national reports to both WHO and FAERS, removal of the duplicates is an important quality procedure at UMC. Duplicate detection in VigiBase is not only limited to the simple check of case identifiers and manual inspection of given case series, but includes also specific statistical algorithms[162]. The suspected ADRs are coded by using the WHO-Adverse Reactions Terminology (WHO-ART) and MedDRA[163]. Drugs are coded by the WHO Drug Dictionary, which offers indexing and retrieval of drugs by the hierarchical ATC classification[163].

Data analysis

For the evaluation of drug-induced hepatic injury in children and adolescents, we used all the records of suspected ADRs occurring in people <18 years old, as registered in VigiBase during the period January 2000 until December 2006. We excluded all the records in which the suspected drug was a topically administered medication (assuming that these would not cause liver injury and would lead to underestimation of risk). For signal detection, we used the records as unit of analysis, which is the normal routine in the WHO-UMC[164]. An ICSR can contain more than one suspected drug and/or more than one ADR, whereas a record is a unique combination of a drug and an ADR. Hence, an ICSR containing two ADRs with one suspected drug will count for two records and an ICSR containing two ADRs with two suspected drugs will count for four records. Information on these records include country of origin, reporter, age at onset, year of onset, gender, reported drug, reported ADR, start and stop date of the drug, start and stop date of the ADR, dosing regimen of the drug, administration route, and causality assessment of the event.

Associations between specific drugs and hepatic ADRs were analysed using the case/non case method[165, 166], a technique which was introduced in 1991 in a study with WHO data on serum sickness to cefaclor[167]. Cases of hepatic injury were records of suspected ADRs in which one of the following preferred terms was indicated: *abnormal hepatic function*, *active chronic hepatitis*, *biliary tract disorder*, *bilirubinaemia*, *bilirubinaemia aggravated*, *bilirubinuria*, *cholangitis*, *cholecystitis*, *cholelithiasis*, *cholestatic hepatitis*, *fatty liver*, *gallbladder disorder*, *gamma-Glutamyl Transferase* (gGT) *increased*, *hepatic cirrhosis*, *hepatic coma*, *hepatic enzymes increased*, *hepatic failure*, *hepatic necrosis*, *hepatitis*, *hepatocellular damage*, *hepatomegaly*, *hepatorenal syndrome*, *hepatosplenomegaly*, *jaundice*, *Alanine* or *Aspartate aminotransferase* (ALT or AST) *increased*. These are all the preferred terms listed in the system-organ class "*liver and biliary diseases*" from the WHO-ART[164, 168]. Records with "*Budd-Chiari syndrome*", "*infectious*" or "*viral hepatitis*" and "*veno-occlusive*

liver disease" were excluded as these hepatic injuries are not drug-related[168]. Non-cases were all non-hepatic suspected ADR records in children and adolescents. The suspected ADR Reporting Odds Ratio (ROR) was calculated as measure of disproportionality for all the drugs that had at least 4 records of hepatic injury[169].

In a first crude approach, we compared the odds of exposure to a specific drug in hepatic injury cases with the odds of exposure to the specific drug in all non-hepatic ADR records. Second, the crude RORs were adjusted for calendar year, gender, country of reporting and type of reporter by using multivariate logistic regression analysis. Third, the analysis was restricted to the drugs belonging to the same therapeutic class (ATC-based, II level). This sensitivity analysis was carried out to limit confounding by indication and by severity and to investigate whether the effect of a specific drug was greater than its class effect. An additional analysis was conducted in which all the records associated with vaccines were excluded, since vaccines may distort reporting odds ratios due to the large number of records of vaccine-related ADR, and the low probability of vaccine-induced hepatic injury. A fourth analysis was conducted which limited the records to those with a reported causality assessment ("*certain*", "*probable*", or "*possible*"). As the last step, we looked at effect modification by age stratifying the analysis in the following age categories: <1 month, 1 month - 2 years, 3-11 years, and 12-17 years. Due to the low number of reports for neonates, these were lumped in the category <3 years for all main analyses.

The statistical package SPSS (version 15.0) was used for all statistical analyses. MICROMEDEX© was used as drug information source to verify whether hepatotoxicity was mentioned as potential adverse drug reaction for those medications which were found to be associated in our study[170].

RESULTS

In the period 2000-2006, VigiBase comprised 226,087 suspected spontaneous ICSRs in the population aged <18 years, corresponding to a total of 867,405 records. The FDA-AERS contributed most of these records (n= 569,701). Stratification by country showed that the highest rate of reporting of hepatic injury was observed in Germany (5% of total German records) (**Table 1**).

After exclusion of all records related to topically administered drugs, 624,673 records of suspected ADRs remained and these were the basis of our analysis. Most suspected ADR records regarded children aged <3 years (47.8% of total records), but vaccine-related reports accounted for large proportion of reports in this age category (**Table 2**).

Table 1. Distribution of suspected ADR records by country from VigiBase*

Country	Total Records# n = 867,405 (%)	Hepatic Injury Records# n = 9,036 (% *of row*)
United States of America	569,701 *(65.7)*	5,363 *(0.9)*
France	24,005 *(2.8)*	968 *(4.0)*
Germany	16,431 *(1.9)*	827 *(5.0)*
United Kingdom	44,004 *(5.1)*	352 *(0.8)*
Canada	86,555 *(10)*	300 *(0.4)*
Australia	27,727 *(3.2)*	262 *(0.9)*
Spain	7,309 *(0.8)*	143 *(2.0)*
Sweden	7,919 *(0.9)*	95 *(1.2)*
Netherlands	4,289 *(0.5)*	64 *(1.5)*
Ireland	5,798 *(0.7)*	46 *(0.8)*
Thailand	17,058 *(2.0)*	44 *(0.3)*
New Zealand	18,833 *(2.2)*	28 *(0.2)*
Italy	4,600 *(0.5)*	18 *(0.4)*

*Data from 2000 until 2006.

#Only the countries with more than 4,000 reports have been listed in the table.

Table 2. Age and gender distribution of suspected ADR records* from VigiBase

	Total Records		Hepatic Injury Records	
	with vaccines n = 624,673 (%)	without vaccines n= 226,266 (%)	with vaccines n= 6,595 *(% of row)*	without vaccines n = 6,147 *(% of row)*
Age groups (years)				
<3	298,718 *(47.8)*	43,465 *(19.2)*	1,360 *(0.5)*	1,104 *(2.5)*
3 to 11	177,029 *(28.3)*	75,345 *(33.3)*	1,962 *(1.1)*	1,882 *(2.5)*
12 to 17	148,926 *(23.8)*	107,456 *(47.5)*	3,273 *(2.2)*	3,161 *(2.9)*
Gender				
Girls	298,209 *(47,7)*	108,431 *(47.9)*	3,136 *(1.1)*	2,947 *(2.7)*
Boys	316,280 *(50.6)*	113,264 *(50.1)*	3,328 *(1.1)*	3,072 *(2.7)*
Unknown	10,184 *(1.6)*	4,571 *(2.0)*	131 *(1.3)*	128 *(2.1)*

*Without topical drugs.

Among 624,673 records, only 1.1% (n. of cases = 6,595) concerned hepatic injury. The rate of hepatic injury reporting in the paediatric population increased with age (from 0.5% of total records among the youngest children up to 2.2% of total records among the oldest) and was highest for children aged 12-17 years. Upon exclusion of vaccine-related ADR records, the age related increase in the rate of reported hepatic injury was less pronounced (**Table 2**).

Ranked by the absolute number of cases (**Table 3**), the top ten most frequently suspected drugs for hepatic injury were isotretinoin (6.4% of total number of

cases), followed by acetaminophen (5.3%), valproic acid (3.2%), carbamazepine (2.1%), methotrexate (2.0%), hepatitis B vaccine (1.9%), minocycline (1.8%), lamotrigine (1.7%), zidovudine, pemoline and ceftriaxone (1.6%). The ROR for hepatic injury was statistically significant for all drugs mentioned above, except for hepatitis b vaccine. After adjustment for calendar year, gender, country of reporting and type of reporter, significant associations remained for all these drugs (**Table 3**).

Ranked by the strength of the crude ROR, the top ten drugs with associations higher than 10 included oxymetholone, norethisterone/ethinylestradiol combination, milrinone, retinol, atazanavir, pemoline, pyrazinamide, isoniazid, naltrexone and troglitazone (**Appendix 1**).

Table 3. ROR for hepatic injury of individual drugs ranked by absolute number of cases (with at least 30 cases) in population <18 years old

Drugs	N. of cases	N. of non-cases	ROR *(95% CI)*		
			with vaccines n. of cases = 6,595		without vaccines n. of cases = 6,147
			crude	adjusted[§]	Adjusted[§]
Isotretinoin	420	12,051	3.4 *(3.1-3.8)*	1.9 *(1.7-2.1)*	1.3 *(1.1-1.5)*
Acetaminophen	347	4,049	8.4 *(7.7-9.3)*	6.0 *(5.4-6.8)*	3.4 *(3.1-3.8)*
Valproic acid	208	3,065	6.5 *(5.8-7.4)*	4.0 *(3.5-4.7)*	2.2 *(1.9-2.6)*
Carbamazepine	140	2,271	5.9 *(5.1-6.8)*	3.6 *(3.0-4.3)*	2.1 *(1.8-2.5)*
Methotrexate	134	1,873	6.8 *(5.9-7.9)*	4.2 *(3.5-5.1)*	2.5 *(2.1-3.0)*
Minocycline	117	959	11.6 *(10.0-13.5)*	4.3 *(3.5-5.3)*	3.5 *(2.9-4.3)*
Lamotrigine	112	3,005	3.5 *(3.0-4.2)*	2.2 *(1.8-2.7)*	1.3 *(1.1-1.6)*
Zidovudine	106	2,446	4.1 *(3.4-4.9)*	4.5 *(3.7-5.5)*	1.2 *(1.0-1.5)*
Pemoline[o]	104	282	35.1 *(30.5-40.4)*	31.6 *(25.0-40.0)*	14.4 *(11.5-18.2)*
Ceftriaxone	104	1,695	5.8 *(4.9-6.9)*	5.0 *(4.0-6.1)*	2.6 *(2.1-3.2)*
Methylphenidate	96	4,199	2.2 *(1.8-2.6)*	1.3 *(1.0-1.6)*	0.7 *(0.6-0.9)**
Bosentan	85	353	22.8 *(19.4-26.9)*	15.0 *(11.8-19. 2)*	7.3 *(5.7-9.2)*
Ciclosporin	71	117	5.7 *(4.6-7.1)*	3.0 *(2.4-3.9)*	1.6 *(1.3-2.1)*
Atomoxetine	64	1,624	3.7 *(2.9-4.7)*	2.0 *(1.5-2.6)*	1.3 *(1.0-1.6)*
Azithromycin	63	2,932	2.0 *(1.6-2.6)*	1.8 *(1.4-2.3)*	0.8 *(0.6-1.0)**
Olanzapine	62	845	6.9 *(5.5-8.7)*	3.1 *(2.4-4.0)*	2.3 *(1.7-2.9)*
Erythromycin	60	1,196	4.7 *(3.7-6.0)*	4.2 *(3.2-5.5)*	2.3 *(1.8-3.1)*
Infliximab	60	2,083	2.7 *(2.1-3.5)*	1.3 *(1.0-1.7)*	0.9 *(0.7-1.1)**
Risperidone	59	2,611	2.1 *(1.7-2.7)*	1.0 *(0.8-1.4)**	0.7 *(0.5-0.9*)*
Phenytoin	57	1,222	4.4 *(3.4-5.6)*	3.0 *(2.3-4.0)*	2.0*(1.5-2.6)*
Voriconazole	52	270	18.2 *(14.7-22.5)*	10.7 *(7.9-14.6)*	6.7 *(5.0-9.1)*
Topiramate	51	1,356	3.5 *(2.7-4.6)*	2.1 *(1.6-2.8)*	1.1 *(0.9-1.5)**

Table 3. ROR for hepatic injury of individual drugs ranked by absolute number of cases (with at least 30 cases) in population <18 years old (continued)

Drugs	N. of cases	N. of non-cases	ROR *(95% CI)*		
			with vaccines n. of cases = 6,595		without vaccines n. of cases = 6,147
			crude	adjusted[§]	Adjusted[§]
Sulfamethoxazole/Trimethoprim	48	3,064	1.5 *(1.1-2.0)*	1.3 *(1.0-1.7)*	0.9 *(0.7-1.2)**
Isoniazid	47	140	31.7 *(25.7-39. 1)*	23.8 *(16.7-33.7)*	14.0 *(9.9-19.7)*
Vincristine	46	1,119	3.9 *(2.9-5.1)*	2.7 *(2.0-3.7)*	1.5 *(1.1-2.0)*
Lamivudine	45	764	5.6 *(4.2-7.3)*	4.9 *(3.6-6.6)*	1.7 *(1.3-2.3)*
Ethinylestradiol/Levonorgestrel	43	928	4.4 *(3.3-5.8)*	1.9 *(1.4-2.6)*	1.5 *(1.1-2.1)*
Oxcarbazepine	43	1,205	3.4 *(2.5-4.5)*	1.9 *(1.4-2.6)*	1.1 *(0.8-1.5)**
Gemtuzumab	42	241	16.4 *(12.9-20.9)*	17.1 *(12.3-24.0)*	6.7 *(4.8-9.4)*
Fluconazole	42	409	9.7 *(7.5-12.5)*	8.6 *(6.2-12.0)*	3.6 *(2.6-5.0)*
Mercaptopurine	41	252	15.3 *(12.0-19.6)*	11.4 *(8.1-16.0)*	6.0 *(4.3-8.4)*
Phenobarbital	41	594	6.5 *(4.9-8.6)*	6.6 *(4.8-9.2)*	3.9 *(2.8-5.4)*
Amoxicillin/Clavulanate	38	1,309	2.7 *(2.0-3.7)*	1.7 *(1.2-2.3)*	0.8 *(0.6-1.2)**
Tioguanine	37	240	14.5 *(11.2-18.9)*	14.5 *(11.2-18.9)*	6.2 *(4.4-8.8)*
Rifampicin	37	243	14.3 *(11.0-18.6)*	8.3 *(5.8-12.0)*	5.1 *(3.6-7.3)*
Nevirapine	37	1,487	2.3 *(1.7-3.2)*	2.8 *(2.0-3.9)*	0.8 *(0.6-1.1)**
Cytarabine	36	885	3.8 *(2.8-5.2)*	2.9 *(2.1-4.1)*	1.5 *(1.1-2.2)*
Clozapine	36	1,646	2.1 *(1.5-2.8)*	0.8 *(0.6-1.1)**	0.7 *(0.5-0.9)**
Clarithromycin	35	1,081	3.0 *(2.2-4.2)*	1.8 *(1.3-2.5)*	1.0 *(0.7-1.4)**
Interferon beta	30	497	5.7 *(4.1-7.9)*	4.4 *(3.0-6.5)*	2.3 *(1.6-3.3)*
Acetylsalicylic Acid	30	1,070	2.6 *(1.9-3.7)*	1.3 *(0.9-1.9)**	0.9 *(0.6-1.3)**

[§]Adjusted for gender, age, country, and type of reporter.

[o]Drugs withdrawn from the market due of hepatotoxicity.

*Adjusted RORs no statistically significant.

The following drugs had more than 30 cases but were not associated with hepatic injury: Hepatitis B vaccine, ibuprofen, poliovirus vaccine live oral, measles, mumps and rubella vaccine, diphtheria and tetanus toxoids and pertussis, sertraline, haemophilus B conjugate vaccine.

When restricting the analysis to the drugs belonging to the same therapeutic class, in most of the cases RORs decreased, pointing to confounding by indication or class effects (**Table 4**). Within the therapeutic groups that were most frequently involved in hepatic ADRs (with at least 100 cases), the following drugs were standing out from their class: sultiame, ethosuximide, phenobarbital, valproic acid and carbamazepine among the antiepileptics (ATC: N03), aztreonam, loracarbef, erythromycin, ceftriaxone, josamycin, minocycline among antibacterial agents (J01), acetaminophen among analgesics (N02), pemoline, nefazodone, atomoxetine among psycho-analeptic drugs (N06) and mercaptopurine, gemtuzumab, tioguanine and methotrexate among antineoplastic drugs (L01).

Table 4. ROR for hepatic injury by therapeutic class*

Therapeutic classes	ATC code (II level)	N. of cases *(% on 6,595)*	Drugs	Adjusted[§] RORs within therapeutic class *(95% CI)*
Antiepileptics	N03	762 *(12)*	Sultiame	3.6 *(1.6-7.9)*
			Ethosuximide	2.8 *(1.6-4.9)*
			Phenobarbital	2.0 *(1.4-2.9)*
			Valproic acid	1.5 *(1.3-1.8)*
			Carbamazepine	1.3 *(1.0-1.5)*
Antibacterials	J01	742 *(11)*	Aztreonam	5.9 *(2.2-15.4)*
			Loracarbef	3.9 *(1.5-10.1)*
			Erythromycin	3.4 *(1.2-10.0)*
			Ceftriaxone	3.1 *(2.5-3.8)*
			Josamycin	2.9 *(1.5-5.7)*
			Minocycline	2.7 *(2.1-3.6)*
Analgesics	N02	472 *(7)*	Acetaminophen	5.6 *(4.5-6.9)*
			Acetaminophen/Hydrocodone	1.8 *(1.1-3.0)*
Psychoanaleptics	N06	457 *(7)*	Pemoline[o]	30.7 *(23.3-40.6)*
			Nefazodone	7.3 *(4.3-12.4)*
			Atomoxetine	1.7 *(1.3-2.3)*
Antineoplastics	L01	421 *(6)*	Mercaptopurine	4.2 *(3.0-6.0)*
			Gemtuzumab	4.2 *(2.9-5.9*
			Tioguanine	3.9 *(2.7-5.7)*
			Methotrexate	3.2 *(2.0-5.3)*
Antivirals	J05	397 *(6)*	Atazanavir	21.5 *(11.3-41.0)*
			Emtricitabine	6.4 *(2.0-21.0*
			Lamivudine	1.7 *(1.3-2.4)*
			Zidovudine	1.5 *(1.1-1.9)*
Psycholeptics	N05	287 *(4)*	Chlorprothixene	4.8 *(1.6-14.2)*
			Olanzapine	3.5 *(2.6-4.7)*
Immunosuppressants	L04	251 *(4)*	Basiliximab	2.6 *(1.6-4.2)*
			Ciclosporin	1.4 *(1.0-1.9)*
Antimycobacterials	J04	120 *(2)*	Pyrazinamide	2.3 *(1.2-4.4)*
			Isoniazid	2.1 *(1.3-3.2)*
Sex hormones	G03	115 *(2)*	Norethisterone ac/Ethinylestradiol	24.5 *(6.4-93.6)*
			Estradiol	6.7 *(2.0-22.1)*
			Norethisterone	5.8 *(2.5-13.5)*
			Ethinylestradiol/Levonorgestrel	2.1 *(1.4-3.2)*
Antimycotics	J02	107 *(2)*	Voriconazole	1.9 *(1.2-3.0)*

For each therapeutic class, only those drugs with statistically significant adjusted RORs for hepatic injury have been reported.

*Only therapeutic classes with at least 100 cases have been considered in this analysis.

[§]Adjusted for age, gender, country of reporting and type of reporter.

[o]Drugs withdrawal from the market because of hepatotoxicity.

After exclusion of records that involving vaccines we retained a total of 226,266 records of suspected ADRs in children and adolescents, and 6,147 of those (2.7%) concerned hepatic injury. Exclusion of vaccine-related records from the analysis resulted in a strong decrease in the association between individual drugs and hepatic injury (**Table 3** and **Appendix 2**).

Drugs that were consistently associated with hepatic injury, upon all sensitivity analyses and adjustments, with the highest number of absolute cases (n. of cases ≥50) were acetaminophen, valproic acid, carbamazepine, methotrexate, minocycline, zidovudine, pemoline, ceftriaxone, bosentan, ciclosporin, atomoxetine, olanzapine, erythromycin and voriconazole. Hepatic injury is already listed in the SmPCs for all these drugs, except for basiliximab. Basiliximab is indicated for prophylaxis of acute rejection in patients receiving renal transplantation, as part of an immunosuppressive regimen that includes also ciclosporin, a known hepatotoxic drug. In all the basiliximab cases ciclosporin was reported as concomitant drug. In order to assess whether basiliximab adds to the hepatic injury risk a sensitivity analysis was done to compare whether the association between hepatic injury and ciclosporin plus basiliximab versus ciclosporin alone (ROR 4.1; 95% CI 0.9-18.1; *P*= 0.06) was different from the association between hepatic injury and ciclosporin plus other immunosuppressant drugs versus ciclosporin alone (ROR 1.1; 0.2-5.3; *P*= 0.94).

Finally, we looked at the records in which the causality assessment was completed (n. of cases =1,224). Causality was considered as "*certain*" in 75 cases, "*probable*" in 897 and "*possible*" in 252. Calculation of the RORs for hepatic injury based on all ADRs with certain, probable or possible causality confirmed our main findings (data not shown).

To inspect effect modification by age, age-specific RORs were calculated for all drugs with at least 30 cases. For each drug, a trend towards a reduction in strength of ROR was observed with increasing age, except for atomoxetine, olanzapine, infliximab, isoniazid and gemtuzumab. Exclusion of vaccine-related records had great impact. The age trend in RORs disappeared mostly with some exceptions. With increasing age, the association between hepatic injury and ciclosporin, phenytoin, topiramate and vincristine gradually decreased, while the association between hepatic injury and erythromycin, gemtuzumab and mercaptopurine progressively increased (**Appendix 2**). Among 6,595 cases in the study population, 287 cases (4.3%) concerned newborns (until 1 month of age). In this specific population, the strongest association with hepatic injury was observed for rifampicin (n. of cases = 6, ROR 22.4; 95% CI 12.0-41.7), acetaminophen (n. of cases = 9, ROR 10.8; 95% CI 6.2-19.0), erythromycin (n. of cases = 4, ROR 5.4; 95% CI 2.2-13.3) and HIV medications (zidovudine, stavudine, didanosine, nelfinavir, lamivudine, nevirapine).

DISCUSSION

This is the first study that explored drug-induced hepatic injury in children and adolescents based on the international WHO-UMC database of suspected ADR reports. There are several important findings from this study. First, hepatic injury is infrequently reported as suspected ADR in children and adolescents (1% of total records). Although we cannot accurately evaluate the absolute risk of hepatic injury from this type of data, it is generally perceived that drug-induced hepatic injury is seldom in the paediatric population. Children use less of the drugs that are known to induce hepatotoxicity and often for much shorter duration[122].

Second, the reporting rate and associations with hepatic injury seems to change with age, although this trend attenuated once vaccine-related reports are excluded. The absolute number of reports may increase with age due to fact that at older age children are more likely to be exposed for a longer time to well-known hepatotoxic drugs, such as retinol and isotretinoin for the treatment of acne, or oestrogens as oral contraceptive pills[163].After stratifying the analyses by three different age categories, we observed some effect modification by age which could be expected based on changes in hepatic maturation, drug pharmacokinetics and pharmacodynamics during childhood[34, 37, 171]. The general trend was that the RORs decreased with increasing age, clear patterns were seen for acetaminophen and valproic acid. Acetaminophen had a higher ROR in younger children, which is contrary to our expectations since in young children the toxic metabolite of acetaminophen is produced much less[158, 171, 172]. An explanation could be that, among toddlers, intoxication from acetaminophen is mainly due to unintentional therapeutic error by inappropriate dosing, unintentional multiple overdosing, ingestion of acetaminophen along with another hepatotoxic drug and use of adult rather than paediatric preparations[173]. The finding of a decreasing association with age for valproic acid is consistent with previous data[81, 171]. Also for ciclosporin and vincristine the associations with hepatic injury decreased. This can be explained by the fact that the isoenzyme CYP 3A4, which plays a fundamental role in the metabolism of these drugs, is less expressed in newborns and infants than in adolescents. This may lead to a reduced capacity in younger children to eliminate these drugs.

The third important but not surprising finding was that the drugs associated with hepatotoxicity in children have also been associated with hepatotoxicity in adults. Interestingly, pemoline and troglitazone, drugs with the highest ROR in our analysis, have been already withdrawn from the market due to their hepatotoxicity[158]. The fact that no (except one) new hepatotoxic drugs were identified in children is reassuring, especially since metabolism and enzyme maturation changes quickly in children and could have impact on toxicity.

Fourth, basiliximab was associated with hepatic injury in this study, and this drug has never been associated with adverse hepatic reactions in adults[170]. Basiliximab is however, always combined with ciclosporin, a well-known hepatotoxic drug, which makes it difficult to investigate whether it is basiliximab or ciclosporin or some interaction. Indirect comparisons of basiliximab/ciclosporin combination versus single use of ciclosporin showed still an increase in risk of hepatic injury for the combination ciclosporin/basiliximab but this may also be caused by severity of disease. It will be important to monitor hepatic safety of basiliximab in the future.

Both strengths and limitations of this study are related to the data source we used in the study, a large database of suspected ADR records. Advantages are that the system covers all drugs and patients from most countries worldwide. The system is sensitive and capable of detecting side effects quickly after market launch[128]. The SRS reflects both real-life events and real-life prescribing, and therefore may comprise drug use patterns that cannot be studied in clinical trials for ethical reasons, such as overdoses and inappropriate co-medication[128]. The use of these data has also limitations. Firstly, drug-related hepatic injury cannot be viewed as a single disease, and many different mechanism and factors lead to hepatotoxicity. On top of that, there is no standardized definition of drug-induced hepatic injury, and collection of spontaneous reports of hepatotoxicity may differ between countries. Also, the frequency with which countries report to the WHO-UMC database varies considerably due to several technical issues: extent of drug use, drug marketing year, general knowledge on the adverse drug effects, public attention to specific safety issues (i.e. specific monitoring programs), and health professionals' attitudes to reporting ADRs[128]. To address confounding due to these factors, we adjusted the main analyses for country of reporting and type of reporter. Secondly, the spontaneous reporting systems contain limited clinical information[128]. Thirdly, these systems may be very vulnerable to selective reporting and its extent is both variable and hard to measure. Selective reporting may lead to distortions in comparisons between drugs[128]. Moreover, only minor part of ADRs are identified and reported, that is a phenomenon known as underreporting[174]. Underreporting leads to two main limitations: 1) underestimation of the frequency of ADRs and, consequently, of the extent of a problem; 2) no cases or very few cases of a true adverse drug reaction might be received from spontaneous reporting system, thus requiring a sensitive and specific methodology for signal detection[128]. Fourthly, causality assessment is frequently not reported, which means that the risk of confounding (especially by indication) is even higher. A sensitivity analysis conducted on the drugs with causality assessment, showed that those with strongest associations remained, which strengthens our conclusion that these drugs may be hepatotoxic in children.

Finally, the high number of vaccine-related reports in specific age categories constitutes a strong confounding effect in signal generation in children. Part of this confounding effect could be removed by age adjustment. Exclusion of vaccine-related reports was more effective since the change in estimates upon exclusion went far beyond the effects observed after age adjustment alone. Although the strength of the associations was attenuated, the main findings still remained statistically significant.

CONCLUSION

In conclusion, hepatotoxicity is infrequently reported as suspected ADR in children and adolescents. Our analysis showed that well-known hepatotoxic drugs in adults, such as acetaminophen, antiepileptic drugs, and anti-tuberculosis agents, are also associated with hepatotoxicity in children. Further pharmacoepidemiological investigations are needed to quantify the risk of drug-induced hepatic injury in the paediatric population.

Appendix 1. ROR for hepatic injury of individual drugs ranked according to the strength of the crude association in the population <18 years old

Drugs	n. of cases	n. of non-cases	ROR *(95% CI)* with vaccines n. of cases = 6,595		ROR *(95% CI)* without vaccines n. of cases = 6,147
			crude	adjusted[§]	adjusted[§]
Oxymetholone	4	4	93.8 *(48.6-180.9)*	40.4 *(9.2-178.4)*	39.0 *(8.0-190.2)*
Norethisterone/Ethinylestradiol	5	6	78.2 *(43.2-141.4)*	31.6 *(9.4-106.2)*	24.9 *(7.6-82.0)*
Milrinone	4	9	41.7 *(20.7-83.7)*	28.2 *(8.3-95.6)*	15.6 *(4.7-52.0)*
Retinol	11	27	38.2 *(25.0-58.5)*	53.7 *(26.2-110.2)*	15.5 *(7.6-31.6)*
Atazanavir	17	44	36.3 *(25.7-51.2)*	33.6 *(18.4-61.4)*	16.9 *(9.6-29.8)*
Pemoline[o]	104	282	35.1 *(30.5-40.4)*	31.6 *(25.0-40.0)*	14.4 *(11.5-18.2)*
Pyrazinamide	17	47	34.0 *(24.0-48.0)*	17.0 *(9.4-30.5)*	17.8 *(9.9-32.0)*
Isoniazid	47	140	31.7 *(25.7-39.1)*	23.5 *(16.6-33.4)*	14.0 *(9.9-19.7)*
Naltrexone	7	22	29.9 *(17.3-51.6)*	12.8 *(5.3-31.1)*	8.6 *(3.6-20.2)*
Troglitazone[o]	10	32	29.3 *(18.5-46.4)*	23.5 *(11.1-49.6)*	11.3 *(5.5-23.3)*
Sultiame	8	27	27.8 *(16.6-46.6)*	11.6 *(5.2-25.7)*	6.1 *(2.7-13.3)*
Nicardipine	4	14	26.8 *(12.9-55.8)*	11.9 *(3.7-38.9)*	7.0 *(2.3-21.8)*
Tetrazepam	6	22	25.6 *(14.0-46.7)*	5.9 *(2.4-14.6)*	4.9 *(2.0-12.0)*
Bosentan	85	353	22.8 *(19.4-26.9)*	15.0 *(11.8-19.2)*	7.3 *(5.7-9.2)*
Halofantrine	4	17	22.1 *(10.4-46.8)*	9.0 *(3.0-27.1)*	5.3 *(1.8-15.7)*
Emtricitabine	4	17	22.1 *(10.4-46.8)*	14.6 *(4.6-46.0)*	10.3 *(3.4-30.9)*
Trimethobenzamide	4	18	20.8 *(9.8-44.4)*	15.7 *(5.1-48.6)*	6.3 *(2.1-18.7)*
Etodolac	5	25	18.8 *(9.4-37.3)*	15.8 *(5.9-42.5)*	9.1 *(3.4-24.1)*
Voriconazole	52	270	18.2 *(14.7-22.5)*	10.7 *(7.9-14.6)*	6.7 *(5.0-9.1)*
Isoniazid/Rifampicin	4	21	17.9 *(8.2-38.7)*	7.5 *(2.4-23.2)*	8.0 *(2.7-24.1)*
Pioglitazone	4	21	17.9 *(8.2-38.7)*	14.7 *(4.9-43.9)*	6.1 *(2.1-17.9)*
Codeine/Pseudoephedrine	5	27	17.4 *(8.7-34.8)*	6.9 *(2.6-18.2)*	6.4 *(2.5-16.6)*
Dapsone	9	49	17.2 *(10.3-28.9)*	12.9 *(6.2-27.0)*	8.5 *(4.1-17.6)*
Propylthiouracil	14	79	16.6 *(11.0-25.3)*	10.9 *(6.1-19.7)*	7.0 *(3.9-12.6)*
Gemtuzumab	42	241	16.4 *(12.9-20.9)*	17.1 *(12.3-24.0)*	6.8 *(4.9-9.4)*
Sevoflurane	5	29	16.2 *(8.0-32.6)*	13.6(*5.2-35.5)*	4.6 *(1.8-12.0)*
Ursodeoxycholic acid	4	24	15.6 *(7.1-34.3)*	19.9 *(6.7-58.6)*	7.1 *(2.4-20.6)*
Estradiol	4	24	15.6 *(7.1-34.3)*	6.4 *(2.1-19.3)*	4.8 *(1.6-14.0)*
Mercaptopurine	41	252	15.3 *(12.0-19.6)*	11.4 *(8.1-16.0)*	6.0 *(4.3-8.4)*
Ethinylestradiol/Dienogest	7	43	15.3 *(8.4-27.7)*	3.3 *(1.5-7.3)*	3.0 *(1.4-6.8)*
Basiliximab	26	162	15.1 *(11.1-20.6)*	11.2 *(7.3-17.1)*	3.8 *(2.5-5.8)*
Chlorprothixene	4	25	15.0 *(6.8-33.1)*	5.4 *(1.9-16.0)*	3.8 *(1.3-11.0)*
Dactinomycin	28	177	14.9 *(11.0-20.1)*	16.7 *(11.1-20.3)*	6.3 *(4.2-9.5)*
Tioguanine	37	240	14.5 *(11.2-18.9)*	14.2 *(9.9-20.2)*	6.2 *(4.4-8.8)*
Rifampicin	37	243	14.3 *(11.0-18.6)*	8.4 *(5.8-12.0)*	5.1 *(3.6-7.3)*

Drugs	n. of cases	n. of non-cases	ROR *(95% CI)* with vaccines n. of cases = 6,595 crude	ROR *(95% CI)* with vaccines n. of cases = 6,595 adjusted[§]	ROR *(95% CI)* without vaccines n. of cases = 6,147 adjusted[§]
Norethisterone	8	53	14.2 *(8.1-24.9)*	7.4 *(3.4-15.7)*	3.5 *(2.9-4.3)*
Loracarbef	5	34	13.8 *(6.7-28.2)*	9.1 *(3.5-23.9)*	3.9 *(1.5-10.1)*
Aztreonam	5	34	13.8 *(6.7-28.2)*	12.3 *(4.6-32.6)*	6.7 *(2.6-17.3)*
Fusidic acid	8	56	13.4 *(7.6-23.7)*	6.1 *(2.9-13.1)*	3.5 *(1.7-7.4)*
Hydroxychloroquine	6	44	12.8 *(6.6-24.7)*	6.0 *(2.5-14.2)*	3.9 *(1.7-9.3)*
Ethosuximide	14	105	12.5 *(8.1-19.3)*	8.0 *(4.5-14.2)*	4.3 *(2.5-7.6)*
Acetazolamide	7	54	12.2 *(6.6-22.5)*	8.7 *(3.9-19.7)*	4.2 *(1.9-9.3)*
Nefazodone	17	136	11.7 *(7.9-17.5)*	6.7 *(4.0-11.2)*	4.5 *(2.7-7.4)*
Levocetirizine	5	40	11.7 *(5.6-24.4)*	4.4 *(1.7-11.3)*	3.0 *(1.2-7.7)*
Minocycline	117	959	11.6 *(10.0-13.5)*	4.3 *(3.5-5.3)*	3.5 *(2.9-4.3)*
Josamycin	10	82	11.4 *(6.8-19.2)*	5.2 *(2.7-10.1)*	2.6 *(1.3-5.0)*
Enoxaparin	4	33	11.4 *(5.0-25.8)*	5.2 *(1.8-15.4)*	3.5 *(1.2-10.0)*
Acetaminophen/Hydrocodone	19	160	11.2 *(7.6-16.3)*	4.2 *(2.6-6.8)*	3.9 *(2.4-6.4)*
Rasburicase	8	68	11.0 *(6.2-19.8)*	5.2 *(2.5-11.0)*	3.7 *(1.8-7.8)*
Ethinylestradiol/Gestodene	6	54	10.4 *(5.3-20.5)*	2.3 *(1.0-5.5)*	2.3 *(1.0-5.2)*
Itraconazole	15	137	10.3 *(6.7-15.8)*	4.2 *(2.5-7.3)*	2.8 *(1.7-4.9)*
Caspofungin	9	84	10.1 *(5.8-17.5)*	4.3 (*2.1-8.6)*	3.0 *(1.5-6.0)*
Zafirlukast	6	56	10.0 *(5.1-19.9)*	6.0 *(2.6-14.1)*	3.5 *(1.5-8.1)*

[§]Adjusted for age, gender, country of reporting and type of reporter.

[o]Drugs withdrawn from the market due to hepatotoxicity.

Appendix 2. Crude ROR for hepatic injury of individual drugs (with ≥30 cases from the main analysis) stratified by age groups

Drugs	Age Group <3 yrs			Age Group 3 to 11 yrs			Age Group 12 to 17 yrs		
	N. of cases	ROR		*N. of cases*	ROR		*N. of cases*	ROR	
		with vaccines *(n= 1,360)*	without vaccines *(n= 1,104)*		with vaccines *(n= 1,962)*	without vaccines *(n= 1,882)*		with vaccines *(n= 3,273)*	without vaccines *(n= 3,161)*
Isotretinoin	*8*	0.1*	1.8*	*2*	2.6*	1.1*	*410*	1.6	1.2
Acetaminophen	*117*	22.6	4.0	*74*	5.1	2.2	*156*	4.9	3.6
Valproic acid	*29*	16.8	2.9	*99*	6.6	2.9	*80*	2.8	2.1
Carbamazepine	*16*	17.5	2.0	*67*	5.6	2.4	*57*	2.6	1.9
Methotrexate	*13*	11.2	2.0	*75*	8.5	3.7	*46*	2.6	1.9
Minocycline	-			*2*	5.6	2.4*	*115*	5.7	4.2
Lamotrigine	*5*	4.2	0.7*	*50*	3.5	1.5	*57*	1.8	1.3
Zidovudine	*99*	10.4	1.8	*7*	3.3	1.5*	-		
Ceftriaxone	*19*	7.2	1.3*	*56*	7.3	3.2	*29*	3.1	2.3
Pemoline°	-	-		*76*	42.7	18.7	*28*	10.7	8.0
Methylphenidate				*51*	1.8	0.8*	*45*	1.3	0.9*
Bosentan	*35*	89.2	15.7	*22*	14.7	6.4	*28*	9.7	7.2
Ciclosporin	*34*	31	5.4	*23*	3.8	1.7	*14*	1.6	1.2*
Atomoxetine	-			*17*	1.7	0.7*	*47*	2.9	2.1
Azithromycin	*10*	2.8	0.5*	*30*	1.9	0.8*	*23*	1.3	1.0*
Olanzapine	-			*5*	2.4	1.0*	*57*	4.2	3.1
Infliximab	-			*4*	0.8*	0.3*	*56*	1.6	1.2*
Erythromycin	*12*	7	1.2*	*30*	4.9	2.1	*18*	3.0	2.3
Risperidone	-			*20*	2.1	0.9*	*39*	1.0	0.7*
Phenytoin	*11*	12.1	2.1	*18*	4.0	1.7	*28*	2.0	1.5
Voriconazole	*4*	67.5	11.8	*19*	14.6	6.4	*29*	9.3	6.9
Topiramate	*11*	11.6	2.0	*23*	3.3	1.4*	*17*	1.5*	1.1*
Sulfamethoxazole/Trimethoprim	*8*	2	0.3*	*13*	0.9	0.4*	*27*	1.4	1.0*
Isoniazid	*8*	10.2	10.7	*15*	30.0	13.1	*24*	16.3	12.1
Vincristine	*12*	15.7	2.8	*21*	3.5	1.5	*13*	1.4*	1.0*
Lamivudine	*25*	10.7	1.9	*17*	7.8	3.4	*3*	2.6*	1.9*
Ethinylestradiol/Levonorestrel	-			-			*43*	2.1	1.6
Oxcarbazepine	*5*	10.3	1.8*	*13*	2.2	0.9*	*25*	2.0	1.5
Fluconazole	*13*	14.6	2.6	*7*	5.9	2.6	*22*	9.3	6.9
Gemtuzumab	*1*	4.2	0.7*	*27*	16.4	7.2	*14*	15.6	11.6
Mercaptopurine	*2*	17.5	3.1*	*16*	11.1	4.8	*23*	10.6	7.9
Phenobarbital	*11*	11.5	2.0	*22*	8.3	3.6	*8*	2.5	1.8*
Amoxicillin/Clavulanate	*9*	4.8	0.8*	*12*	1.8	0.8*	*17*	2.7	2.0
Rifampicin	*11*	43.3	7.6	*11*	17.0	7.4	*15*	5.2	3.9

Drugs	Age Group <3 yrs			Age Group 3 to 11 yrs			Age Group 12 to 17 yrs		
	N. of cases	ROR		*N. of cases*	ROR		*N. of cases*	ROR	
		with vaccines *(n= 1,360)*	without vaccines *(n= 1,104)*		with vaccines *(n= 1,962)*	without vaccines *(n= 1,882)*		with vaccines *(n= 3,273)*	without vaccines *(n= 3,161)*
Tioguanine	*3*	34.6	6.1	*28*	15.5	6.8	*6*	4.6	3.4
Nevirapine	*29*	5.3	0.9*	*3*	1.9*	0.8*	*5*	1.8*	1.4*
Clozapine	-			*1*	1.0*	0.4*	*35*	1.1	0.8*
Cytarabine	*10*	15.6	5.7	*16*	3.1	1.4*	*10*	1.6*	1.2*
Clarithromycin	*6*	7.3	1.3*	*13*	2.4	1.1*	*16*	1.7	1.2*
Acetylsalicylic acid	*2*	2.6	0.5*	*15*	3.4	1.5*	*13*	1.1*	0.8*
Interferon beta	-			*20*	13.4	5.9	*10*	1.5*	1.1*

*No statistically significant crude RORs.

[o]Drugs have been ranked by number of cases. Topical drug-related records have been excluded.

CHAPTER 4

Electronic Healthcare Record Databases

4.1. The Power of Electronic Healthcare Databases for Active Drug Safety Surveillance in Children and Adolescents

Submitted for publication

ABSTRACT

Background

Traditional pharmacovigilance activities do not focus specifically on children and medicines in children are frequently being prescribed off-label based on extrapolating experience in adults to children. In Europe, the EU-ADR Project (*Exploring and Understanding Adverse Drug Reactions by Integrative Mining of Clinical Records and Biomedical Knowledge*), aims to use information from various EHR databases to produce a computerized integrated system for the early detection of drug safety signals. This might also prove to be a useful tool in paediatric pharmacovigilance.

Objective

To provide estimates on the number of drugs and incidence rates (IRs) of adverse events that can be monitored in children and adolescents in the EU-ADR database network.

Methods

Demographic, clinical events and outpatient drug prescription/dispensing data were obtained for children and adolescents (0 to 18 years), from seven population-based EHR databases of the EU-ADR network from Denmark, Italy, and the Netherlands. Data were analysed for the period January 1st 1996 through December 31st 2008. We estimated the number and types of drugs for which specific adverse events can be monitored as a function of actual drug use, minimally detectable relative risk (RR), and empirically-derived incidence rates for 10 events deemed to be important in pharmacovigilance. The same was done for adverse events frequently reported in children, using age-dependent IRs described in literature.

Results

The paediatric population (0-18 years) of the EU-ADR network comprised 4,838,146 individuals contributing 25,575,132 person years (PYs) of follow-up during the study period. Within this study population, a total of 2,170 drugs (i.e., distinct chemical substances) were prescribed during the study period with a total drug exposure of 1,610,631 PYs. Eighteen of the 2,170 drugs (0.8%) comprised half of the total drug exposure while 90% of the total drug exposure in PYs was represented by 158 drugs (7.3%). For a relatively frequent event such as upper gastrointestinal bleeding (IR= 14.4/100,000 PYs), there were 39 drugs (comprising 66% of total exposure in PY) for which an association with a RR≥4, if present, can be investigated. For rare events such as anaphylactic shock and bullous eruptions, there were 8 drugs (comprising 35% of total exposure) and 9 drugs (comprising

37% of total exposure) respectively, for which an association of same magnitude can be investigated. Based on literature-derived IR, there was a higher number of drugs that can be monitored for the events febrile convulsions, suicide attempt, and epilepsy at the same magnitude of risk.

Conclusion

Drug use in children is rare and shows little variation; only 18 out of the total 2,170 prescribed drugs make up half of the total exposure time to drugs in the paediatric population. The number of drugs with enough exposure to detect safety signals within EHRs for rare events in children and adolescents using EHRs from EUADR network is limited. Mining within EHR databases seems especially promising for events that have a high background incidence in the paediatric populations and for drugs with a large amount of exposure. Intercontinental collaboration will be necessary gain enough statistical power for paediatric drug safety detection.

BACKGROUND

Medicines in children are frequently being prescribed off-label as little information is available from clinical trials and data is extrapolated from adults to children[43]. The number of clinical trials is expected to increase with the introduction of new legislation with respect to the approval of drugs used in children in the US (2002) and in Europe (2007)[95, 133, 175]. However, clinical trials are primarily designed to assess therapeutic efficacy and have well known limitations for the assessment of risks since the number of included children is often too limited to draw firm conclusions with respect to safety. These limitations underline the importance of monitoring potential safety signals of drugs with a paediatric indication during the post-marketing phase. Currently, spontaneously reported ADRs and post-marketing safety studies are the most important source for identifying such safety signals both in children and adults[176, 177].

Although there is a fair amount of experience with using SRSs to study vaccine safety in children[178-183], the usefulness of such systems for routine safety surveillance of conventional medicines in children is limited. Studies on ADR-reporting primarily focus on the number of ADR-related hospital admissions or are descriptive in nature[100, 103, 108, 184]. Little is known on the capability of prospective monitoring for signal detection in the paediatric population. And, although safety signal detection in SRS have proven their value, mainly as a hypothesis-generating tool in adults, there are well-recognized limitations and biases such as selective underreporting, stimulated reporting and the lack of exposure data[150, 185, 186].

To complement SRS and other traditional pharmacovigilance systems, several initiatives, both in the US and in EU have set up population-based surveillance systems that make use of longitudinal healthcare data[187-189]. In Europe, the EU-ADR Project was initiated in 2008 and is funded by the European Commission (EC). The EU-ADR project is a collaboration of 18 public and private institutions representing academic research, general practice, healthcare services administration, and the pharmaceutical industry. EU-ADR aims to exploit information from various EHR and other biomedical databases in Europe to produce a computerized integrated system for the early detection of drug safety signals[187]. In a previous study, using data from the EU-ADR project, Coloma *et al.*[190] provided estimates on the number and types of drugs that can be monitored for safety surveillance in the general population using EHR databases. It was concluded that signal detection within these data was possible, but that the statistical power might be low for infrequently used drugs or for rare outcomes. Although this study included paediatric data, no specific analyses were performed on the paediatric sub-population. As disease prevalence, pharmacokinetics, pharmacodynamics and

drug exposure are different for children compared to adults[122, 191, 192], paediatric ADR-data should be analysed separately. Many EHR databases contain data on large numbers of children, which make such databases a good source for safety monitoring.

In this study, we first aimed to provide estimates of the number of drugs that have enough exposure to be monitored in children and adolescents based on actual drug use, minimal detectable RRs and empirically-derived IRs for ten adverse events currently being investigated within the EU-ADR project. Second, the same estimation of the number of drugs to be monitored was done for adverse events reported as frequently occurring in children, using age-dependent IRs described in literature. Third, we aimed to provide information on the range of IRs that can be monitored in children and adolescents based on the actual drug-exposure in the paediatric cohort and the minimal detectable RR.

METHODS

Data sources and setting

We used data from the EU-ADR network, of which a detailed description has been published earlier[92, 187]. In summary, the EU-ADR platform currently comprises data from eight EHR databases in four EU countries. For the current study we used paediatric data from the seven of the databases from three European countries: Health Search/CSD LPD (HSD, Italy), Integrated Primary Care Information (IPCI, the Netherlands) and Pedianet (Italy) are population-based general practice databases, in which clinical information and medication prescriptions are recorded. Aarhus University Hospital Database (Aarhus, Denmark), PHARMO Network (the Netherlands), and the regional Italian databases of Lombardy's and Tuscany's *Agenzia Regionale di Sanità* (ARS) are all comprehensive record-linkage systems in which drug dispensing data of regional/national catchment area are linked to a registry of hospital discharge diagnoses and other registries. The majority of healthcare services, including pharmaceutical services, are provided for, or subsidized by the State in Italy and Denmark and covered by obligatory health insurance in the Netherlands. In all of these countries general practitioners function as gatekeepers of the healthcare system. Children aged 0 to 18 years included in these databases were included in the current study.

The study period ran from January 1st 1996 to December 31st 2008. Follow up started after a run-in period of 365 days. This run-in period was required to determine if an event was incident. The run-in period was omitted for children younger than one year at the start of observation; these children started to contribute

follow-up person time from the date of birth or the date of registration on, whichever came first.

Data from the different databases were pooled using a distributed network approach, in which data holders maintain control over their original data and only aggregated data are shared with the rest of the network. This was done through generation of the data into a common format followed by local aggregation using custom-built software, Jerboa©[187].

Drug exposure

Drug use was categorized using the ATC classification system[193]. Drug exposure was measured in terms of PYs. We further analysed drug use by anatomical main groups (ATC 1st level), and by chemical substances (ATC 5th level). Drug exposure was stratified according to one year age-categories.

Drugs were subsequently categorized based on the total amount of drug exposure in PYs as follows: <10 PYs; >10 - ≤50 PYs; >50 - ≤100 PYs; >100 - ≤500 PYs; >500 - ≤1,000 PYs; >1,000 - ≤5,000 PYs; >5,000 - ≤10,000 PYs; and >10,000 PYs. Furthermore, the number of drugs (distinct chemical substances) that accounted for 50% and 90% of the total drug exposure in the population were calculated.

Events

The identification of the events of interest in EU-ADR has been described in detail in an earlier publication[187]. Only those events considered to be most serious and most relevant (generally within the context of pharmacovigilance in adults) were included. In summary, events of interest were identified in the databases using an iterative process that included definition of events based on clinical criteria established from literature, using diagnosis codes and free text as well as laboratory findings when available. Since the databases included in EU-ADR use in total four nomenclature systems to code the events, these different terminologies were first mapped using the Unified Medical Language System1 (UMLS1), a biomedical terminology integration system handling more than 150 terminologies[194]. The processes of terminology mapping, harmonization, and benchmarking of event extractions from the various databases have been described in more detail in other publications[91, 92, 195].

The following events have been mapped and harmonized in the EU-ADR platform: (1) acute liver injury; (2) acute myocardial infarction; (3) acute renal failure; (4) anaphylactic shock; (5) bullous eruptions; (6) cardiac valve fibrosis; (7) hip fractures; (8) neutropenia; (9) acute pancreatitis; (10) pancytopenia; (11) progressive multifocal leukoencephalopathy; (12) rhabdomyolysis; and (13) upper gastrointestinal bleeding. Not all events selected within the EU-ADR platform

were considered relevant to study in children and adolescents. Therefore, only events occurring in children with an annual incidence rate of >1/100,000 PYs were included. For children and adolescents the following 10 events were considered relevant and were included in this study: (1) acute liver injury, (2) acute renal failure (3) anaphylactic shock; (4) bullous eruptions; (5) cardiac valve fibrosis; (6) hip fractures; (7) neutropenia; (8) acute pancreatitis; (9) pancytopenia; and (10) upper gastrointestinal bleeding.

The events as currently monitored in the EU-ADR project are not paediatric-specific and therefore the analyses were extended to include events that are recognized as posing risk in children and adolescents. The following serious events were chosen based on studies on AE related hospital admissions in children and reviews of spontaneous reported AEs in children[108, 196, 197]: *(1) completed suicide; (2) suicide attempt; (3) febrile convulsions; and (4) epilepsy.*

Statistical analysis

Required amount of drug exposure to detect safety signals

Given the pooled population-based IR of the 10 events that were evaluated directly within EU-ADR, we calculated the total amount of PYs of exposure that would be required to detect an association between a particular drug and a particular event over varying magnitudes of RR of 2 (weak signal), 4 (moderate signal), and 6 (strong signal) using one-sided significance level $\alpha = 0.05$ and a power of 80% ($\beta = 0.2$). To estimate what the required amount of exposure would be for specific strengths of signals to be detected we used the formula as described and discussed previously by Coloma *et al*[190]. We subsequently determined the number of drugs for which there would be sufficient data for safety monitoring. The number of drugs was expressed as the number of unique chemical substances (ATC 5th level). For the drugs with enough exposure to detect the RR of interest, the proportion of the PYs of exposure to these drugs, compared to the total PYs of exposure for all drugs, was calculated.

Based on the actual exposure and hypothetical incidences of AEs it was also calculated for how many drugs within the anatomical main groups (ATC 1st level) there was enough exposure to detect associations with varying magnitudes. The following, (hypothetical), incidences were considered: 1/100,000 PYs; 10/100,000 PYs; 50/100,000 PYs; 100/100,000 PYs; and 500/100,000 PYs.

Age-specific IRs for the additional events were obtained from the literature. (1) completed suicide[198-200] (2) suicide attempt[198-200] (3) febrile convulsions[201] (4) seizures and convulsions (epilepsia)[202] (**Appendix**).

Required incidence based on the actual drug exposure to detect safety signals
Analogous to the analysis described under the heading 'Required amount of drug exposure to detect safety signals,' we calculated the range of IRs of events that can be monitored to detect weak (RR=2), moderate (RR=4) or strong (RR=6) associations based on the actual drug exposure within the cohort. These results were stratified within categories of drug exposure (as specified under 'drug exposure') and age.

Stratification by age
Results were stratified in one-year age categories and according to four age-categories based on the guidelines of the International Conference of Harmonization (ICH): 0-<2 years, 2-≤5 years; 6-≤11 years and 12-<18 years[203].

RESULTS

The paediatric population of the EU-ADR network, comprised 4,838,146 children and adolescents (0 to 18 years) contributing 25,575,132 PYs of follow-up between 1996 and 2008. Of these PYs of follow-up, 12.8% were for children aged 0 to

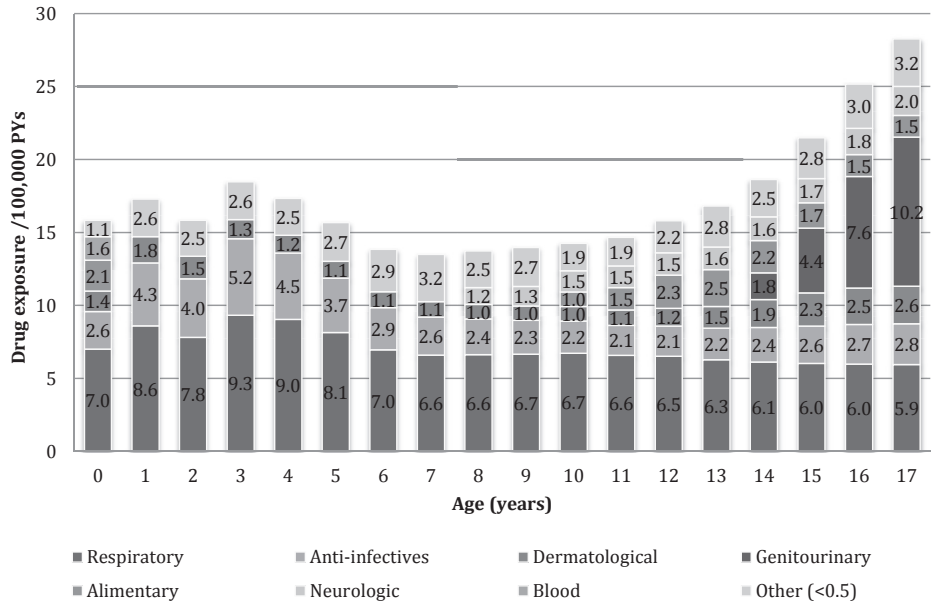


Figure 1. Drug exposure in person-years/100,000 person-years by age.
Drug exposure is aggregated on the 1st ATC level (anatomical main group). 'Other' represents all groups with an exposure of <1.0 /100,000 PYs.

<2years, 22.2% for children aged 2 to ≤5 years, 32.7% for children aged 6 to ≤11 years and 32.3% for adolescents aged 12 to <18 years.

A total of 2,170 drugs (i.e., distinct chemical substances) were prescribed or dispensed to this population during the study period with a total exposure of 1,610,631 PYs. An overview of drug exposure, at the anatomical level of the ATC classification across different age categories is illustrated in **Figure 1**. Up to 12 years of age, the drug classes with the highest exposure are respiratory drugs (6.5-9.3 PYs of exposure/100,000 PYs of follow up) and anti-infective drugs (2.1-5.2 PYs of exposure/100,000 PYs of follow up). From 14 years on the genitourinary drugs were increasingly prescribed up to 10.2 PYs of exposure/100,000 PYs of follow up.

Required exposure for monitoring of pre-defined events within EU-ADR and for events frequently occurring in children

The number of drugs on a chemical substance level (ATC 5th level) that have enough exposure to detect weak (RR=2), moderate (RR=4) or strong (RR=6) associations for the 10 EU-ADR events are presented in **Table 1**. Since the numbers are low, these results were not further stratified by age. The stronger the association to be studied, the higher is the number of drugs that can be studied, which is expected from the power calculations. Conversely, the higher the IR of the event the higher the number of drugs that can be studied. Considering the IR of upper gastro-intestinal bleeding (UGIB) within our paediatric population of 14.4/100,000 PYs,

Table 1. Amount of required amount drug exposure to identify potential safety signals

Event Type	IR/100,000 PYs	Weak association (RR≥2)			Moderate association (RR≥4)			Strong association (RR≥6)		
		Required exposure (PY)	Drugs N	% of Exp	Required exposure (PY)	Drugs N	% of Exp	Required exposure (PY)	Drugs N	% of Exp
Hip fracture	15.31	52,501	6	29.5	8,039	42	67.8	3,589	81	80.4
Upper GI bleeding	14.42	55,725	5	26.2	8,532	39	66.3	3,810	79	79.9
Neutropenia	8.10	99,259	2	13.0	15,198	25	56.9	6,786	48	70.5
Acute liver injury	3.96	202,733	0	0	31,041	9	37.3	13,860	26	57.8
Pancytopenia	3.73	215,469	0	0	32,991	9	37.3	14,730	25	56.9
Bullous eruption	3.58	224,394	0	0	34,358	9	37.3	15,341	24	56.0
Anaphylactic shock	3.23	248,526	0	0	38,053	8	35.0	16,990	20	52.1
Cardiac valve fibrosis	2.91	275,840	0	0	42,235	8	35.0	18,858	15	46.6
Acute renal failure	1.55	517,050	0	0	79,168	3	17.9	35,348	9	37.3
Acute pancreatitis	1.55	519,664	0	0	79,568	3	17.9	35,527	9	37.3

Drugs (N): Number of drugs at 5th ATC, chemical substance level that have enough PY of exposure to detect a potential signal. (total 2,170).

% of Exp: Proportion of PYs of exposure of the drugs with enough exposure compared to the total PYs of exposure for all drugs.

which was relatively high compared to the other events included in the study; a minimal exposure of 55,725 PYs was required to detect a weak association (RR≥2). Within our population five drugs fulfilled this criterion. These five drugs made up 26.2% of the total drug exposure in PYs. To detect a moderate association (RR≥4) with UGIB, a minimal exposure of 8,532 PYs was required; 39 drugs, covering 66.3% of the total drug exposure had this minimal exposure. Finally, to assess a strong association (RR≥6), a total of 79 drugs (79.9% of the total exposure) had enough exposure. The IR of pancreatitis was low, 1.6/100,000 PYs. Since the IR was low, none of the drugs had enough exposure to detect a weak signal (RR≥2), 3 drugs (17.9% of the total exposure) had enough exposure to detect a moderate signal (RR≥4), and 9 drugs (37.3% of the total exposure) had enough exposure to detect a strong signal (RR≥6).

The number of drugs, stratified at the anatomical level of the ATC classification, with enough exposure to study hypothetical IRs to detect weak, moderate or strong associations is given in **Table 2**. For drugs rarely prescribed in the study population, such as antineoplastic, anti-parasitic, and cardiovascular drugs, no drug had enough exposure to monitor an association with RR≥2 for any of the hypothetical incidences ranging from 1 to 500/100,000 PYs. Respiratory drugs and anti-infective agents were, however, included in those drugs having exposure to monitor associations of RR≥2, RR≥4 and RR≥6 for events having (hypothetical) IRs 10/100,000 PYs and higher.

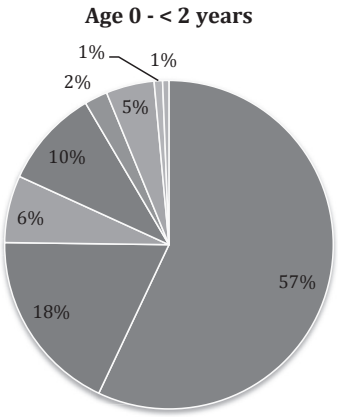
As illustrated in **Figure 2**, only a small number of the drugs have a high exposure in the paediatric population, 53% of the drugs have a total exposure of less than 10 PYs. This is most pronounced in the youngest children, for which 75% of the drugs have a total exposure of less than 10 PYs. In the table accompanying **Figure 2**, the minimal detectable IRs for the exposure-categories for each RR is given: for drugs with an exposure of less than 10 PYs, IRs of maximal 765/1,000 PY can be detected for RR≥2, maximal 12/1,000 PY for RR≥4 and maximal 5.2/1,000 PY for RR≥6. The proportion of the drugs with an exposure of more than 1,000 PYs is less than 5% for all age-categories, and is only 8.4% for the total paediatric population. An exposure of more than 1,000 PYs is necessary to detect IRs of more than 1.6/1,000 PYs with a RR≥2.

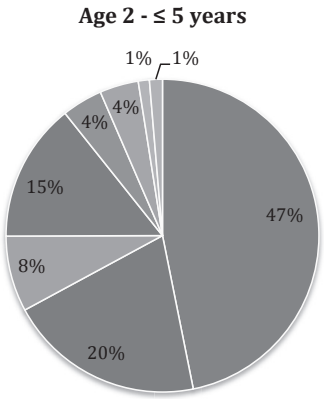
We estimated the power of the EU-ADR system to detect events frequently occurring in children based on the literature-derived IRs for 'completed suicide', 'suicide attempt', febrile seizures', and 'seizures and convulsions' (**Appendix**). For events with a high incidence rates like febrile convulsion (estimated IR: 1,400/100,000 PYs; children ≤5 years), a large number of drugs have enough exposure to detect a potential safety signal. Within the age-category 0 to <2 years, 50 drugs had enough exposure in PYs to detect an association with RR≥2, 132 drug

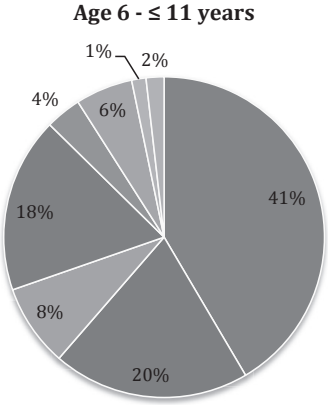
Table 2. Number (ATC 5th level) and proportions within class of drugs with enough exposure to study the given incidences with the given RRs

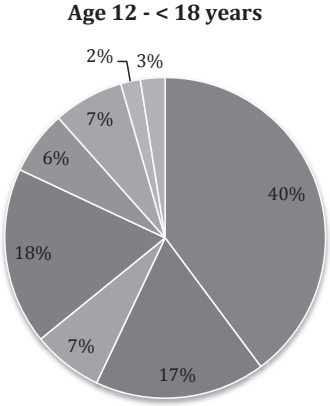
	≤1/100,000 PYs			≤10/100,000 PYs			≤50/100,000 PYs			≤100/100,000 PYs			≤500/100,000 PYs		
1st ATC class *	**RR=2**	**RR=4**	**RR=6**	**RR=2**	**RR=4**	**RR=6**	**RR=2**	**RR=4**	**RR=6**	**RR=2**	**RR=4**	**RR=6**	**RR=2**	**RR=4**	**RR=6**
Alimentary [N=391]	-	-	-	-	1 (0.3)	2(0.5)	1 (0.3)	11 (2.8)	21 (5.4)	2 (0.5)	20 (5.1)	37 (9.5)	14 (3.6)	52 (13.3)	66 (16.9)
Respiratory [N=160]	-	-	3 (1.9)	2 (1.3)	15 (9.4)	21 (13.1)	12 (7.5)	29 (18.1)	37 (23.1)	18 (11.3)	35 (21.9)	47 (29.4)	30 (18.8)	54 (33.8)	63 (39.4)
Dermatological [N=203]	-	-	-	-	2 (1.0)	8 (3.9)	1 (0.5)	14 (6.9)	23 (11.3)	5 (2.5)	23 (11.3)	35 (17.2)	19 (9.4)	48 (23.6)	62 (30.5)
Anti-infectives [N=232]	-	-	2 (0.9)	1(0.)	5 (2.2)	8 (3.4)	3 (1.3)	12 (5.2)	22 (9.5)	6 (2.6)	19 (8.2)	28 (12.1)	17 (7.3)	40 (17.2)	58 (25.0)
Neurologic [N=269]	-	-	-	-	2 (0.7)	2 (0.7)	1 (0.4)	9 (3.3)	19 (7.1)	2 (0.7)	19 (7.1)	27 (10.0)	14 (5.2)	41 (15.2)	57 (21.2)
Sensory organs [N=169]	-	-	-	-	-	1 (0.6)	-	5 (3.0)	12 (7.1)	-	12 (7.1)	19 (11.2)	11 (6.5)	34 (20.1)	42 (24.9)
Cardiovascular [N=192]	-	-	-	-	-	-	-	-	5 (2.6)	-	3 (1.6)	9 (4.7)	-	18 (9.4)	33 (17.2)
Genitourinary [N=153]	-	-	1 (0.7)	-	2 (1.3)	4 (2.6)	2 (1.3)	5 (3.3)	8 (5.2)	3 (2.0)	8 (5.2)	11 (7.2)	8 (5.2)	24 (15.7)	32 (20.9)
Hormones [N=72]	-	-	-	-	2 (2.8)	8 (11.1)	-	10 (13.9)	14 (19.4)	4 (5.6)	14 (19.4)	18 (25.0)	14 (19.4)	24 (33.3)	30 (41.7)
Musculoskeletal [N=104]	-	-	-	-	-	-	-	2 (1.9)	5 (4.8)	-	4 (3.8)	7 (6.7)	3 (2.9)	8 (7.7)	18 (17.3)
Blood [N=97]	-	-	-	-	-	2 (2.1)	-	3 (3.1)	6 (6.2)	2 (2.1)	5 (5.2)	7 (7.2)	3 (3.1)	11 (11.3)	16 (16.5)
Anti-parasitic [N=35]	-	-	-	-	-	-	-	-	1 (2.9)	-	1 (2.9)	1 (2.9)	-	5 (14.3)	11 (31.4)
Antineoplastics [N=76]	-	-	-	-	-	-	-	-	2 (2.6)	-	2 (2.6)	5 (6.6)	-	6 (7.9)	10 (13.2)
Total [N=2,170]	-	-	6 (0.3)	3 (0.1)	29 (1.3)	56 (2.6)	20 (0.9)	100 (4.6)	175 (8.1)	42 (1.9)	165 (7.6)	251 (11.6)	133 (6.1)	365 (16.8)	498 (22.9)

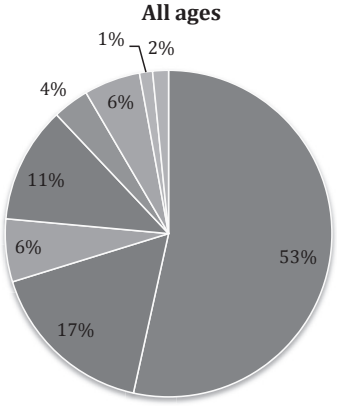
*First ATC level: 'Various' [N=35] had no drugs with enough exposure for any of the incidences and is not presented.











Range PY	Incidence /1,000 RR=2	Incidence /1,000 RR=4	Incidence /1,000 RR=6
<10	<765	<12	<5.2
>10 - ≤50	<160	<2.4	<1.1
>50 - ≤100	<80	<1.2	<0.55
>100 - ≤500	<16	<0.25	<0.11
>500 - ≤1,000	<8.0	<0.12	<0.055
>1,000 - ≤5,000	<1.6	<0.02	<0.011
>5,000 - ≤10,000	<0.8	<0.01	<0.0055
>10,000	≥0.8	≥0.01	≥0.0055

Figure 2. Distribution of exposure in PY by age-groups (5th ATC level, chemical subgroup).

The range in PY is given with the corresponding incidence rates of events that can be monitored. PY=person-years.

had enough exposure for RR≥4, and 188 drugs had enough exposure to detect RR≥6. However, for very rare events such as Reye's syndrome, only one drug had enough exposure to detect a strong association (RR≥6) in children aged 2 to ≤ 5 years.

Range of incidence rates of events that can be monitored within the network

Eighteen of the 2,170 drugs (0.8%) make up 50% of the total drug-exposure in PYs (**Table 3**). For 0 to <2 years, 2 to ≤5 years; 6 to ≤11 years and 12 to <18 years there were 8 (0.6%), 8 (0.5%), 14 (0.9%) and 20 (1.0%) drugs prescribed/dispensed. These drugs had corresponding exposures of ≥7,024 PY (0 to <2 years), ≥10,951 PYs (2 to ≤5 years), ≥6,822 PYs (6 to ≤11 years) and ≥7,227 PYs (12 to <18 years). Based

Table 3. Drugs* that cover 50% of the total drug exposure in person years by age-categories

Age 0 to < 2 years	Age 2 to ≤ 5 years	Age 6 to ≤ 11 years	Age 12 to< 18 years	Total
Beclametasone [R03BA01] (13.1)	Beclametasone [R03BA01] (12.5)	Salbutamol [R03AC02] (6.8)	Levonorgestrel/ estrogen [G03AA07] (11.9)	Beclametasone [R03BA01] (6.8)
Salbutamol [R03AC02] (10.5)	Salbutamol [R03AC02] (9.1)	Beclametasone [R03BA01] (6.4)	Sodium fluoride [A01AA01] (3.9)	Salbutamol [R03AC02] (6.2)
Amoxicillin [J01CA04] (6.5)	Amoxicillin/Clavulanate [J01CR02] (7.9)	Amoxicillin/Clavulanate [J01CR02] (5.2)	Amoxicillin/Clavulanate [J01CR02] (3.4)	Amoxicillin/Clavulanate [J01CR02] (4.9)
Amoxicillin/Clavulanate [J01CR02] (4.9)	Amoxicillin [J01CA04] (5.0)	Fluticasone [R03BA05] (4.8)	Salbutamol [R03AC02] (3.2)	Levonorgestrel/estrogen [G03AA07] (4.6)
Phytomenadione (vitamin K) [B02BA01] (4.4)	Fluticasone [R03BA05] (5.0)	Cetirizine [R06AE07] (4.1)	Cyproterone/estrogen [G03HB01] (3.2)	Amoxicillin [J01CA04] (3.6)
Fluticasone [R03BA05] (3.8)	Budesonide [R03BA02] (4.3)	Budesonide [R03BA02] (3.5)	Cetirizine [R06AE07] (2.5)	Fluticasone [R03BA05] (3.4)
Budesonide [R03BA02] (3.6)	Clarithromycin [J01FA09] (3.8)	Amoxicillin [J01CA04] (3.4)	Beclametasone [R03BA01] (2.4)	Budesonide [R03BA02] (2.9)
Fluisolide [R03BA03] (3.6)	Fluisolide [R03BA03] (3.1)	Methylphenidate [N06BA04] (3.2)	Amoxicillin [J01CA04] (2.3)	Cetirizine [R06AE07] (2.6)
		Salmeterol/other drugs for obstructive airway diseases [R03AK06] (2.7)	Ferrous sulfate [B03AA07] (2.1)	Clarithromycin [J01FA09] (2.2)
		Clarithromycin [J01FA09] (2.7)	Methylphenidate [N06BA04] (1.8)	Sodium fluoride [A01AA01] (1.9)
		Desmopressin [H01BA02] (2.3)	Salmeterol and other drugs for obstructive airway diseases [R03AK06] (1.7)	Fluisolide [R03BA03] (1.7)
		Montelukast [R03DC03] (1.7)	Desloratadine [R06AX27] (1.6)	Methylphenidate [N06BA04] (1.6)

Table 3. Drugs* that cover 50% of the total drug exposure in person years by age-categories (continued)

Age 0 to < 2 years	Age 2 to ≤ 5 years	Age 6 to ≤ 11 years	Age 12 to< 18 years	Total
		Fluticasone (nasal) [R01AD08] (1.7)	Budesonide [R03BA02] (1.6)	Salmeterol/other drugs for obstructive airway diseases [R03AK06] (1.6)
		Terbutaline [R03AC03] (1.6)	Fluticasone [R03BA05] (1.6)	Terbutaline [R03AC03] (1.5)
			Levocetirizine [R06AE09] (1.4)	Cyproterone/estrogen [G03HB01] (1.2)
			Gestodene/estrogen [G03AA10] (1.4)	Fluticasone [R01AD08] (1.1)
			Clarithromycin [J01FA09] (1.3)	Montelukast [R03DC03] (1.1)
			Fluticasone (nasal) [R01AD08] (1.3)	Salbutamol and other drugs for obstructive airway diseases [R03AK04] (1.1)
			Terbutaline [R03AC03] (1.2)	
			Mometasone [R01AD09] (1.1)	

* drug [5th ATC level] (% of total exposure in PY)

on these exposure data, for the age 0 to <2 years, events with IR >114/100,000 PYs (at RR≥2), IR >18/100,000 PYs (at RR≥4) and IR >7.8 (at RR≥6) can be detected. For the age 2 to ≤5 years, events with IR >73/100,000 PYs (at RR≥2), IR >11/100,000 PYs (at RR≥4) and IR >5.4 (at RR≥6) can be detected. For the age 6 to ≤11 years, events with IR >118/100,000 PYs (at RR≥2), IR >18/100,000 PYs (at RR ≥4) and IR >8.1 (at RR≥6) can be detected. Finally for the age 12 to <18 years, events with IR >111/100,000 PYs (at RR≥2), IR >17/100,000 PYs (at RR≥4) and IR >7.6/100,000 PYs (at RR≥6) can be detected (*data not shown*).

Data from 90% of the total drug exposure (represented by 158 drugs) will allow detection of events with IR >387/100,000 PYs (at RR≥2 and RR≥4) and IR >173/100,000 PYs (at RR≥6) for 0 to <2 years (67 drugs); IR >281/100,000 PYs (at RR≥2 and RR≥4) and IR >125/100,000 PYs (at RR≥6) for 2 to ≤5 years (86 drugs); IR >313/100,000 PYs (at RR≥2 and RR≥4) and IR >140/100,000 PYs (at RR≥6) for 6 to ≤11 years (125 drugs); and IR>258/100,000 PYs (at RR≥2 and RR≥4) and IR>115/100,000 PYs (at RR≥6) for the age 12 to <18 years (165 drugs) (*data not shown*).

DISCUSSION

There is a growing number of initiatives evaluating the use of EHR databases as a source for safety signal detection[188, 189, 204, 205]. Although some of these include data on children and adolescents, we are not aware of any specific analyses that have been carried out regarding the paediatric population. To our knowledge, this is the first study that explores the feasibility of using EHR databases as a source for prospective safety signal detection in children and adolescents.

Despite the large number of children and adolescents included in the EU-ADR system, the number of drugs that have enough exposure to study weak, moderate or strong associations with the events currently monitored in EU-ADR network is limited. For a rare event like anaphylactic shock (IR: 3.2/100,000 PYs) there were no drugs with enough exposure to study a weak association (RR≥2), there were 8 drugs to study a moderate association (RR≥4), and 20 drugs to study a strong association (RR≥6). These numbers are low compared to the total of 2,170 different drugs prescribed in the paediatric population. There was enough exposure to monitor a wide range of IRs for varying magnitudes of risks mainly for drugs that are known to be chronically used in children (e.g. anti-infective drugs, respiratory drugs and hormones)[122]. An important group of drugs for which safety alerts concerning the use in children and adolescents have been issued in recent years are central nervous system drugs: ADHD drugs, anti-epileptics, antidepressants and analgesic drugs[197]. Methylphenidate was the only neurological drug within the group of drugs that covered 50% of the total drug exposure in PYs.

This study showed that within the paediatric population of the EU-ADR database network, drug exposure is low and that a limited number of drugs cover the majority of the prescriptions. The 1.6 million PYs of exposure were distributed over 2,170 individual drugs, compared to 2,289 for the overall population (all ages) in EU-ADR (95%). Of these 2,170 drugs, only 18 represented 50% of the entire exposure time and 158 drugs covered 90% of the total drug exposure time. This knowledge places the number of drugs having enough exposure to detect weak, moderate or strong associations in another context. In view of the total exposure time in PYs of all drugs, the number of drugs that have enough exposure to study anaphylactic shock is therefore relatively high. The 20 drugs that have enough exposure to study a strong association with anaphylactic shock (at RR≥6) represent 52.1% of the total drug exposure. As illustrated in the current study, moderate associations can be studied for half of the total drug exposure, for events having IRs of ≥10/100,000 (29 drugs, covering 60% of the total exposure), while for events having IRs of ≥50/100,000 also weak associations can be studied (20

drugs, covering 52% of the total exposure). It should be noted that these results have not been corrected for multiple testing.

The study is also limited by the low IRs of the 10 adverse events as directly derived from the EU-ADR data within the paediatric population. The low IRs were expected, since the events were chosen based on safety issues, which were more relevant in adults. Furthermore, the mechanisms of action of certain adverse events differ between children and adults. For example, hip fractures, which have the highest IR within this population (15.3/100,000 PYs), is caused by a high-energetic trauma in 85-90% of the cases in children and are likely unrelated to drug use[206, 207], while in adults the main causes are falls and osteoporosis, which may be associated with the use of certain drugs. The causal pathway for this particular event, make it less important to study in children. Also, since the symptoms of the same condition can differ between children and adults, there is a higher chance of misclassification if this is not accounted for in the selection of cases. In future initiatives to set up a drug surveillance systems for the paediatric population using EHRs, it is very important to choose age-appropriate events with age-appropriate symptoms because, as we demonstrated, events with a higher incidence in children (such as fever convulsions), require less PYs of exposure to study associations.

As emphasized in the recently published CIOMS (Council for International Organizations of Medical Sciences) VIII report, an important unaddressed question is whether the positive predictive value of mining longitudinal EHR database as a source for signal detection will be higher than data mining in SRSs[150]. Trifirò and colleagues have tried to address this issue in a study reporting where potential signals derived from the EU-ADR network were compared with signals derived from SRSs[208]. The SRSs were more likely to detect potential signals for events with a low incidence in the general population and commonly regarded as drug-induced like bullous eruptions and anaphylactic shock. At the same time, it was noted that systems like EU-ADR may complement traditional SRS in the detection of adverse events that are frequent in the general population and are not commonly regarded to be drug-induced. This is in line with the results we obtained specifically for the paediatric population. For events with a low IR and a high probability to be drug-induced only a small number of drugs had enough exposure to detect potential safety signals. For events with a high IR a larger number of drugs could be studied. This makes studies and networks like EU-ADR an important supplement to the existing SRS. It is also important to note that although the number of drugs that can be studied for rare events is low the drugs that can be studied have a relatively large exposure within the population and hence, EHR databases appear to be able to detect associations for drugs that are

frequently used. It is known that ADRs have the highest chance to be detected (and reported) at the beginning of the drug therapy, since at this time both the treating physician and the patients are most aware of potential AEs. Because of the longitudinal nature of the data collection in EHR databases, signals may also be detected after long-term use of drugs, even for rare diseases, and may thereby further complement SRSs.

Limitations and Future directions

Our study illustrates that the capacity of EHR databases as a source for safety signal detection is not primarily only limited by the size of the population, but is mainly hampered by the fact that the majority of the drugs are prescribed very rarely in this population and the variation is small; 53% of the drugs had an exposure of less than 10 PYs and 88% of the drugs had an exposure of less than 500 PYs. We emphasize that the results should be interpreted within the context of the data sources which gave rise to these results. Since the databases are primary care-based, specialist prescriptions (e.g., for antineoplastic drugs) are only captured in the system if continued by the GP. Expansion of the database network to include other populations would be necessary to capture all drugs prescribed in the population, not only to increase the size of the studied population, but also to increase the variation in prescribing patterns.

Global collaboration will be necessary for further development of paediatric drug safety monitoring systems using EHRs, although such collaborations may still be incapable of studying the majority, if not all, drugs used in children and adolescents.

CONCLUSION

Drug use in children is rare and shows little variation; only 18 out of the total 2,170 prescribed drugs make up half of the total exposure to drugs in the paediatric population in EU-ADR. The number of drugs with enough exposure to detect safety signals using EHR for rare events in children and adolescents is limited. Mining within EHR databases seems especially promising for events that have a high background incidence in the paediatric population and for drugs with a large amount of exposure. Inter-continental collaboration will be necessary to gain enough statistical power for paediatric safety signal detection.

Appendix

Incidence rates of important events in terms of drug safety in children and adolescents and number of drugs at 5th ATC (chemical substance level) that have enough PY of exposure to study a potential signal.

Event	Estimated incidence based on literature	RR≥2	RR≥4	RR≥6
Suicide				
6-≤11 years	2/100,000 PY	0	0	1
12-<18 years	10/100,000 PY	0	9	25
Suicide attempt				
6-≤11 years	150/100,000 PY	15	80	129
12-<18 years	750/100,000 PY	188	288	423
Febrile convulsions				
0-<2 years	1,400/100,000 PY	50	132	188
2-≤5 years	1,400/100,000 PY	70	206	294
Epilepsy				
0-<2 years	75/100,000 PY	3	24	44
2-≤5 years	75/100,000 PY	8	33	59
6-≤11 years	75/100,000 PY	10	50	83
12-<18 years	75/100,000 PY	10	73	121

Age-specific incidence rates for the additional events were obtained from the literature. (1) Incidence for completed suicide: Incidence rates in literature for completed suicide range from 1.7-3.5 / 100,000 PYs under the age of 15 and from 9.9-20.3 for the age between 15 and 17-24.[1-3] For the current study we explored the power of the EU-ADR system for detecting (i) an incidence of 2 / 100,000 PYs for the age-category 6-≤11 years, and (ii) for an incidence of 10 / 100,000 PYs for the age-category 12-<18 years. (2) Incidence for suicide attempt: Incidence rates for suicide attempt are more difficult to quantify. It has been estimated that suicide attempts have a 50 to 100 times higher incidence than suicide. We therefore multiplied the incidence of suicide with 75 and explored the power of the EU-ADR system for detecting (i) an incidence of 150 / 100,000 PYs for the age- 6-≤11 years, and (ii) for an incidence of 750 / 100,000 PYs for the age-category 12-<18 years (3) Incidence of febrile convulsions: Febrile seizures are common up to the age of 4 with a reported incidence of 14 per 1,000 person-years up in Finland.[4] For the current study we explored the power of the EU-ADR system for detecting an incidence of 1,400 / 100,000 PYs for the age-categories 0-<2 years and 2-≤5 years only. (4) Incidence of seizures and convulsions: The incidence of epilepsy, defined as recurrent unprovoked seizures) in children and adolescents is estimated at 50-100/100,000 PYs.[5] The incidence is highest in the first year of life, but no exact incidences are known. For the current study we explored the power of the EU-ADR system for detecting an incidence of 75 / 100,000 PYs for all age-categories.

CHAPTER 5

Signal Detection and Verification using Electronic Health Care Databases

5.1. Signal Detection of Potentially Drug-Induced Acute Liver Injury in Children using a multi-country Healthcare Database Network

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ABSTRACT

Background
Data mining in spontaneous reporting databases has shown that hepatic injury is infrequently reported as ADR in children.

Objectives
i) To identify drugs potentially associated with acute liver injury (ALI) in children and adolescents using EHR data; and ii) to evaluate the significance and novelty of these associations.

Methods
We identified potential cases of ALI during exposure to any prescribed/dispensed drug for individuals <18 years old from the EU-ADR network, which includes 7 databases from three countries, covering the years 1996-2010. Several new methods for signal detection were applied to identify all statistically significant associations between drugs and ALI. A drug was considered statistically significantly associated with ALI, using all other time as reference category, if the 95% CI lower band of the RR was >1 and in the presence of ≥3 exposed cases of ALI. Potentially new signals were distinguished from already known associations concerning ALI (whether in adults and/or in the paediatric population) through manual review of published literature and drug product labels.

Results
The study population comprised 4,838,146 individuals ≤18 years, who contributed overall 25,575,132 person-years of follow-up. Within this population, we identified 1,015 potential cases of ALI. Overall, 20 positive drug-ALI associations were detected. The associations between ALI and domperidone, flunisolide, and human insulin were considered as potentially new signals. Citalopram and cetirizine have been previously described as hepatotoxic in adults but not in children, while all remaining associations were already known in both adults and children.

Conclusion
Data mining of multiple EHR databases for signal detection confirmed known associations between ALI and several drugs and identified some potentially new signals in children, that require further investigation through formal epidemiologic studies. This study shows that EHRs may complement traditional SRSs for signal detection and strengthening.

BACKGROUND

Drug-induced acute liver injury is more likely to occur in the post-marketing rather than in pre-marketing setting as the incidence is low. This is particularly true in children, since they are not frequently included in clinical trials and, if included, follow-up is usually short. In an earlier publication, based on data from the WHO-UMC SRS database, we showed that liver toxicity in children is infrequently reported as adverse drug reaction (only 1% of total reports in the paediatric population) with acetaminophen, anticonvulsants, and anti-tuberculosis agents being the most frequently implicated drugs[101]. It is however well known that the reporting of ADRs is strongly underestimated[101, 209].

In the aftermath of the rofecoxib safety concerns, several initiatives in both the US and EU started to explored the use of routinely-collected data from HER databases as source for signal detection/refinement[90, 91, 188, 189, 210]. The EU-ADR Project is a collaboration of 18 public and private institutions in the EU representing academic research, general practice, healthcare services administration, and pharmaceutical industry that has produced an integrated system of EHR databases for drug safety surveillance[92]. The large population coverage of the EU-ADR network make it particularly suitable for drug safety signal detection in special subpopulation such as the paediatric one, but its potential in this setting has not been studied yet.

The aim of this study was to identify drugs associated with ALI in children and adolescents in the EU-ADR database network. We further investigated these potential signals by scrutinizing whether these drug-ALI associations have been previously reported in the adult and/or paediatric population.

METHODS

Setting

Healthcare data from January 1st 1996 to December 31st 2010 were retrieved from the EU-ADR database network, which has been described in depth in previous publications[92, 187]. For this study we used only paediatric data from seven European EHR and claims databases originating from three countries. Health-Search/CSD LPD (HSD) and Pedianet from Italy and Integrated Primary Care Information (IPCI) from the Netherlands are population-based electronic medical record databases, which include demographic and clinical information. The Aarhus University Hospital Database (Aarhus, Denmark), PHARMO research database (Netherlands), and the regional Italian databases of ARS of Lombardy and Tus-

cany are all comprehensive record-linkage systems in which drug dispensing data of a well-defined population are linked to a hospital discharge diagnoses and other registries collecting clinical information (e.g., laboratory tests). Most healthcare services, including pharmacy services and hospitalizations are covered by the national health system in Italy and Denmark and by obligatory health insurance in the Netherlands. In all of these countries GPs or FPs serve as "gatekeepers" of the healthcare system.

Cohort definition and follow-up time

The study population included all children and adolescents younger than 18 years who were registered within one of the above databases for at least one year. This one-year requirement was waived for newborns and infants younger than one year during the study period whose follow up started immediately at date of birth or at their respective registration. Follow-up of all other patients started from cohort entry until the first occurrence of any of the following events, whatever came first: first diagnosis of ALI; transferring out of the practice; 18th birthday; death; or end of the study period (December 31st 2010).

Acute liver injury ascertainment

Both diagnostic codes and laboratory values, whenever available, were used for potential case of ALI ascertainment in different databases. As databases participating in the study use different terminologies for coding medical diagnoses and contain different types of information, a process of translation of coding algorithms in different databases was set up, which was based on identification of ALI-related medical concepts in the Unified Medical Language System1 (UMLS1) and projection of these codes into different terminologies: (a) International Classification of Primary Care (ICPC) for IPCI; (b) International Classification of Diseases 9th revision-Clinical Modification (ICD-9 CM) for ARS, HSD, Lombardy, Pedianet and PHARMO; and (c) ICD-10th revision for Aarhus. IPCI, HSD, and Pedianet also explored free text within the clinical narratives, using specific key words relevant to ALI, as well as pertinent laboratory examinations, whenever available[195, 211]. The UMLS1 codes and coding algorithm for ALI are listed in **Appendix**.

To prevent finding spurious associations, ALI potential cases due to other specified causes, such as viral infections, hepatic neoplasm, autoimmune hepatitis, genetic and metabolic disorder-related hepatopathy (e.g. hemochromatosis, α1-antitrypsin deficiency, Wilson Disease, Gilbert Syndrome) and biliary tract diseases, were not included.

Drug exposure

Drug exposure was assessed using data from prescriptions or pharmacy dispensing. Because of the nature of these databases, exposure to over-the-counter medications, OTC, such as acetaminophen, is not captured. All databases code drugs using the ATC classification. The number of PYs of exposure was calculated per single compound (ATC 5th level). The duration covered by each prescription/dispensing was estimated by the legend duration (if dosing regimen was available) or based on the defined daily dose (DDD)[212].

Data analysis

Data on patient demographics, clinical events (i.e. ALI), and prescriptions were locally generated from each database and formatted towards a simple common data model[213]. Based on the common data model, data were aggregated by databases using a custom-built software Jerboa©[187].

In EU-ADR, several statistical methods have been developed and tested for signal detection. Based on a reference set (i.e. surrogate gold standard) and performance analysis, the best performing method was the Longitudinal Gamma Poisson Shrinker (LGPS), which is an adaptation of a method used for signal detection in SRS, i.e. Gamma Poisson Shrinker[214]. LGPS estimates the age- and sex-adjusted incidence rate ratios, during the exposure of interest against all other follow-up time (on other drugs and off drugs) as reference while additionally applying Bayesian shrinkage (RR_{LGPS})[214, 215]. All the drugs for which we observed at least 3 exposed cases of ALI and with a lower 95% CI of RR_{LGPS} >1 were considered as potential signals[216]. In a sensitivity analysis, we compared the risk estimates derived from LGPS with the estimates from the self-controlled case series (SCCS) method. Confounding by indication (or contraindication) may arise when a drug treatment serves as a marker for a clinical characteristic or medical condition that triggers the use of the treatment and that, at the same time, increases the risk of the outcome under study. In SCCS analysis, cases serve as their own controls, thereby allowing time-fixed confounding factors, known and unknown (e.g. confounding by indication), to be controlled for implicitly[214]. As in the main analysis, a drug-ALI association was considered as statistically significant if the lower 95% CI of the RR, calculated using SCCS, was >1 with at least 3 exposed cases.

In addition, for each potential drug-ALI signal, we evaluated the possible role of protopathic bias by applying LEOPARD (Longitudinal Evaluation of Observational Profiles of Adverse events Related to Drugs)[214, 217]. Since protopathic bias occurs when a drug is prescribed for an early manifestation of a disease that has not yet been diagnosed, the number of the specific drug prescriptions initiated increases after the event date (relative to the period prior to the event date),

indicating that the drug is used to treat prodromal symptoms of the event, rather than cause it. Accordingly, for every suspect drug-ALI association, LEOPARD compared the prescription rates within a fixed window of 25 days prior to and 25 days after the occurrence of ALI. Thus, the method flagged the drug-ALI associations as potentially due to protopathic bias if the prescription rate after the event was higher than the prescription rate before the event[215].

For all signals that could not be explained by protopathic bias, based on current scientific evidence, we assessed whether the association was known, unknown or incompletely documented and we evaluated also the possible biological plausibility. For this purpose, we reviewed main drug-related information sources: a) Micro-

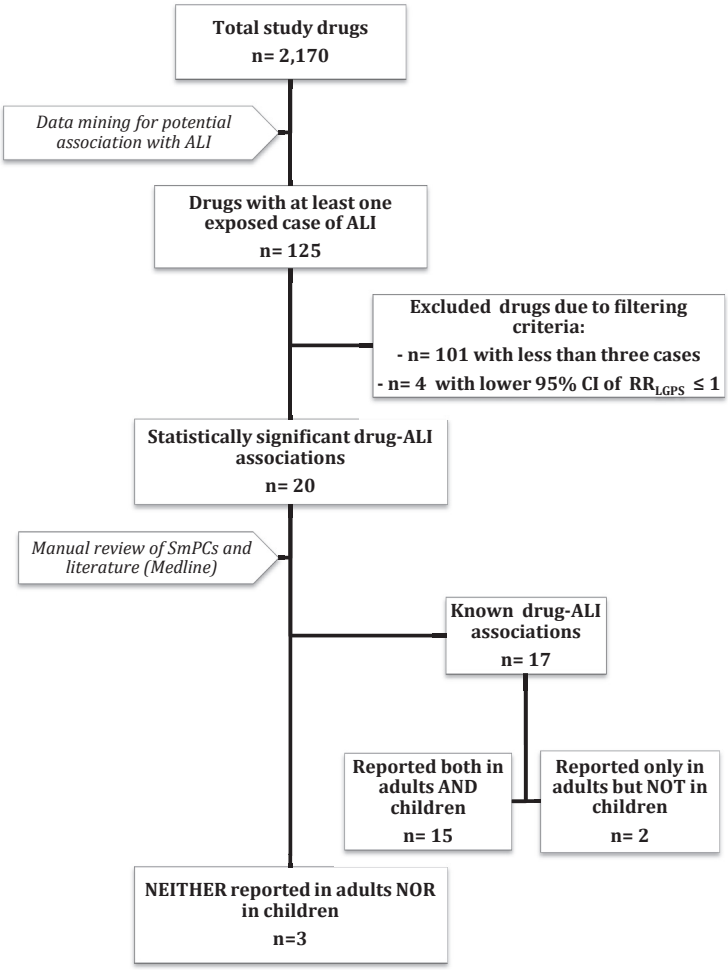

Figure 1. Schematic diagram for the identification of potentially new signals of acute liver injury in children and adolescents.
Statistically significant association means drugs with ≥3 exposed cases of ALI and a lower band of 95% CI of RR >1 when applying LGPS method.

medex (http://www.thomsonhc.com/hcs/librarian), and b) the SmPCs, derived from the following two databases: i) electronic Medicines Compendium (eMC, http://www.medicines.org.uk/emc); and ii) FarmaDati (www.farmadati.it). With regard to the SmPCs, we specifically looked at the sections "*Undesirable/adverse effects*" and "*Special warnings and precautions*". We additionally explored whether the drug-ALI associations were already described in the published literature (via MEDLINE: web.ebscohost.com) in adults and/or more specifically in children.

RESULTS

The paediatric population of the EU-ADR network comprised overall 4,838,146 children and adolescents (<18 years) contributing 25,575,132 PYs of follow-up in the period 1996-2010. Among 1,015 potential cases of ALI identified in this database network, 251 potential cases (24.7%) occurred during exposure to any drug (1,032,899 PYs) accounting for a crude incidence of 2.4/10,000 PYs (2.1-2.7) among exposed.

The stepwise procedure leading to identification of potential signals is schematically shown in **Figure 1**.

Table 1. Drugs used in paediatric population and those associated with ALI

Main anatomic group (ATC 1st level)	Study Drugs N= 2,170	No. of drugs with statistically significant associations* (% within anatomic group)
(A) Alimentary & metabolism	391	5 (1.3)
(N) Nervous system	269	2 (0.7)
(J) Anti-infectives for systemic use	232	6 (2.6)
(D) Dermatologicals	203	-
(C) Cardiovascular system	192	-
(S) Sensory organs	169	-
(R) Respiratory system	160	2 (1.3)
(G) Genito-urinary & sex hormones	153	-
(M) Musculo-skeletal system	104	1 (1.0)
(B) Blood and blood forming organs	97	-
(L) Antineoplastics & immunomodulators	76	2 (2.6)
(H) Systemic hormonal preparations	72	2 (2.8)
(P) Antiparasitic	35	-
(V) Various	17	-

*drugs with ≥3 exposed cases and a value and with lower band of 95% CI of RR_{LGPS}>1

Table 2. Comparison of different methods applied for signal detection concerning acute liver injury

ATC	Drugs	No. of cases	Exposure (PYs)	Crude IR/10,000 PY (95% CI)	RR_{LGPS} (95% CI)	RR_{SCCS} (95% CI)	LEOPARD[#]
A02	Ranitidine	7	3,833.86	18.3 (8.14-35.8)	43.7 (17.7-87.6)	12.9 (4.9-34.0)	yes
	Omeprazole	7	5,583.97	12.5 (5.6-24.6)	29 (9.5--60.9)	13.3 (4.9-35.6)	yes
A03	Metoclopramide	4	282.27	141.7 (47.4-336.9)	262.8 (85.7-636.2)	449.1 (104.0-1,938.7)	no
	Domperidone	3	2,903.41	10.3 (2.1-30.2)	22.9 (3.5-89.2)	35.9 (10.5-122.1)	no
A10	Insulin, human*	3	3,344.73	9.0 (1.8-26.2)	9.2 (2.3-41.2)	4.1 (0.8-20.4)	no
H02	Prednisolone	5	1,699.56	29.4 (11.2-64.5)	45.1 (12.3-104.2)	6.0 (1.9-19.6)	yes
	Prednisone	3	5,647.87	5.3 (1.5-14.2)	8.9 (2.3-39.4)	5.6 (1.8-17.9)	yes
J01C	Amoxicillin	10	59,842.54	1.7 (8.6-29.6)	3.9 (2.1-6.8)	4.2 (2.3-7.5)	no
	Penicillin V	14	6,623.26	21.1 (12.1-34.5)	17 (8.6-30.0)	16.7 (9.9-28.1)	no
	Amoxicillin/clavulanate	9	81,268.6	1.1 (0.5-2.0)	3.5 (1.8-6.3)	2.7 (1.4-5.0)	yes
J01D	Cefaclor*	3	15,857.28	1.9 (0.5-5.1)	3.6 (1.2-8.8)	2.6 (0.8-9.3)	no
J01F	Erythromycin	4	3,722.42	10.7 (3.6-25.5)	6.9 (2.4-21.1)	12.3 (4.1-37.2)	no
	Clarithromycin	5	36,597.8	1.4 (0.5-3.0)	4 (1.7-8.3)	6.8 (3.3-13.9)	no
L04	Methotrexate	8	840.8	35.7 (9.9-95.2)	211.3 (98.8-401.5)	180.1 (20.7-1,568.3)	yes
	Azathioprine	3	618.99	129.2 (61.0-243.9)	48.6 (5.8-153.3)	4.8 (1.1-21.1)	yes
M01	Diclofenac	5	2,290.91	21.8 (8.3-47.8)	31.2 (7.3-76.8)	39.8 (16.7-94.6)	yes
N03	Valproic acid	4	12,502.01	3.2 (1.1-7.6)	5.1 (1.9-12.3)	24.4 (5.4-111.0)	no
N06	Citalopram	3	2,878.31	10.4 (2.9-27.8)	5.6 (1.8-17.6)	7.6 (1.2-50.5)	yes
R03	Flunisolide*	4	27,548.87	1.5 (0.5-34.5)	3.4 (1.3-7.6)	2.7 (0.9-8.1)	no
R06	Cetirizine	5	43,255.13	1.2 (0.4-2.5)	2.5 (1.0-5.1)	3.0 (1.2-7.7)	yes

Drugs with ≥3 exposed cases of ALI and a lower band of 95% CI of RR >1 when applying LGPS method.

*not statistically significant association when using SCCS method;

[#]Yes= protopathic bias is likely to be present, No= protopathic bias is unlikely to be present.

Of 2,170 drugs prescribed/dispensed to the paediatric population during the study period, almost 6% (N= 125) were involved in at least one potential case of ALI. However, only for 20 drugs were at least three exposed cases observed across all databases (**Table 1**). ALI potential cases occurred most often during use of anti-infectives for systemic use (N= 6) and drugs for alimentary tract and metabolism (N= 5) (**Table 1**).

Detection of statistically significant drug-ALI associations

The drugs that were associated with ALI, using unexposed period to the drug of interest as reference, are given in **Table 2**. Ranked by the strength of the RR_{LGPS} for ALI, the top 10 drugs included metoclopramide, methotrexate, azathioprine, prednisolone, ranitidine, diclofenac, omeprazole, domperidone, phenoxymethylpenicillin (i.e. penicillin V), and human insulin. Applying the SCCS method, most of these drugs remained statistically significantly associated with ALI, except for insulin, cefaclor, and flunisolide. The magnitude of risk decreased substantially for the anti-ulcer drugs (ranitidine and omeprazole), for the corticosteroids (prednisolone and prednisone), and the immunosuppressants (azathioprine and methotrexate) pointing towards confounding by indication. The association became stronger for metoclopramide, domperidone, diclofenac, valproic acid and erythromycin (**Table 2**). Notably, the associations for insulin, cefaclor and flunisolide were not confirmed using this method only because of lack of power, although the potential risk remained high. Based on the results derived from LEOPARD, 10 of the drugs with statistically significant associations using LGPS and SCCS were classified as potentially due to protopathic bias (**Table 2**).

Evaluation of the significance and novelty of the signals

Table 3 describes the available knowledge on the 20 statistically significant drug-ALI associations we observed. Three associations were identified as potentially new signal, i.e. they had not been previously described in the literature either in adults or in children: domperidone, human insulin and flunisolide. For these drugs, there is currently no mention of ALI as possible adverse event either in the SmPCs or in the published literature, irrespective of the age group. In addition, two other drugs, citalopram and cetirizine, have never been described to be associated with hepatotoxicity in the paediatric population so far, although ALI has been documented in adults and is reported in the SmPCs.

Table 3. Novelty of statistically significant drug-ALI associations

Drugs	SmPCs[*]	Literature (Medline)[#]		Use not authorised in children[$]
		Adults	Children[&]	
Ranitidine	yes	yes	yes	
Omeprazole	yes	yes	yes	
Metoclopramide	no	yes	yes	<15 years
Domperidone	no	no	no	
Insulin (human)	no	no	no	
Prednisolone	yes	yes	yes	
Prednisone	yes	yes	yes	
Amoxicillin	yes	yes	yes	
Phenoxymethylpenicillin	yes	yes	yes (in co-therapy with erythromycin)	
Amoxicillin/clavulanate	yes	yes	yes	
Cefaclor	yes	no	yes	
Erythromycin	yes	yes	yes	
Clarithromycin	yes	yes	yes	
Azathioprine	yes	yes	yes	
Methotrexate	yes	yes	yes	
Diclofenac	yes	yes	yes	
Valproic acid	yes	yes	yes	
Citalopram	yes	yes	no	<18 years
Flunisolide	no	no	no	
Cetirizine	yes	yes	no	

[*]SmPCs reviewed: i) Micromedex (http://www.thomsonhc.com/hcs/librarian); ii) electronic Medicines Compendium (eMC, http://www.medicines.org.uk/emc); and iii) FarmaDati (www.farmadati.it).

[#] literature (via MEDLINE: web.ebscohost.com).

[&] yes= drug-ALI association was reported also or only in children

[$]use not approved for the indicated age range.

DISCUSSION

In this study, three not previously documented signals of acute liver injury in children and adolescents were identified using "real–world" data from a combination of multiple European healthcare databases.

Among all the drugs being prescribed/dispensed in children and adolescents during the study period, around 1% (20/2,170) was potentially associated with ALI. ALI occurred most frequently during use of antibacterial agents and drugs for peptic ulcer and gastro-oesophageal reflux disease. These data are very much in line with previous findings from an analysis of the WHO spontaneous reporting database[101].

According to the definition of safety signals by Hauben and Aronson[218], not all statistically significant associations should be regarded as potential new signals. In a way to detect whether the detected drug-ALI associations (i.e. possible signal) was newly discovered, we performed a manual review of the SmPCs and other main drug information sources including Micromedex and Medline and scientific literature. To the best of our knowledge, three of the drug-ALI associations were not previously described in the literature, neither in adults nor in children, and also not labelled in the SmPCs. These drugs were domperidone, flunisolide and insulin (human). Two other drug-ALI associations (citalopram, cetirizine) were not previously described in children but have already been described in adults.

Potentially new signals

The association between domperidone and ALI was identified as a potential new signal. However, prodromal signs/symptoms of liver injury such as nausea and vomiting, represent the main indication for domperidone intake. Therefore, although LEOPARD did not automatically flag this as protopathic bias, we cannot exclude that this is a spurious association due to protopathic bias. If indeed, the interval between incriminated prodromal symptoms and onset of event is larger than 25 days, protopathic bias will not be detected by LEOPARD[214].

Flunisolide is a synthetic inhaled corticosteroid with potent topical anti-inflammatory activity, with an oral bioavailability ranging from 7% to 20%[219, 220]. After gastrointestinal and lung absorption, the drug undergoes rapid and extensive first-pass metabolism by the liver to an inactive 6-beta-hydroxylated metabolite. Systemic effects have not been reported for the commonly used doses. Higher doses of flunisolide, as well as the other inhaled corticosteroid, may develop adverse events similar to those occurring during the corticosteroid systemic use, due to an increased oral adsorption of the medicine. Although signs/symptoms of liver injury are reported as undesirable side class effects with systemic use of overall corticosteroids, liver injury is not specifically mentioned for inhaled corticosteroid, including flunisolide. Different pharmacokinetic characteristics across inhaled corticosteroids might suggest differences in the occurrence of adverse drug reactions[221]. A comparison among inhaled corticosteroid specifically concerning hepatotoxicity needs to be further investigated.

Insulin therapy is indicated in children and adolescents with type 1 diabetes and in children with ketosis or diabetic ketoacidosis when the distinction between type of diabetes is unclear. There are no reports in the literature associating human insulin with (acute) liver injury. It is, however, possible that insulin induces undesired weight gain from hunger triggered by insulin-induced hypoglycemia. Comparative trials of patients with type 2 diabetes found that weight gain and

risk of hypoglycemia might occur during the use of human insulin more than the analogues[222, 223]. Moreover, weight gain could lead to hepatic steatosis, an aetiopathologic sign of non-alcoholic fatty liver disease (NAFLD), explaining the potential role of human insulin in this type of liver injury[224]. On the other hand, since NAFLD has been reported to increase the risk of type 2 diabetes[225], and potentially the use of insulin, it is unclear the role of insulin in this association.

The associations identified for flunisolide and human insulin deserve a separate discussion because these associations were not confirmed by SCCS analysis, suggesting the influence of potential confounder factors on the estimations. This seems particularly true for insulin, for which the nature (causal inference) of the connection to NAFLD remains a matter of speculation[226]. However, although not significant (meaning that we had insufficient power for this statistical test only because of low prevalence of exposure), the associations were high for flunisolide (RR_{SCCS}= 2.7) and human insulin (RR_{SCCS}= 4.1). Accordingly, we still cannot rule out that these drugs are truly associated with increased risk of ALI and the associations of ALI, but these drugs should be investigate in a formal pharmacoepidemiological study in a wider paediatric setting in order to confirm or confute these potential signals.

Potentially new signals in paediatrics

Citalopram is a selective serotonin reuptake inhibitor and widely used antidepressant. Rare instances of acute, clinically apparent episodes of liver injury with moderate or marked liver enzyme elevations with or without jaundice have been described in less than 1% of adults within 6 to 10 weeks, or earlier, of citalopram therapy. One study (RCTs or pharmacoepidemiological studies) investigated the use of central nervous system agents and risk of idiosyncratic drug-induced liver injury in children but did not identify citalopram as a suspect drug[82].

Cetirizine is a second generation antihistamine used for the treatment of allergic rhinitis, angioedema and chronic urticaria. Cetirizine and analogues have been related to rare, isolated cases of clinically apparent acute liver injury with a pattern ranging from cholestatic hepatitis to hepatocellular jaundice in adults[227-230]. Nevertheless, as urticaria may represent a prodromic sign of underlying liver disease, potential protopathic bias as alternative explanation for the association cannot be excluded and is supported by the results from LEOPARD.

Although the association between ALI and metoclopramide was already described in two epidemiologic studies, including within the paediatric population, showing a risk of liver injury for metoclopramide ranging from moderate to low[70, 231], we do believe that here as well, the potential of protopathic bias holds similar to what we described for domperidone.

Finally, data mining on EHR databases detected associations for several drugs already widely known as hepatotoxic in both adults and children[99]. The application of the LGPS as main analysis and the SCCS as sensitivity analysis suggested that the system can produce reliable results.

In fact, when applying SCCS method, the associations from LGPS were amplified or reduced, but still remained for all drugs already known to be hepatotoxic, except for cefaclor. Similarly, using LEOPARD to filter signal due to protopathic bias improved the overall performance of signal detection. LEOPARD flagged associations with anti-acid drugs, as ranitidine or omeprazole as spurious. Indeed, these drugs might have been prescribed for gastric discomfort which is one of the prodromal signs of hepatic injury. On the other hand, it was rather unexpected that LEOPARD was not able to detect this bias for metoclopramide or domperidone. This might be explained by the ±25 day-window that LEOPARD (by default) applies as described above. Protopathic bias was correctly captured for azathioprine, methotrexate, diclofenac and systemic corticosteroids. Indeed, these drugs are prescribed for conditions (such as rheumatic disease) associated with ALI.

Strengths and limitations

The main strength of this study is its capability to (retrospectively) observe a large number of children and adolescents in a "real-world" setting by combining data from multiple longitudinal healthcare databases. While we were able to investigate the associations between ALI and the most frequently used drugs in children in Europe[122], the system did not allow to explore over-the-counter medications, such as acetaminophen, in-hospital used drugs or less frequently prescribed drugs such as anti-tubercolosis agents and other anti-convulsants, which are well-known to be hepatotoxic in children. For instance, we found a non-statistically significant increase in risk of ALI related to the use of acetaminophen, or anti-convulsants (i.e. carbamazepine, phenobarbital, vigabatrin, or gabapentin) or other nervous system agents (i.e. atomoxetine, risperidone, sertraline, tramadol, or methadone). This result does not imply the absence of association, but rather the low prevalence of exposure of these drugs in such prescribing/dispensing registries.

We used harmonised database-specific disease codes and free text search to automatically identify liver injury from the database network. Individual causality assessment of the identified associations was not conducted. However, a previous study using US database network demonstrated that outcome misclassification does not influence the results concerning signal detection[232]. Usually, exclusion of alternative causes for the potential signal is part of an aetiology-based approach for the evaluation of a physician-reported ADR. To reproduce this process using

EHR databases, we used SCCS method to control for time-fixed confounders such as genetic factors, socio-economic status, individual frailty, and severity of underlying disease[214].

To investigate the potential of protopathic bias, we used the LEOPARD method. Despite using filtering criteria for significance and sensitivity analyses, the likelihood to obtain false positive results cannot be excluded and further validation of the newly identified associations needs to be carried out[233].

CONCLUSION

We found potentially new signals concerning ALI for flunisolide, domperidone and insulin. There was also a signal of acute liver injury in children for citalopram and cetirizine, but this association was already described in adults. All potential new signals require further evaluation in hypothesis testing studies (e.g., formal pharmacoepidemiologic studies) to better account for bias and confounding. Our findings highlight the potential of EHR databases to complement traditional SRS for drug safety signal detection and strengthening in a paediatric setting. However, combining data from other longitudinal healthcare and paediatric-specific databases would be meaningful to gain sufficient statistical power to investigate a large range of drugs specifically used in children and adolescents.

Appendix 1

Disease Codes used for the identification of the Acute Liver Injury events in the EU-ADR network

UMLS CUI	Medical term	ICD9CM	ICD10	ICPC2005	Supplementary information
C0151766	Liver function tests abnormal finding	794.8	R94.5	B85001, A91017	ALT≥3x ULN (60 IU/L) OR AST≥3x ULN (40 IU/L) AND Total bilirubin≥2x ULN (1.2 mg/dL or 40 µmol/L)
C0001308	Acute and subacute liver necrosis (disorder)	570			
C0162557	Liver Failure, Acute	570			
C0267795	Subacute hepatic necrosis	570			
C0400929	Subacute hepatic failure				
C0019151	Hepatic Encephalopathy	572.2			
C0085605	Liver Failure		K72.9	D97007	
C0019158	Hepatitis	573.3	K75.9	D97008, D72002	
C0019193	Hepatitis, Toxic	573.3			
C0348754	Toxic liver disease		K71.9, K71		
C0451707	Toxic liver disease with cholestasis		K71.0		
C0451708	Toxic liver disease with hepatic necrosis		K71.1		
C0451709	Toxic liver disease with acute hepatitis		K71.2		
C0451713	Toxic liver disease with fibrosis and cirrhosis of liver		K71.7		
C1531701	Acute hepatic failure due to drugs				
C0400887	Nonspecific reactive hepatitis		K75.2		
C0348750	Other specified inflammatory liver diseases		K75.8		
C0400927	Hepatic failure as a complication of care				

Abbreviations: UMLS CUI= Unified Medical Language System Concept Unique Identifier; ICPC= International Classification of Primary Care; ICD= International Classification of Diseases; ALT= Alanine Aminotransferase; AST= Aspartate Aminotransferase; IU= International Unit.

Coding algorithm for acute liver injury:

a) Occurrence of at least one diagnostic code/key words corresponding to the selected UMLS concepts (as reported in GP's medical records or primary hospital discharge diagnoses)

OR

b) Occurrence of the following lab tests and test results within 7 day period:

Alanine aminotransferase ≥ 3 times the upper limit of normal (40 IU/L) OR Aspartate aminotransferase ≥ 3 times the upper limit of normal (40 IU/L)

AND

Total bilirubin ≥ 2 times the upper limit of normal (1.2 mg/dL).

5.2. Idiopathic Acute Liver Injury in Paediatric Outpatients: Incidence and Signal Detection in Two European Countries

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ABSTRACT

Background
Acute liver failure is idiopathic and drug-related in, respectively, around 50 and 15% of children. Population-based, epidemiological data about the pattern of disease manifestation and incidence of less severe acute liver injury, either idiopathic or potentially drug-attributed are limited in children and adolescents.

Objectives
(i) To assess the incidence of idiopathic acute liver injury (ALI) and its clinical features in children and adolescent outpatients; and (ii) to investigate the role of the drug as a potential cause of ALI which is considered idiopathic.

Methods
A retrospective cohort study was performed during the years 2000–2008. Data were retrieved from three longitudinal electronic healthcare databases in two European countries: Pedianet and Health Search/CSD Longitudinal Patient Database from Italy and the Integrated Primary Care Information database from The Netherlands. Cases of idiopathic acute liver injury in population aged <18 years were identified by exclusion of all competing causes of liver injury (e.g. viral, autoimmune hepatitis), according to CIOMS criteria. The potential role of drug exposure as actual underlying cause of idiopathic ALI was detected through signal detection mining techniques. Both pooled and country-specific incidence rates (IR/100,000 PYs) of idiopathic ALI and pooled adjusted rate ratios (RR) of drugs identified as a potential cause of idiopathic ALI, plus 95 % confidence intervals (CI) were estimated using the custom-built software Jerboa®.

Results
Among 785 definite cases of idiopathic ALI, the pooled IR was 62.4/100,000 PYs (95 % CI 58.1–66.8). The country-specific IR was higher in Italy (73.0/100,000 PYs, 95 % CI 67.8–78.4) than in The Netherlands (21.0/100,000 PYs, 95 % CI 16.0–27.2) and increased with age in both countries. Isolated elevations of liver enzymes were reported in around two-thirds of cases in Italy, while in The Netherlands the cases were more often identified by a combination of signs/symptoms. Among drugs detected as potential underlying cause of idiopathic ALI, clarithromycin (RR 25.9, 95 % CI 13.4–50), amoxicillin/clavulanic acid (RR 18.6, 95 % CI 11.3–30.6), and amoxicillin (RR 7.5, 95 % CI 3.4–16.8) were associated with the highest risk compared to non-use.

Conclusion

The incidence of idiopathic ALI in paediatrics is relatively low and comparable with adults. Clinical presentations differ between the two European countries. Signal detection in healthcare databases allowed identifying antibiotics as the drugs mostly associated with ALI with apparently unknown aetiology.

BACKGROUND

A previous epidemiologic study performed on a liver disease registry documents that the aetiology of acute liver failure is not determined, i.e. idiopathic, in almost 50% of paediatric patients, is drug-related in around 15%, while other causes (i.e., autoimmune hepatitis or hepatopathy due to metabolic diseases or viral infections) account for less than 10% each[80]. Published data on the incidence and characteristics of less severe acute liver injury are scarce because initially the disease is asymptomatic and, thus, difficult to recognize[65]. So far, information on clinical features of ALI are only available from disease registries[73, 82, 234].

The increasing availability of EHR and claims databases allows for the conduct of population-based epidemiologic studies in larger populations, including children and adolescents[91]. To date, no data are available on the pattern of disease manifestation, severity of the features, and incidence of ALI, either idiopathic or potentially drug-attributed, in children and adolescents.

In this retrospective, population-based study we explored the incidence of idiopathic ALI in a general paediatric population (<18 years old) from three longitudinal EHR databases in two European countries, Italy and the Netherlands. Our main aims were to quantify the incidence of idiopathic ALI and to describe its clinical features. Additionally, we investigated the role of drug exposure as underlying potential cause of apparently idiopathic ALI by applying data mining techniques on the pooled data from the three databases.

PATIENTS AND METHODS

Source Population

A retrospective cohort study was conducted combining data from three longitudinal EHR databases in two European countries: Pedianet (from 2000 to 2008) and HSD (from 2002 to 2008) from Italy and the IPCI database (from 2001 to 2007) from the Netherlands[235]. In the Netherlands, GPs serve as gatekeepers to medical care for all patients, including children and adolescents. In Italy, children receive medical care by FPs up to the age of 14 years, and thereafter by GPs. All these databases have been proven valid data sources for pharmacoepidemiological studies[122, 236-240]. Pedianet was set up in 2000 and comprises healthcare records of around 150,000 children 0-14 years old provided by 150 FPs distributed all over Italy. HSD is a GP database that was established in 1998 by the Italian College of General Practitioners. HSD currently contains records of around 1.4 million patients (190,772 patients <18 years) from over 900 GPs throughout Italy. Pedianet

and HSD were combined together in the analysis to represent the whole Italian paediatric population (≤18 years). IPCI is a Dutch GP database that was set up in 1992 and currently contains records from 600 GPs, covering approximately 1 million patients(93,294 patients ≤18 years), with an age and gender distribution that is representative for the Netherlands.

All three databases contain anonymous data on patient demographics, reasons for visits, diagnoses from GPs/FPs and specialists, hospitalizations, drug prescriptions, laboratory and other diagnostic findings for the paediatric population. Symptoms and medical diagnoses are either registered as free text or coded using ICPC in IPCI and ICD-9-CM in HSD and Pedianet. Drug prescription data include details on product name, formulation, dosing regimen and indication of use. All drugs are coded according to the ATC classification system[241].

Study Population

The retrospective study population comprised a dynamic cohort of all children and adolescents aged <18 years, who were registered with GPs/FPs in any of the three databases for at least 6 months. Newborns in the study period immediately entered the cohort (no prior history required). Follow-up of patients started from cohort entry until one of the following events, whichever occurred first: diagnosis of liver injury, death, transferring out of the practice, end of the study period (December 31st, 2008) or 18 years of age. Patients with a diagnosis of liver injury (irrespective of the cause) prior to the study entry were excluded from the study. The study period ran from 2000 to 2008.

Outcome Definition and Case Ascertainment

According to the CIOMS criteria and previous evidence[70, 242-246], a liver injury was defined as an increase of more than two times the upper limit of the normal (ULN) range in alanine aminotransferase (ALT) or a combined increase in aspartate aminotransferase (AST), alkaline phosphatase (AP) and total bilirubin, provided that one of them was twice the upper limit of the respective normal range. Jaundice and hepatomegaly, which are suggestive of liver injury but are not sufficient by themselves for the diagnosis, were considered only in association with other specific symptoms/signs (e.g. abnormal liver enzyme values, steatosis). Patients with elevation of biochemical liver tests ≤2 ULN only in presence of isolated increase of gamma-GT or neonatal jaundice were excluded. According to our aims, we excluded all clear competing causes of liver injury: (i) viral infections (Hepatitis A virus; Hepatitis B virus; Hepatitis C virus; Cytomegalovirus; and Epstein-Barr virus); (ii) hepatic neoplasm; (iii) autoimmune hepatitis; (iv) genetic and metabolic disorders-related hepatopathy (e.g., hemochromatosis, α1-

antitrypsin deficiency, Wilson Disease, Gilbert Syndrome, etc.); (v) biliary tract diseases (i.e., biliary atresia, gallstones, cholangitis, and cholecyctitis); and (vi) abdominal trauma documented with imaging. Cases with drugs as potential cause were not excluded, as it is difficult to assess causality[244, 247].

ALI was identified through a similar stepwise approach across all three databases. First, all the potential cases of liver injury were extracted through a very broad automated search using both free text and diagnostic codes for hepatitis, liver failure, hepatopathy, hepatic steatosis, hepatic cirrhosis, chronic liver disease, or hepatic necrosis. Second, all of these records were independently validated by four medically trained investigators, two for each country, who were blinded to the drug exposure and native speakers of the language of the concerned database. For each potential case, the whole clinical diary history, including results of laboratory data, ultrasound and other diagnostic test, as well as hospital discharge summaries and specialists' letters were reviewed. All cases of chronic liver injury were excluded. Based on validation, cases were classified as: *definite*, whit a diagnosis confirmed by a specialist or laboratory data/ultrasound evidence; and *possible*, with a diagnosis made by a GP/FP. In addition, cases of isolated hepatomegaly were assessed separately. In case of disagreement between the two assessors, a third expert medical doctor arbitrated.

All cases were further characterized, whenever possible, based on the source of diagnosis. According to the CIOMS criteria[242] and to Benichou *et al.*[243], with the respect to biochemical hepatic function, the pattern of liver injury was defined as (a) *hepatocellular*, with an increase of more than twice the ULN of ALT; (b) *cholestatic*, with an increase of AP; or (c) *mixed*, when both ALT (or AST) and AP increased. Similarly, we categorized the degree of severity as *mild* (more than twice ULN), *moderate*, (more than three times ULN), *severe* (more than five times ULN), and *most severe* (more than eight times ULN)[76].

The index date was defined as the onset of the first symptom/sign related to liver injury (i.e., fatigue, weakness, anorexia, nausea, jaundice, dark urine, light stools, itching, bloating and abdominal pain). In our analysis, we only included incident cases, i.e. newly diagnosed ALI during the study period and no diagnosis in the past six months.

Drug Exposure

Drug exposure was evaluated based on prescription and dispensing data from healthcare databases. As drug prescriptions and/or dispensing are locally coded using the national product codes, which differ among countries, we defined drug exposure based on the ATC classification. The ATC code was used as the drug code in the input files for Jerboa©. Cases were considered as exposed to certain

drug if the prescription for that drug overlapped the date of onset/ascertainment of liver injury, based on the estimated duration of exposure. Each database owner estimates the duration covered by each prescription or dispensing according to the legend duration (if dosing regimen is available), or is otherwise based on the defined daily dose (DDD)[187].

Statistical Analysis

Our main analyses focused on definite cases. Jerboa© software was used to estimate the incidence rates of cases of idiopathic ALI, as both pooled and country-specific. The validity of the software has been previously described[187, 208, 214]. Briefly, Jerboa© elaborates input files with common data model (including patient unique identifier and demographics, follow-up time, exposure and outcome information) from different databases and produces as output several parameters [e.g. incidence rates (IRs), relative risks (RRs), prescription rates (Rx/PYs)], stratified by age, gender and calendar years[91, 187]. Age was categorized into four categories, based on guidelines of the ICH: <2 years, 2-≤5 years, 6-≤11 years and 12-<18 years.

To calculate the incidence rates, we considered the number of first event of ALI after a one year run-in period as numerator and the number of cumulated person-years of the study population at risk of developing first event of ALI, as denominator. IRs were expressed as rate/100,000 PYs plus 95% CI. In addition, we compared IRs between the two countries and calculated by direct method the standardized IR in Italy using the distribution of Dutch population as a reference.

To study the effect of outcome misclassification, we conducted a sensitivity analysis combining definite and possible cases as well as cases of isolated hepatomegaly.

In order to investigate the associations between drug exposure and apparently idiopathic ALI, we linked prescribing data from all combined databases to case occurrence in a temporal manner, by estimation of IRs of ALI during exposure and comparing the drug specific rates with background rates. We only considered the definite cases for this analysis. Based on the background IR of ALI, using Mantel–Haenszel method, we calculated age- and gender-adjusted rate ratios (RRs, with CI 95%), for each drug associated with at least two event occurring its use (ATC 5th level) using the non-use of the drug of interest as comparator[91, 187].

RESULTS

During the period 2000-2008, the study cohort comprised 429,772 subjects (of which 68% from Italy and 32% from the Netherlands) aged <18 years.

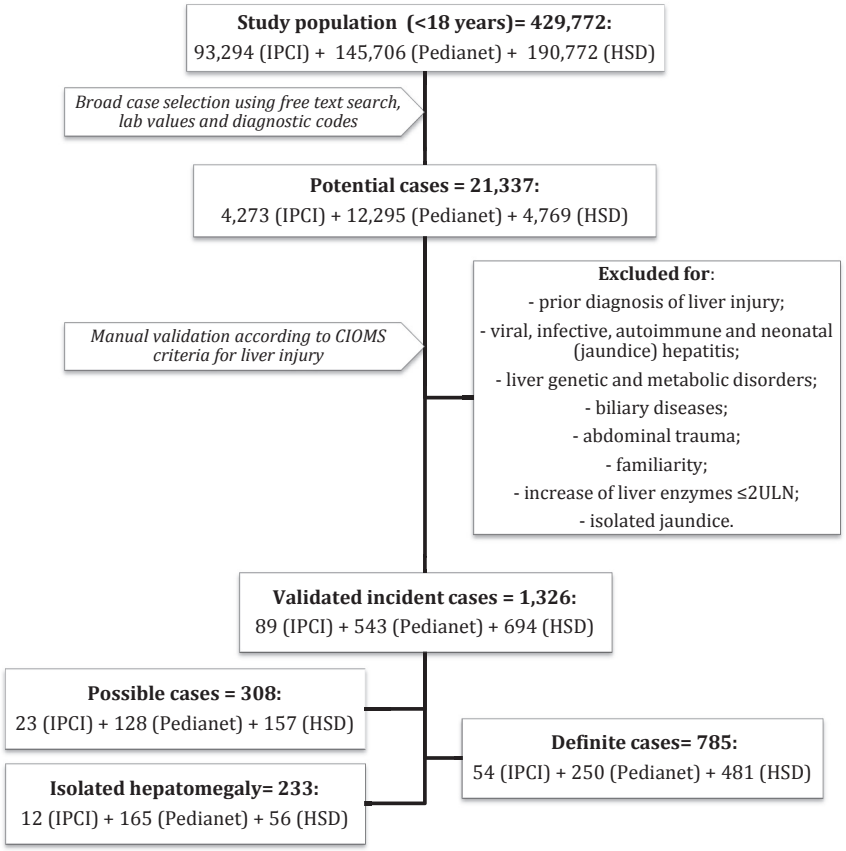

Figure 1. Flowchart of cohort and cases selection.

Abbreviation: CIOMS = Council for International Organizations of Medical Sciences; ULN = Upper Limit of Normal.

After exclusion of all clear causes of liver injury and of chronic hepatitis through case by case validation (**Figure 1**), we identified 1,326 cases of idiopathic ALI of which the majority originated from Italy (1,237 cases, 93.3%) (**Table 1**). In the two Italian databases, 731 cases (59.1% of 1,237) were classified as definite, 285 (23.0%) as possible, while 221 (17.9%) were cases of isolated hepatomegaly. In the Netherlands, 54 (60.7% of 89) were classified as definite, 23 (25.8%) as possible cases, and 12 (13.5%) as isolated hepatomegaly.

Features of Idiopathic Acute Liver Injury

An overview of the clinical features of idiopathic ALI in the general population aged 18 years or younger is described in **Table 1**. In Italy, asymptomatic liver enzyme elevations were reported in around two-thirds of definite cases (n= 487, 66.6%), followed by cases of hepatitis (n= 141, 19.3%) and hepatic steatosis (n=

Table 1. Features of idiopathic acute liver injury by countries

	Italy (HSD+Pedianet) Total cases = 1,237			the Netherlands (IPCI) Total cases = 89		
	Definite N=731 (59.1%)	**Possible N=285 (23.0%)**	**Hepatomegaly N=221 (17.9%)**	**Definite N=54 (60.7%)**	**Possible N=23 (25.8%)**	**Hepatomegaly N=12 (13.5%)**
Diagnosis						
Abnormal liver enzymes	487 (66.6)	83 (29.1)	-	25 (46.3)	15 (65.2)	-
Hepatitis	141 (19.3)	166 (58.2)	-	-	-	-
Steatosis	66 (9.0)	14 (4.9)	-	3 (5.6)	2 (8.7)	-
More than one	35 (4.8)	21 (7.4)	-	26 (48.1)	5 (21.7)	-
Other diagnosis[a]	2 (0.3)	1 (0.4)	-	-	1 (4.3)	-
Source of diagnosis						
GP or FP	29 (4.0)	268 (94.0)	201 (91.0)	2 (3.7)	2 (8.7)	9 (75.0)
Specialist/hospital discharge	182 (24.9)	6 (2.1)	8 (3.6)	8 (14.8)	2 (8.7)	2 (16.7)
Specific diagnostic tests[b]	513 (70.2)	10 (3.5)	12 (5.4)	26 (48.1)	16 (69.6)	1 (8.3)
More than one	7 (0.9)	1 (0.4)	-	18 (33.3)	3 (13.0)	-
Type of liver injury[c]						
Hepatocellular	256 (52.6)	5 (6.0)	-	19 (76.0)	13 (86.7)	-
Cholestatic	192 (39.4)		-	-	-	-
Mixed	4 (0.8)	-	-	1 (4.0)	-	-
NA	35 (7.2)	78 (94.0)	-	5 (20.0)	2 (13.3)	-
Degree of severity[c]						
Mild (> 2 to ≤3ULN)	139 (28.5)	1 (1.2)	-	12 (48.0)	9 (60.0)	-
Moderate (>3 to ≤5 ULN)	245 (50.3)	2 (2.4)	-	4 (16.0)	4 (26.7)	-
Severe (>5 to ≤8 ULN)	36 (7.4)	1 (1.2)	-	3 (12.0)	-	-
Most severe (>8 ULN)	33 (6.8)	-	-	1 (4.0)	1 (6.7)	-
NA	34 (7.0)	79 (95.2)	-	5 (20.0)	1(6.7)	-

Abbreviation: GP= general practitioner, FP= family paediatrician, NA= non-assessable, ULN= Upper Limit of Normal.[a]Other diagnosis included fatigue, weakness, anorexia, nausea, jaundice, dark urine, light stools, itching, bloating and abdominal pain.[b]Including laboratory data and hepatic ultrasounds.[c]Only in case of abnormal liver enzymes diagnosis.

66, 9.0%). Diagnoses were confirmed by diagnostic tests (i.e. laboratory data and hepatic ultrasounds) in 70.2% and by specialists in 24.9 % of the cases. In The Netherlands, most of the definite cases were combinations of multiple signs/symptoms of liver injury (n= 26, 48.1%) and asymptomatic liver enzyme elevations (n= 25, 46.3%). The diagnoses were confirmed by diagnostic tests in 48.1% of the cases. According to liver enzyme elevations, idiopathic ALI presented hepatocellular in 52.6% and cholestatic in 39.4% of the cases in Italy. Around 80 % of those were considered mild or moderate. In The Netherlands, the pattern of idiopathic ALI seems to be similar, but the contribution of hepatocellular cases (76.0%)

was higher compared to Italy. Likewise, the degree of severity of ALI is likely consistent with the Italian degree of severity, because most of the Dutch cases (64.0%) presented a mild to moderate degree of severity. In The Netherlands, the number of cases is too low to conduct a formal comparison with the Italian data.

Epidemiology of Idiopathic Acute Liver Injury

Among the pooled 785 definite cases, the crude IR of idiopathic ALI was 62.4 (95% CI 58.1–66.8) per100,000 PYs. Per country, the IR was 3.5 times greater in Italy (73.0/100,000 PYs; 95 % CI 67.8–78.4) than in The Netherlands (21.0/100,000 PYs; 95 % CI 16.0–27.2). Standardized to the population distribution of The Netherlands, the incidence in Italy was 81.1/100,000 PYs (95 % CI 70.7–92.7). Age distribution was similar in the two countries (mean age: 12.1 ± 4.6 in Italy

Table 2. Age and gender standardized IRs per 100,000 PYs of cases of idiopathic acute liver injury distributed by countries

	Definite cases			Total cases[#]	
	N (%)	PYs[*]	IR (95% CI)	N (%)	IR (95% CI)
Italy (HSD+Pedianet)	731	1,000,720	73.0 (67.8-78.4)	1,237	123.6 (116.9-130.6)
Mean age (± SD)	12.1(±4.6)			10.6 (±5.4)	
Gender					
Male	445 (60.9)	524,123	84.8 (77.2-93.0)	704 (56.9)	134.3 (124.7-144.5)
Female	286 (39.1)	476,597	59.9 (53.3-67.2)	533 (43.1)	111.8 (102.6-121.6)
Age category					
<2 years	38 (5.2)	105,585	36.0 (25.8-48.8)	140 (11.3)	132.6 (112.0-156.0)
2-5 years	55 (7.5)	221,454	24.8 (18.9-32.0)	138 (11.2)	62.3 (52.6-73.4)
6-11 years	166 (22.7)	346,007	47.9 (41.0-55.6)	310 (25.0)	89.6 (80.0-100.0)
12-17 years	472 (64.6)	327,675	143.9 (131.3-157.3)	649 (52.5)	198.1 (183.3-213.7)
the Netherlands (IPCI)	54	256,762	21.0 (16.0-27.2)	89	34.7 (28.0-42.4)
Mean age (± SD)	12.9 (±4.5)				12.4 (±5.3)
Gender					
Male	24 (44.4)	131,218	18.3 (12.0-26.8)	40 (44.9)	27.4 (19.5-37.5)
Female	30 (55.6)	125,544	23.9 (16.4-33.6)	49 (55.1)	32.7 (23.8-43.8)
Age category					
<2 years	4 (7.4)	26,836	14.9 (5.0-35.4)	8 (9.0)	29.8 (14.1-56.3)
2-5 years	3 (5.6)	57,332	5.2 (1.4-14.0)	9 (10.1)	15.7 (7.8-28.7)
6-11 years	9 (16.6)	86,835	10.4 (5.1-18.9)	14 (15.7)	16.1 (9.2-26.3)
12-17 years	38 (70.4)	85,759	44.3 (31.8-60.1)	58 (65.2)	67.6 (51.9-86.8)
SIR *ref.NL*			81.1 (70.7-92.7)		133.8 (120.2-148.5)

Abbreviation: IR = incidence rate; PYs= Person-years; SD = standard deviation; SIR ref. NL= Standardized Incidence Rate on the population from the Netherlands.

[*]For the calculation of disease specific incidence rates, censoring was done upon disease occurrence: person-time may differ slightly.

[#]Including definite and possible cases and hepatomegaly.

versus 12.9 ± 4.5 in The Netherlands, P =0.105), while a statistically significantly higher proportion of boys with idiopathic ALI was found in Italy as compared to The Netherlands (60.9 *vs.* 44.4%, $P \leq 0.001$). Males showed a higher incidence than females in Italy (84.8/100,000 *vs.* 59.9/100,000 PYs, respectively), whereas the opposite trend was observed in the Netherlands (18.3/100,000 *vs.* 23.9/100,000 PYs, respectively). In both countries, the IRs of idiopathic ALI increased with age, even if a slightly increase of the rates was observed among the neonates up to 1 years. In particular, among the total cases, also including hepatomegaly, a notable peak of the incidence rate was observed in very young Italian children rather than in Dutch newborns (**Table 2**).

Associations between Drugs and Idiopathic Acute Liver Injury

Out of the 785 definite cases of idiopathic ALI, there were 110 cases (14 %) where the index date occurred during the time window of the prescription. Such a

Table 3. Drugs* associated with acute liver injury among definite idiopathic cases.

Drugs	ATC	Exposure time (in days)	n. of events	Crude IR/100,000 person-days (CI 95%)	Adjusted RR# (CI 95%)	*p value*
Clarithromycin	J01FA09	156,763	9	5.7 (2.8-10.5)	25.9 (13.4-50.0)	0.001
Amoxicillin/clavulanate	J01CR02	437,708	16	3.6 (2.2-5.8)	18.6 (11.3-30.6)	0.001
Amoxicillin	J01CA04	759,972	6	0.8 (0.3-1.7)	7.5 (3.4-16.8)	0.001
Other drugs with only 2 events						
Rifampicin/isoniazid	J04AM02	114	2	1,754.4 (349.9-5,623.5)	4858.2 (1214.0-19442.4)	0.001
Acetaminophen combination	N02BE51	9,740	2	20.5 (4.1-65.8)	94.2 (23.4-378.3)	0.001
Rokitamycin	J01FA12	13,329	2	15.0 (1.9-54.2)	52.3 (13.0-209.6)	0.001
Co-trimoxazole	J01EE01	42,325	2	4.7 (0.9-15.1)	28.6 (7.1-114.7)	0.002
Phenobarbital	N03AA02	23,337	2	8.6 (1.7-27.5)	25.8 (6.4-103.4)	0.003
Ketoprofen	M01AE03	49,585	2	4.0 (0.8-12.9)	11.1 (2.8-44.5)	0.015
Carbamazepine	N03AF01	76,355	2	2.6 (0.3-9.5)	9.5 (2.4-38.2)	0.019
Valproic acid	N03AG01	134,725	2	1.5 (0.3-4.8)	6.9 (1.7-27.6)	0.035

Abbreviation: ATC = Anatomical Therapeutic Chemical classification.

*Only drugs for systemic use with at least 2 events and statistically significantly (P value < 0.05) associated with liver injury have been reported.

#To estimate the association between event and drug use, the age- and gender-adjusted rate ratios, with non-use of the drug of interest as reference was calculated using Mantel–Haenszel method.

proportion differed between the two countries (Italy: n= 95/731, 13%; the Netherlands: n= 15/54, 28%). Clarithromycin was associated with the highest risk of ALI compared to non-use (n= 9, adj. RR 25.9; 95 % CI 13.4–50.0; *P* ≤0.001), followed by amoxicillin/clavulanic acid (n= 16, adj. RR 18.6; 95 % CI 11.3–30.6; *P* ≤0.001), and amoxicillin (n= 6, adj. RR 7.5; 95 % CI 3.4–16.8; *P* ≤0.001). Statistically significant increases in the rates of ALI were also found during current use of rifampicin/isoniazid combination, acetaminophen, rokitamycin, sulfamethoxazole/trimethoprim (i.e. co-trimoxazole), phenobarbital, ketoprofen, carbamazepine and valproic acid, despite a very low number of exposed cases (n≤3; **Table 3**).

DISCUSSION

To our knowledge, this is the first population-based study that estimated the incidence and the features of the idiopathic acute liver injury in a paediatric outpatient population by combining three longitudinal EHR databases from Italy and the Netherlands.

Acute liver injury of which aetiology is not identified (i.e. idiopathic) or potentially attributed to the drug is relatively rare in children and adolescents, with approximately an annual incidence of 63/100,000 person-years. There are no paediatric studies to which we can directly compare our data because of different age-range setting and/or methodology. The most similar is a study conducted in the United Kingdom (UK), which was restricted however to the population over 15 years. Using healthcare records as data source, Duh *et al.*[244] found 66 cases per 100,000 PYs of liver injury defined as liver enzyme abnormalities of whom 41 potentially drug-attributable and 25 with uncertain aetiology. In both studies, the outcome was identified by similar searching and diagnostic criteria (e.g., through ICD-9-CM referring to hepatic disorders and by a set of international CIOMS criteria based on serum liver enzymes). On the other hand, our rate is substantially higher compared that previously estimated by de Abajo et al. among UK outpatients, namely 2.4/100,000 PYs. However, this lower rate could be attributed to the differences in study population (4-65 years old patients) and study outcome (acute and clinically relevant drug-induced liver injury)[70]. In 2003, moreover, the prospective observational study, Drug-Induced Liver Injury Network (DILIN) was established to create a registry and bio-sample repository for clinical and mechanistic studies of DILI among patients > 2 years old.[82] However, these data, as well as the most published studies concerning clinical features of DILI, as described by Bell *et al*[248], only reported the frequency of disease and thus, the absolute number of cases rather than rates; moreover, they only included the most

severe cases of liver injury which makes comparison difficult[71, 73, 80, 234, 249, 250]. In our study, we identified all cases of liver injury, even less severe, that might be theoretically idiopathic because of exclusion of all the competing well-known aetiologies. However, as differential diagnosis of idiopathic liver injury versus liver injury trigger by drugs can be extremely complicated, even through manual case by case evaluation, we estimated the incidence of acute and idiopathic liver injury, including even those potentially drug exposed.

The incidence of idiopathic ALI is much higher (roughly 4 times) in Italy than in the Netherlands. In Italy, the most of the liver enzyme elevations were asymptomatic with mild/moderate degree of severity, whereas in the Netherlands were reported in combination with other typical sign/symptoms of hepatotoxicity. This discrepant pattern may be explained by differences in healthcare systems and healthcare seeking behaviour of patients and parents. In Italy children are being inspected and monitored systematically during growth by the paediatrician, whereas GPs in the Netherlands only see children with complaints. Moreover, laboratory tests are requested more frequently in Italy than in the Netherlands, which also leads to a higher chance to detect asymptomatic cases of ALI. A similar phenomenon has already been described for Wilson disease. Compared to the other European countries, many Italian children have a Wilson disease's diagnosis when they are completely asymptomatic and present only mild hypertransaminasemia[251]. Additionally, we cannot exclude that the discrepancy is influenced by different drug-prescribing patterns between the two countries. The prevalence of the use of well-known hepatotoxic drugs, such as anti-infective drugs and drugs for musculoskeletal disorders, is indeed much higher in Italy than in the Netherlands[122].

In line with recent evidence[82], the mean age of children and adolescents with idiopathic and potential drug-induced liver injury is 12 years in both countries. Moreover, the rates progressively increase with age, confirming our previous signal detection analysis on global spontaneous reports of liver injury in children[101]. As a matter of fact, susceptibility to drug toxicity changes across different age groups and may differ largely among newborns, toddlers, adolescents and adults, because of age-dependent metabolic activities of the hepatic CYP P450 isoenzymes[35]. In our study, the rates of ALI were slightly higher in the first year of life as compared to 2-5 years. This might be a reflection of varying activity of CYP P450 in early life. On the other hand, a determinant for the slightly high rates in early stage of the life might be represented by a better observation of the parents, medical doctors and paediatricians to the neonates. This finding is supported by the highest observed rate in the youngest Italian children, among the total cases. At this age, indeed, any extension of the liver below the right costal margin has been noted as 'hepatomegaly' by Italian paediatricians, despite mostly physiological.

In an attempt to see whether longitudinal EHRs may be a useful source for drug safety signal detection concerning ALI in paediatric outpatients, we identified several drugs as potential underlying cause. In our study, antibiotics are associated with the highest risk of ALI in children and adolescents. Compared with literature, amoxicillin (with or without clavulanic acid) has been already described as hepatotoxic drug in paediatrics,[81, 82, 101, 252] whereas we failed to find evidence of clarithromycin-included liver injury in children. Nevertheless, paediatrics cases of liver injury have also been reported for other macrolides, such as erythromycin, azithromycin and roxithromycin[82, 252] suggesting that the hepatotoxicity might be an effect of therapeutic class. Noteworthy, the combination amoxicillin/clavulanic acid displays a higher risk of ALI than amoxicillin alone. Our finding is in line with the results from two previous population-based studies in adults on drug-induced liver injury, even if the risk estimated was lower.[70, 253] In addition, a better hepatic safety profile for amoxicillin alone compared to the combination with clavulanic acid was also reported in two studies on spontaneous reporting systems, one of them specifically addressing paediatrics.[101, 254] A recent study confirmed that susceptibility to amoxicillin/clavulanic acid-induced liver injury is influenced by genetic multiple variability[255]. We do believe that the hepatotoxicity related to the combination of amoxicillin-clavulanic acid in children is not rare as assumed until now and, as the number of cases is low, further investigations are needed to quantify this risk.

Noteworthy, we found only two events of acute liver injury related to acetaminophen, despite its well-known hepatotoxicity in adults as well as in children[252] and its wide use in paediatric patients[256]. Low exposure and number of cases regarding acetaminophen are due to the study data source that does not collect information about over the counter product and drugs not reimbursed by Healthcare System.

Overall, because all of these drugs were already known to be hepatotoxic in adults, this analysis could be considered a proof of concept that EHRs might be useful for estimation of incidence rates and for safety signal detection in paediatrics[122]. To gain sufficient statistical power to detect drug safety signals concerning a wide range of drugs exposure in paediatric population, combining data from multiple longitudinal EHR databases was necessary. Other initiatives such as OMOP and EU-ADR may also be explored for signal detection and validation in children and adolescents[91, 257].

The strength of this population-based study is the availability of detailed information on several clinical variables and drug use for a well-defined cohort of large size from two Countries. In particular, using electronic medical record databases allowed us to identify asymptomatic and less severe liver injury, which

cannot be fully captured in prospective disease registries[82]. A very careful stepwise approach for case ascertainment has been performed to ensure the accuracy of the outcome data extraction by conducting an initial broad search of potential cases of acute liver injury and, subsequently, through manual inspection of all the medical records of potential cases by expert medical doctors.

Limitations

Being observational in nature, our study is also vulnerable to confounding and bias. To reduce the effect of misclassification of the outcome, the main analyses have been performed among the cases confirmed by a specialist or laboratory data/ultrasound evidence. Nevertheless, because of lack of consistent diagnostic criteria for liver injury that are specific for children, residual misclassification cannot be completely excluded. In particular, serum liver enzyme activity is a marker of liver injury but no data are available on the sensitivity and specificity of different thresholds and the normal reference range for liver enzymes has not been clearly established in children[258-261]. Part of this potential misclassification has been tackled by excluding children with enzymes values below than twice of ULN. Finally, in the second part of our research, we identified drugs that were associated with ALI. As proper drug-event causality assessment cannot be performed based on the available information, our findings should be considered as purely hypothesis-generating.

CONCLUSION

The incidence rate of idiopathic ALI is rather low in children and adolescents consistently with the adults. The differences observed between Italy and the Netherlands are likely due to the variability in the use of healthcare resources as well as drug prescribing pattern. Data mining of EHRs allowed us to identify antibiotics as the drugs with highest and most frequent associations with ALI with apparently unknown aetiology. Combination of several healthcare databases is necessary to gain the statistical power to investigate the association of liver injury with a much larger range of drugs in the paediatric population.

5.3. Antibiotics and Hepatotoxicity in Paediatrics? A Case-Control Study using Primary Care Databases

Submitted for publication

ABSTRACT

Objective
To assess the association between antibiotic use and hepatotoxicity in paediatric outpatients.

Methods
We performed a population-based case-control study in children (<18 years old) between 2000 to 2008 by using multiple electronic primary care databases from two European countries. After exclusion of all competing causes, cases of hepatotoxicity were defined as cases with a medical diagnosis of liver disease or an increase of more than twice the upper limit of the normal range for any liver function test or. Up to 100 controls for each case were matched on index date, age, gender and database. Exposure to antibiotics was defined as *current* if prescription duration lasted until the index date or ended within 15 days before, as *recent* if it ended within 16-90 days before, while as *past* if it ended more than 90 days before. Conditional logistic regression analysis was used to calculate odds ratios (ORs) for hepatotoxicity with antibiotic exposure.

Results
Among 938 cases of hepatotoxicity, current use of any antibiotic was associated with a 3-fold increased risk of hepatotoxicity as compared to past use (adjusted ORs: 3.2 [95% confidence interval: 2.6-4.0]). The risk of hepatotoxicity was statistically significantly increased following current use of any antibiotic with ≥3 exposed cases, except for azithromycin. Odds ratios varied between 1.9 [1.1-3.2] for amoxicillin to 24.2 [11.8-49.5] for co-trimoxazole, 26.7 [12.1-59.0] for ceftriaxone, and 31.8 [14.7-69.0] for rokitamycin. Sensitivity analyses confirmed the associations between hepatotoxicity and use of ceftriaxone, co-trimoxazole, clarithromycin and rokitamycin.

Conclusion
In the paediatric population, current use of ceftriaxone, co-trimoxazole and some macrolides increased the risk of hepatotoxicity. Based on our results, it might be advisable that paediatricians monitor the liver function in children who are prescribed the above mentioned antibiotics.

BACKGROUND

Antibiotics represent the most commonly prescribed drug class related to hepatotoxicity in the US and in the EU[99, 101, 262, 263]. As documented in a previous analysis of the WHO-UMC SRS, Vigibase, hepatic adverse drug reactions, in children, were mainly reported for antibiotics.[101] However, this finding could be also due to the widespread prescription of these drugs in children and adolescent.[122]. Actually, antibiotic-induced hepatotoxic reactions occur with an estimated frequency of 1-10 per 100,000 drug prescriptions in the overall population, and the absolute risk for most antibiotics is thought to be low[264]. Nevertheless, in our previous research where we investigated whether a longitudinal electronic healthcare database may be a useful source to assess hepatic drug safety in paediatric outpatients, antibiotics were associated with the highest risk of acute liver injury as compared to no use (age and gender-adjusted RRs: 25.9, [95% CI: 13.4-50.0] for clarithromycin; 18.6, [11.3-30.6] for amoxicillin/clavulanic acid; 7.5, [3.4-16.8] for amoxicillin[99].

So far, no studies investigated the risk of hepatotoxicity in association with the antibiotic use specifically in a paediatric population. Given the low frequency of the event and based on previous estimates[99], we conducted a large multi-database, population-based, case-control study to explore the risk of hepatotoxicity associated to individual antibiotics in children and adolescents.

METHODS

Study population

We identified paediatric patients from three longitudinal electronic primary care databases from two European countries: a) Pedianet, a FP database, and b) Health Search/CSD Longitudinal Patient Database (HSD), a GP database in Italy; and c) the Integrated Primary Care Information (IPCI), which is a Dutch GP database.

All the three databases contain anonymous data on patient demographics, reasons for visits, medical diagnoses from GPs/FPs and specialists, hospitalizations, drug prescriptions, laboratory and other diagnostic findings. Symptoms and medical diagnoses are either registered as free text or coded using ICPC in IPCI and ICD-9-CM in HSD and Pedianet. Drug prescription data include details on product name, formulation, dosing regimen and indication for use. The respective databases captured data for children and adolescents (<18 years old) who receive medical care by GPs in the Netherlands and by FPs (up to 14 years of age) and by GPs (over 14 years) in Italy. These databases are representative of the Italian and

Dutch paediatric population and have been proven to be valid data sources for pharmacoepidemiological studies. The study period ran from January 1st, 2000 to December 31st, 2008.

Case and control selection

We selected all cases of hepatotoxicity in children and adolescents aged <18 years. Details on the identification and validation of these patients have been published elsewhere[99]. In brief, we applied a similar stepwise approach across all three databases. First, all potential cases were extracted through a very broad automated search by using: 1) diagnostic codes for liver diseases/signs (i.e. hepatitis, liver failure, hepatic steatosis, hepatic cirrhosis, hepatic necrosis, hepatomegaly, or jaundice); 2) specific key words for free text search, and 3) laboratory findings on liver function tests (i.e. ALT, AST, AP and total bilirubin). Of each potential case, the complete electronic medical record including results of laboratory data, ultrasound and other diagnostic tests, as well as hospital discharge summaries and specialists' letters was manual reviewed validation by four medically trained investigators, blinded to the drug exposure, and doubtful cases were reviewed by expert medical doctors to reach a consensus. Specific terms, i.e., jaundice and hepatomegaly, which are suggestive of liver injury but are not sufficient by themselves for the diagnosis, were considered only in association with other specific symptoms/signs (e.g. abnormal liver enzyme values, steatosis). We excluded all potential cases with clear competing causes of liver injury: (i) viral infections (Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Cytomegalovirus, and Epstein-Barr virus); (ii) hepatic neoplasm; (iii) autoimmune hepatitis; (iv) genetic hepatopathy (e.g., hemochromatosis, α1-antitrypsin deficiency, Wilson Disease, Gilbert Syndrome, etc.); (v) biliary tract diseases (i.e., biliary atresia, gallstones, cholangitis, and cholecyctitis); and (vi) abdominal trauma documented with imaging. Patients with small elevation of biochemical liver tests (≤2 upper limit of normal range, ULN), only in presence of isolated increase of gamma-GT or neonatal jaundice, were excluded as well[101, 243].

Based on CIOMS criteria and previous evidence[243, 246], any hepatotoxicity was defined as i) any increase of more than two times of ULN for ALT, AST, AP or total bilirubin, or ii) a diagnosis of liver disease confirmed either by specialist or GP/FP or via ultrasound evidence. As secondary outcome, definite liver injury was specified as an increase over twice of ULN for ALT, or a combined increase of AST, AP and total bilirubin, provided that one of them was at least twice ULN, confirmed by specialist or via laboratory data[242, 243].

The index date of the event was defined as the earliest date of the first symptom/sign of liver injury (i.e. fatigue, weakness, anorexia, nausea, jaundice, dark urine,

light stools, itching, bloating and abdominal pain) or, in absence of these, the date of abnormal liver test preceding the diagnosis.

We sampled up to 100 control participants for each case, matched on index date, year of birth, gender, and database. The mechanism of control sampling used in our study (namely *incidence density sampling*) means that the likelihood of being selected as a control is proportional to the person-time[265].

Exposure definition

To estimate the association between hepatotoxicity and antibiotic use, we created exposure categories based on timing of use, dose and duration of use. Exposure to antibiotics was categorized as *current* if a prescription duration of the single compound of interest lasted until or beyond the index date or ended within 15 days prior to the index date (i.e. carry-over period), as *recent* if last prescription ended within 16-90 days prior to the index date, or as *past* if it ended more than 90 days before. *No use* was defined if there was no prescription prior to the index date.

Patient comorbidities

Information on the presence of several potential risk factors for liver injury was extracted from the computerized patient record: age, sex, and database of origin as matching factors; comorbidity, such as diabetes mellitus, hypoglycemia, obesity, dyslipidaemia, nutrition-related disturbances, thyroid disorders, hypertension, alcohol intake, smoking (within one year prior the index date) and congenital diseases (from the birth). We evaluated also the use at the index date of other hepatotoxic medications (i.e., anti-mycotics, drugs for the treatment of tubercolosis, acid-suppressants, anti-convulsants, anti-asthmatics, acetaminophen and its combinations, psycholeptics and psychoanaleptics, non-steroidal anti-inflammatory drugs (NSAIDs), immunosuppressants).

Statistical Analysis

To study the association between exposure to antibiotics and risk of any hepatotoxicity, we used a conditional logistic regression analysis. All covariates associated with hepatotoxicity in the univariate analysis at P <0.10 and that changed the point estimate of the association between antibiotics and hepatotoxicity by more than 10% were included in the final model of multivariate conditional logistic regression analysis to estimate the risk as OR and 95% CI[266]. Under this design, the OR is an unbiased estimator of the rate ratio as measure of the probability to develop hepatotoxicity in children and adolescents who receive antibiotics.

To control for confounding by indication, past use of any antibiotic served as reference category. Several multivariate models were computed in the primary analyses to estimate the associations between any hepatotoxicity and current use of i) antibiotics as a whole; ii) different therapeutic subgroups; iii) individual antibiotic.

Sensitivity and restricted analyses

To explore the possible effects of outcome misclassification, we restricted all the analyses to cases of definite liver injury (i.e. confirmed by specialists or diagnostic tests).

In addition, we checked for effect modification in the association between antibiotic use and hepatotoxicity.

To address confounding by indication, a sensitivity analysis was conducted using current use of amoxicillin as a reference group. Amoxicillin was chosen since amoxicillin is the most commonly prescribed antibiotic in children and is considered to be "non-hepatotoxic" when used as single ingredient[122, 253].

To investigate misclassification of exposure, we conducted a sensitivity analysis in which we removed the carry-over period.

All analysis were conducted using SPSS, version 20 (SPSS Chicago, Illinois). We set up P value <0.05 as the threshold of statistical significance, except for the selection of the covariates to be included in the final multivariate models (P value < 0.10).

RESULTS

In a study population of around 430,000 children and adolescents (< 18 years old) from two countries, we identified 938 (0.2%) cases of idiopathic hepatotoxicity after exclusion of all clear causes of liver injury. Cases were overall matched to 93,665 controls. Demographic and clinical characteristics of cases and controls are reported in **Table 1**. Most cases were boys (58.2%), with a mean age of 11.3 (SD: 5.1) years and identified in the Italian databases. Cases had a greater burden of comorbidities such as diabetes, hyperlipidaemia, obesity, thyroid disorders or congenital disease. Children with hepatotoxicity were more likely to be exposed at the index date to acid-suppressant drugs, anti-convulsants, NSAIDs, psycholeptic agents, acetaminophen, and anti-asthmatics than controls.

Table 1. Demographic and clinical characteristics of hepatotoxicity cases and matched controls from paediatric population.

	Cases N=938 (%)	Controls N=93,665 (%)	OR (95% CI)	p-value[&]
Boys	546 (58.2)	54,559 (58.2)		
Mean age (±SD)	113 (5.1)	11.4 (5.2)	Matching factor	
Age category (yrs)				
<2	88 (9.4)	8,811 (9.4)		
2-5	101 (10.8)	9,704 (10.4)		
6-11	260 (27.8)	26,060 (27.7)		
12-18	489 (52.1)	49,090 (52.4)		
Database			Matching factor	
HSD (IT)	478 (51.0)	47,480 (51.0)		
Pedianet (IT)	382 (40.7)	38,159 (40.7)		
IPCI (NL)	78 (8.3)	7,706 (8.2)		
Comorbidities[*]				
Diabetes mellitus	16 (1.7)	264 (0.3)	6.2 (3.7-10.3)	<0.001
Hypoglycemia	-	27		
Obesity	57 (6.1)	1,767 (1.9)	3.5 (2.6-4.5)	<0.001
Hyperlipidaemia	7 (0.7)	177 (0.2)	4.0 (1.9-8.5)	<0.001
Thyroid imbalance	9 (1.0)	395 (0.4)	2.3 (1.2-4.5)	0.014
Nutrition-related disorders	10 (1.1)	762 (0.8)	1.3 (0.7-2.5)	0.390
Hypertension	1 (0.1)	89 (0.1)	NA	
Congenital diseases	18 (1.9)	871 (0.9)	2.1 (1.3-3.4)	0.002
Alcohol consumption	-	22 (0.0)	NA	
Smoking (data available only from HSD)	1 (0.1)	238 (0.3)	NA	
Other hepatotoxic medications[#] [ATC]				
Acid related disorders drugs [A02]	8 (0.9)	141 (0.2)	5.8 (2.8-11.9)	<0.001
Antimycotics for systemic use [J02]	1 (0.0)	41 (0.1)	NA	0.375
Antimycobacterials [J04]	2 (0.2)	9 (0.1)	NA	<0.001
Sex hormones [G03]	10 (1.1)	678 (0.7)	1.8 (0.8-3.8)	0.133
Immunosuppressants [L04]	-	113	NA	
NSAIDs [M01]	10 (1.1)	320 (0.3)	3.4 (1.8-6.3)	<0.001
ASAP and its combinations [N02BE]	4 (0.4)	128 (0.1)	3.2 (1.2-8.7)	0.022
Anticonvulsivants [N03]	12 (1.3)	323 (0.3)	3.7 (2.1-6.7)	<0.001
Psycholeptics [N05]	3(0.3)	93 (0.1)	3.3 (1.0-10.4)	0.043
Psychoanaleptics [N06]	3 (0.3)	107 (0.1)	2.9 (0.9-9.1)	0.075
Anti-asthmatic agents [R03]	37 (3.9)	1,859 (2.0)	2.4 (1.7-3.3)	<0.001

Abbreviations: OR= Odds Ratio; 95% CI= 95% of confidential interval; SD= standard deviation; NA= Not available; HSD= Health Search/CSD database; IPCI=Integrated Primary Care Information.

[*]all the covariates for comorbidity have been assessed within 365 years before the index date, except for congenital defects (cardiovascular, hematologic, pregnancy-childbirth and puerperium complications) that have been evaluated from birth.

[#]Use of other potentially hepatotoxic medications has been assessed at the index date.

Estimates only provided in case of at least 3 exposed cases.[&]wald's test

Main analyses

Current use of antibiotics (adjusted OR 3.2 [95% CI: 2.6-4.0]) was significantly (*P* <0.001) associated with increased risk for hepatotoxicity as compared with past use of any antibiotic (**Table 2**). An association, although less strong, was also observed for recent use of any antibiotics (adjusted OR 1.5 [1.2-1.9]; *P*= .043). The analyses of the therapeutic classes of antibiotics showed some heterogeneity. Considering only those classes with at least 3 exposed cases, the increased risk for hepatotoxicity ranging from 2.8 [2.1-3.9] for current use of penicillins to 13.9 [4.8-40.0] for current use of fluoroquinolones. As the number of exposed cases to fluoroquinolones was low, this association could not be further investigated in detail.

Table 3 shows the hepatotoxicity risk for individual antibiotics versus past use of any antibiotics. Except for azithromycin, the risk of hepatotoxicity was statistically significantly increased (*P* <0.005) for current use of each antibiotic, varying

Table 2. Associations between use of therapeutic classes of antibiotics[*] and risk of any hepatotoxicity or definite liver injury only in paediatric population.

Antibiotic exposure	Any hepatotoxicity				Definite liver injury		
	Cases n= 938 (%)	Controls n=93,665 (%)	OR $_{matched}$ (95% CI)	OR $_{adjusted}^{\#}$ (95% CI)	Cases n=485 (%)	Controls n=48,500 (%)	OR $_{matched\ and\ adjusted}^{\&}$ (95% CI)
Past use of any antibiotic	417 (44.5)	40,740 (43.5)	**ref.**		211 (43.5)	21,200 (43.7)	**ref.**
Recent use of any antibiotic	138 (14.7)	8,044 (8.6)	1.7 (1.4-2.1)	1.5 (1.2-1.9)	69 (14.2)	4,198 (8.7)	1.7 (1.3-2.3)
Current antibiotic use	117 (12.5)	3,398 (3.6)	3.5 (2.8-4.3)	3.2 (2.6-4.0)	59 (12.2)	1,749 (3.6)	3.5 (2.6-4.8)
Tetracyclines (J01A)	3 (0.3)	68 (0.1)	4.1 (1.3-13.1)	4.0 (1.2-13.2)	-	36 (0.1)	NA
Amphenicols (J01B)	-	12 (0.4)	NA	NA	-	8 (0)	NA
Penicillins (J01C)	46 (4.9)	1,600 (1.7)	2.9 (2.1-4.0)	2.8 (2.1-3.9)	17 (3.5)	822 (1.7)	2.2 (1.-3.6)
Cephalosporins (J01D)	26 (2.8)	719 (0.8)	3.8 (2.5-5.7)	3.5 (2.3-5.3)	15 (3.1)	369 (0.8)	4.5 (2.5-7.5)
Sulfonamides (J01E)	5 (0.5)	55 (0.1)	8.8 (3.5-22.1)	12.4 (5.5-28)	2 (0.4)	32 (0.1)	NA
Macrolides (J01F)	21 (2.2)	695 (0.7)	3.0 (1.9-4.7)	2.9 (1.8-4.5)	12 (2.5)	351 (0.7)	3.5 (1.9-6.4)
Aminoglycosides (J01G)	-	5 (0.1)	NA	NA	-	5 (0)	NA
Quinolones[$] (J01M)	3 (0.3)	29 (0)	10.1 (3.0-33.3)	13.9 (4.8-40)	3 (0.6)	16 (0)	19.0 (5.4-66.9)
Other Abs	-	44 (1.3)	NA	NA	-	22 (0)	NA
More than one AB	13 (1.4)	171 (0.2)	7.7 (4.3-13.7)	9.4 (5.5-16)	10 (2.1)	88 (0.2)	12.2 (6.2-24.)
No antibiotic use	266 (28.4)	41,483 (44.3)	0.6 (0.5-0.7)	0.8 (0.6-0.9)	146 (30.1)	21,353 (44.0)	0.7 (0.5-0.8)

[*]all classes of antibiotics are reported in the table. However, risk estimates have been considered in the analysis for antibiotic classes having more than three exposed cases.

[#]OR adjusted for concomitant use of anti-asthmatics and drugs for the treatment of tubercolosis, as potential confounders since they changed in the univariate analysis the point estimate of the association between antibiotics and hepatotoxicity by more than 10%;

[&]any covariate changed the point estimate of the association between antibiotics and definite liver injury.

[$]No further analyses fit within the group because of low number of cases.

from the 1.9 [1.1-3.2] for amoxicillin to 24.2 [11.8-49.5] for co-trimoxazole, 26.7 [12.1-59.0] for ceftriaxone, and 31.8 [14.7-69.0] for rokitamycin.

Sensitivity and restricted analyses

In restricted analysis, the association between antibiotics current use and risk of definite liver injury did not change substantially (Table 2). When considering individual antibiotics, the increased risk was confirmed for co-amoxicillin/clavulanate and ceftriaxone and became stronger for cefixime and clarithromycin. This analysis was limited by statistical power given the few cases exposed to cefaclor, ceftibuten, co-trimoxazole and rokitamycin (**Table 3**).

When considering current use of amoxicillin as the reference group, we found a significantly increased risk of hepatotoxicity for ceftriaxone (adjusted OR 14.3

Table 3. Associations between individual antibiotics* and risk of any hepatotoxicity or definite liver injury only in paediatric population

Antibiotic exposure	Any hepatotoxicity				Definite liver injury		
	Cases n=938 (%)	Controls n= 93,665 (%)	OR $_{matched}$ (95% CI)	OR $_{adjusted}^{\#}$ (CI 95%)	Cases n=485 (%)	Controls n= 48,500 (%)	OR $_{matched\ and\ adjusted}^{\#}$ (95% CI)
Past use of any antibiotic	417 (44.5)	40,740 (43.5)	**Ref.**	**Ref.**	211 (43.5)	21,200 (43.7)	**Ref.**
Penicillins							
Amoxicillin	19 (2.0)	842 (0.9)	2.3 (1.4-3.7)	1.9 (1.1-3.2)	6 (1.2)	424 (0.9)	1.5 (0.7-3.4)
Amoxicillin/clavulanate	22 (2.3)	697 (0.7)	3.2 (2.1-4.9)	2.8 (1.7-4.5)	10 (2.1)	365 (0.8)	2.8 (1.5-5.4)
Cephalosporins							
Cefuroxime	1 (0.1)	40 (0.0)	NA	NA	1 (0.2)	23 (0.0)	NA
Cefaclor	8 (0.9)	199 (0.2)	4.4 (2.1-9.0)	4.3 (2.0-9.2)	2 (0.4)	93 (0.2)	NA
Ceftriaxone	3 (0.3)	37 (0.0)	8.4 (2.6-27.5)	26.7 (12.1-59)	3 (0.6)	22 (0.0)	14.7 (4.4-49.4)
Cefixime	8 (0.9)	192 (0.2)	4.3 (2.1-8.9)	4.4 (2.1-9.3)	5 (1)	88 (0.2)	6.1 (2.4-15.3)
Cefpodoxime	2 (0.2)	65 (0.1)	NA	NA	1 (0.2)	44 (0.1)	NA
Ceftibuten	3 (0.3)	82 (0.1)	3.8 (1.2-12.3)	3.6 (1-12.6)	2 (0.4)	40 (0.1)	NA
Sulfonamides							
Co-trimoxazole	4 (0.4)	49 (0.1)	8.1 (2.9-22.6)	24.2 (11.8-49.5)	2 (0.4)	30 (0.1)	NA
Macrolides							
Clarithromycin	12 (1.3)	293 (0.3)	4.1 (2.3-7.4)	4.3 (2.3-7.8)	8 (1.6)	147 (0.3)	5.6 (2.7-11.6)
Azithromycin	4 (0.4)	262 (0.3)	1.5 (0.6-4.1)	1.2 (0.4-3.9)	3 (0.6)	128 (0.3)	2.4 (0.8-7.7)
Rokitamycin	3 (0.3)	35 (0.0)	8.7 (2.7-28.4)	31.8 (14.7-69.0)	1 (0.2)	21 (0)	NA

*Risk estimates are reported for all antibiotics with at least one exposed case. However, in the analysis we considered only the antibiotics having at least three cases exposed.

#OR adjusted for potential confounders only if in the univariate analysis they changed by more than 10% the point estimate of the association between antibiotics and hepatotoxicity (i.e. concomitant use of anti-asthmatics and drugs for the treatment of TBC) or between antibiotics and definite liver injury (no covariate).

Table 4. Association between individual antibiotics* and risk of any hepatotoxicity or definite liver injury in paediatric outpatients using current use of amoxicillin as comparator

Antibiotic exposure	Any hepatotoxicity				Definite Liver Injury		
	Cases n=938 (%)	Controls n= 93,665 (%)	OR matched (95% CI)	OR adjusted# (CI 95%)	Cases n=485 (%)	Controls n= 48,500 (%)	OR matched and adjusted# (CI 95%)
Amoxicillin	19 (2.0)	842 (0.9)	REF	REF	6 (1.2)	424 (0.9)	REF
Amoxicillin/clavulanate	22 (2.3)	697 (0.7)	1.4 (0.7-2.6)	1.5 (0.7-3.0)	10 (2.1)	365 (0.8)	1.9 (0.7-5.2)
Cefaclor	8 (0.9)	199 (0.2)	1.9 (0.8-4.4)	2.3 (0.9-5.8)	2 (0.4)	93 (0.2)	NA
Ceftriaxone	3 (0.3)	37 (0.0)	3.6 (1.0-12.8)	14.3 (5.6-36.9)	3 (0.6)	22 (0.0)	9.7 (2.3-41.2)
Cefixime	8 (0.9)	192 (0.2)	1.9 (0.8-4.3)	2.4 (0.9-5.9)	5 (1)	88 (0.2)	4.0 (1.2-13.5)
Ceftibuten	3 (0.3)	82 (0.1)	1.7 (0.5-5.7)	2.0 (0.5-7.5)	2 (0.4)	40 (0.1)	NA
Co-trimoxazole	4 (0.4)	49 (0.1)	3.5 (1.1-10.7)	13.0 (5.3-31.5)	2 (0.4)	30 (0.1)	NA
Clarithromycin	12 (1.3)	293 (0.3)	1.8 (0.8-3.7)	2.3 (1.0-5.1)	8 (1.6)	147 (0.3)	3.7 (1.3-10.9)
Rokitamycin	3 (0.3)	35 (0.0)	3.8 (1.1-10.7)	17.1 (6.7-43.4)	1 (0.2)	21 (0)	NA

*Risk estimates are reported for all antibiotics significantly associated with any hepatotoxicity in the main analysis. However, in the analysis we considered only the antibiotics having at least three cases exposed

#OR adjusted for potential confounders only if in the univariate analysis they changed by more than 10% the point estimate of the association between antibiotics and hepatotoxicity (i.e. concomitant use of anti-asthmatics and drugs for the treatment of TBC) or between antibiotics and definite liver injury (any covariate).

[5.6-36.9]), co-trimoxazole (adjusted OR 13.0 [5.3-31.5]), clarithromycin (adjusted OR 2.3 [1.0-5.1]), and rokitamycin (adjusted OR 17.1, [6.7-43.4]) (**Table 4**). The association was still observed for all other explored antibiotics, such as co-amoxicillin/clavulanate, cefaclor, cefixime, ceftibuten, however did not achieve statistical significance, due to low numbers. When we restricted this sensitivity analysis to definite cases of liver injury only, the association was confirmed for current use of ceftriaxone (9.7, [2.3-41.2]), and clarithromycin (3.7, [1.3-10.9]) and become significant for cefixime (4.0, [1.2-13.5]). Here as well, the association was less strong compared to the analysis where past use of antibiotics was used as reference category. The number of cases for the other antibiotics was too small to allow risk estimate calculation. (**Table 4**)

To estimate the effect of potential misclassification of exposure, we removed the carryover period in a sensitivity analysis which yield an increased risk of hepatotoxicity by around 20% for current use of amoxicillin/clavulanate (adjusted OR 3.3, [1.7-6.4]), by 50% for current use of amoxicillin (adjusted OR 2.8 [1.5-5.2]), ten-fold for current use of clarithromycin (adjusted OR 46.4 [30.9-69.8]) and twenty-five-fold for ceftibuten (adjusted OR: 93.2 [43.6-199.4]). The association remained for current use of amoxicillin, amoxicillin/clavulanate and clarithromycin when considering cases of definite liver injury only. (**Table 5**)

Table 5. Effect of exclusion of carry-over period on the association between individual antibiotics* and risk of any hepatotoxicity or definite liver injury only in paediatric population (past use of any antibiotic as comparator)

Antibiotic exposure	Any hepatotoxicity				Definite liver injury			
	Cases n=938 (%)	Controls n= 93,665 (%)	OR $_{matched}$ (95% CI)	OR $_{adjusted}$ [#] (95% CI)	Cases n=485 (%)	Controls n= 48,500 (%)	OR $_{matched}$ (95% CI)	OR $_{adjusted}$ [#] (95% CI)
Past use of any antibiotic	417 (44.5)	40,740 (43.5)	REF	REF	211 (43.5)	21,200 (43.7)	REF	REF
Amoxicillin	13 (1.4)	413 (0.4)	3.2 (1.9-5.2)	2.8 (1.5-5.3)	6 (1.2)	206 (0.4)	3.1 (1.3-7.0)	2.9 (1.3-6.6)
Amoxicillin/ clavulanate	11 (1.2)	311 (0.3)	3.2 (1.8-5.6)	3.3 (1.7-6.4)	8 (1.6)	161 (0.3)	5.1 (2.5-10.6)	4.8 (2.3-10.0)
Ceftibuten	3 (0.3)	27 (0)	7.1 (2.2-22.9)	93.2 (43.6-199.4)	2 (0.4)	7 (0)	NA	NA
Clarithromycin	11 (1.2)	114 (0.1)	7.6 (4.2-13.8)	46.4 (30.9-69.8)	8 (1.6)	57 (0.1)	14.2 (6.7-30.2)	13.0 (6.1-27.8)
Rokitamycin	3 (0.3)	9 (0.0)	23.4 (6.6-83.5)	NA	1 (0.2)	6 (0)	NA	NA

*Risk estimates have been reported only for antibiotics significantly associated with increased risk of any hepatotoxicity in the main analysis and at least three exposed cases.

[#]adjusted for R03

DISCUSSION

In this research, we found that overall, the use of antibiotics in children and adolescents was associated with a 3-fold increased risk of hepatotoxicity and this association was confirmed for the following individual drugs: amoxicillin, amoxicillin/clavulanate, ceftibuten, cefixime, ceftriaxone, cefaclor, co-trimoxazole, clarithromycin and rokitamycin. For the other antibiotics, it was difficult to draw strong conclusions due to limited number of exposed cases. The associations remained for ceftriaxone, or amoxicillin/clavulante, clarithromycin, and cefixime when restricting the main analysis to cases of definite liver injury only, meaning that the outcome misclassification could have overestimated the risk for the other explored antibiotics in the main analysis. Co-trimoxazole, ceftriaxone, clarithromycin and rokitamycin still remained associated with an increased risk of any hepatotoxicity or definite liver injury even with current use of amoxicillin as reference group, in the sensitivity analysis. The association between use of amoxicillin, amoxicillin-clavulanic acid, ceftibuten, and clarithromycin and risk of any hepatotoxicity became stronger for a strict definition of current use (use on index date only thus no carry over effect). Finally, several concomitant diseases were also recognized as risk factor for hepatotoxicity in childhood as well as previously documented in adulthood[267].

Comparison with other studies

To the best of our knowledge, no other population-based studies addressing the association between hepatotoxicity and antibiotic use in children have been conducted. Our results thus need to be viewed in relation to observational data on adults, and results generated from spontaneous reporting systems. Fluoroquinolones, sulfonamides, tetracyclines, cephalosporins, macrolides and penicillins have all been associated to hepatotoxicity[268-272]. Obviously, variations on risk estimates across antibiotic classes depend on different pharmacodynamics and pharmacokinetics which play a crucial role in their manifestations of hepatotoxicity, as already documented in adults[70, 253, 268, 271].

Confounding by indication is a main concern when studying the association between antibiotics and risk of hepatotoxicity. For this reason, we better control for this factor by using current use of amoxicillin as a reference category in a sensitivity analysis, that reinforced our main findings for ceftriaxone, co-trimoxazole, and some macrolides. Specifically, our results confirmed the high risk of ceftriaxone-induced toxic hepatitis or elevated liver enzymes already described in few case reports in children/adolescents[273-275]. Moreover, the high risk is also supported by our previous findings from signal detection analysis on the WHO SRS in children[101]. The clinical manifestation of the ceftriaxone-induced hepatitis may represent a direct toxic effect, an idiosyncrasy, or a cholestatic reaction, associated with its calcium precipitates typically after 9-11 days to the treatments[268-270]. Co-trimoxazole-induced hepatotoxicity is well-described[276-279]. The typical presentation is sudden development of fever and rash followed by jaundice within a few days or weeks of starting the medication and the typical pattern of serum enzyme elevations is mixed or cholestatic and often asymptomatic. This is also confirmed by restricting analysis to the elevation of liver enzymes, (i.e. ALT, implying hepatocellular type of liver injury) when the number of exposed cases decreased for co-trimoxazole.

Conversely to previous evidence[70, 280, 281], different hepatotoxic profiles among macrolides have been showed in our results. While azithromycin was not associated with hepatotoxicity in the paediatric population, an increased risk was observed for rokitamycin and clarithromycin. There are no evidence on rokitamycin-induced hepatotoxicity, due to the limited use in Italian children. On the other side, the findings on clarithromycin, confirmed in restricted and sensitivity analyses, are supported by our previous signal detection study[99]. As documented in several case reports, the hepatic reaction to clarithromycin, sharing the hepatotoxic profile of the macrolides, is characterized by transient and asymptomatic elevation in serum aminotransferases which occurs in 1-2% of patients treated for short periods and a somewhat higher proportion of patients given clarithromycin for a long-term

period[70]. The effect of exclusion of carry–over on clarithromycin risk is consistent with the proposed mechanism suggesting the rapidity of onset[281, 282].

Our results also point toward an association between hepatotoxicity and amoxicillin. According to previous studies, amoxicillin/clavulanate showed a higher risk than amoxicillin alone, supporting the potential role of the clavulanic acid in the hepatotoxic pathway[70, 99, 253]. Conversely, we cannot exclude any hepatotoxic effect of amoxicillin alone, especially as we observed an almost 3-fold increased risk when restricting the analysis to current use only (thus no carry-over effect). This risk may be explained by the difference latency period among the antibiotic alone and its combination, that has not been observed in previous studies because of different methods. However, potential confounding by indication, that cannot be ruled out for this antibiotic, could explain the observed association.

Strengths and limitations of this study

The strengths of our study are the following. First, given the large, heterogeneous study population by combination of three longitudinal, nationally representative, GP and FP databases from two European countries, the results can be largely generalizable to young people. Secondly, in these registries that are maintained for daily routine healthcare purposes, the exposure is prospectively collected, limiting the possibility of recall bias. Thirdly, we were able to adjust the analyses for many potential confounders because of the availability of clinically relevant information in the study databases. Misclassification of the outcome was unlikely, because all cases of hepatotoxicity were retrieved by searching electronic medical records, free text, and supplementary information such as hospital discharge and laboratory data –whenever possible - for evidence of hepatotoxicity and all data was reviewed by medically trained researchers who were blinded to the exposure. Inclusion of only definite cases of liver injury increased the risk estimates in current users of cefixime and clarithromycin. Finally, the sensitivity analysis in which current exposure to amoxicillin was used as reference category, allowed us to compare all the other antibiotics with a drug with similar indication of use and considered less hepatotoxic than other antibiotics[70, 253], and reduced the potential for confounding by indication.

This study has some potential limitations, because of its observational nature. Due to the limited number of exposed cases we cannot explore the association within antibiotic classes with high risk observed in the macro-analysis (e.g. fluoroquinolones, tetracyclines), within countries, as well as within each age category to assess potential effect modification. Residual confounding due to unmeasured severity of disease cannot be excluded. Moreover, although we carefully excluded viral infections as underlying disease, they still may represent the non-documented indication for antibiotic prescription[283-285].

Misclassification of the exposure may have occurred because we used outpatient prescription data and no information on actual filling or administration of the medication was available. However, such a bias would be likely non-differential between case and controls, thus eventually underestimating the actual risk. The increased risk for penicillins and macrolides when we restricted the current use window by excluding the carry over effect might be explained by misclassifying non-exposed period as exposed due to carry-over period as well as the fact that the liver injury risk may decrease over time.

CHAPTER 6

Need for Sharing Data in Paediatric Pharmacoepidemiology

6.1. Healthcare Databases for Paediatric Studies: a Report from the GRiP Network Global Survey

Submitted for publication

ABSTRACT

Background
A global federation of available healthcare databases on infants, children, and adolescents could provide the power and heterogeneity in drug exposure to improve evidence on the effects of drugs in children.

Objectives
To identify and describe electronic population based health care databases to create a global collaborative network.

Methods
In the framework of the Global Research in Paediatric (GRiP) network (http://www.grip-network.org), we performed a web-based survey to all electronic databases that were identified through manual review of the pharmacoepidemiology/pharmacovigilance conference abstracts, the 'Bridge.to.Data' database or by direct knowledge of the GRiP network members. The survey solicited information on the database contact and custodian, available population, exposure and outcome information, as well as access, governance, and sharing possibilities.

Results
A total of 125 population based databases were identified around the globe (in Europe, North- and South-America, in Asian-Pacific area, and Africa), all were invited to participate. To date, 64 answers were received (49%), with 53% of respondents (n= 34) agreeing to collaborate with the GRiP network in future pharmacoepidemiology studies. Databases willing to collaborate are located in 8 different European countries (n= 22), in 4 Asian/Pacific area countries (n= 6), in Canada (n= 4) and in the US (n= 2); one is available in more than one country. The data sources comprise a total of more than 40 million children (<18 years). According to available data for the survey, thirty databases provide drug/vaccine exposure information, including prescribing/dispensing drug data (n= 8), immunisation data (n= 4) or both (n= 18); three databases capture only clinical data, without exposure information. Concerning the study population, 18 databases capture outpatient records, 10 capture both inpatients and outpatients and 1 only inpatient data.

Conclusion
Identified databases willing to collaborate in the GRiP network hold are spread around the world and hold enormous potential for improving paediatric phar-

macoepidemiological studies. A first step towards a collaborative approach is being made by characterizing available databases. Identification and participation of missing response databases will continue, while first proof of concept studies will start.

BACKGROUND

Pre-marketing RCTs are able to identify the most frequent adverse reactions of a medicine. However, because of inclusion of small and selective groups of individuals or of short trial duration[60], tolerability of drugs/vaccines is not fully recognized prior to marketing. Passive and active drug safety surveillance both contribute important information for hypothesis generation and/or testing. Currently, the use of electronic health care databases is recognized as state-of the art for rapid assessment and verification of safety signals that may be generated by different routes. Although the use of health care databases is well inserted in adult pharmacoepidemiology, and pooling is recognized as important tool to improve power, very little is done in paediatric pharmacoepidemiology.

Combining data from different databases and countries is crucial in paediatric pharmacoepidemiology to increase the sample size and the heterogeneity of population setting and to perform long-term follow-up studies[286]. The *Global Research in Paediatrics Network of Excellence* (GRiP) is an EC-funded consortium, developed to implement an infrastructure to stimulate and facilitate the development and safe use of medicine in children. (http://www.grip-network.org/). GRiP promotes sharing of best practices in research, including methodologies and research tools that can be globally used. Central to these efforts are activities that evaluate methodologies and research tools according to GRIP recommendations on the needs of researchers (including industry) and patients.

Currently, some obstacles need to be overcome. First, the lack of collaboration and federation of healthcare databases is a missed opportunity for meaningful investigations in paediatrics. Second, the lack of shared methodologies to specifically retrieve paediatric information hinders access to valuable information.

In this paper, we describe the approach and the results of the identification and characterization of existing health care databases that may be accessed to develop a global paediatric pharmacoepidemiology infrastructure.

METHODS

Procedure for identification of healthcare databases

The procedure employed for the identification of the global population-based automated healthcare databases is outlined in **Figure 1**. Three different methods were combined to complete the total list of database contacts which were invited to participate in the on-line survey.

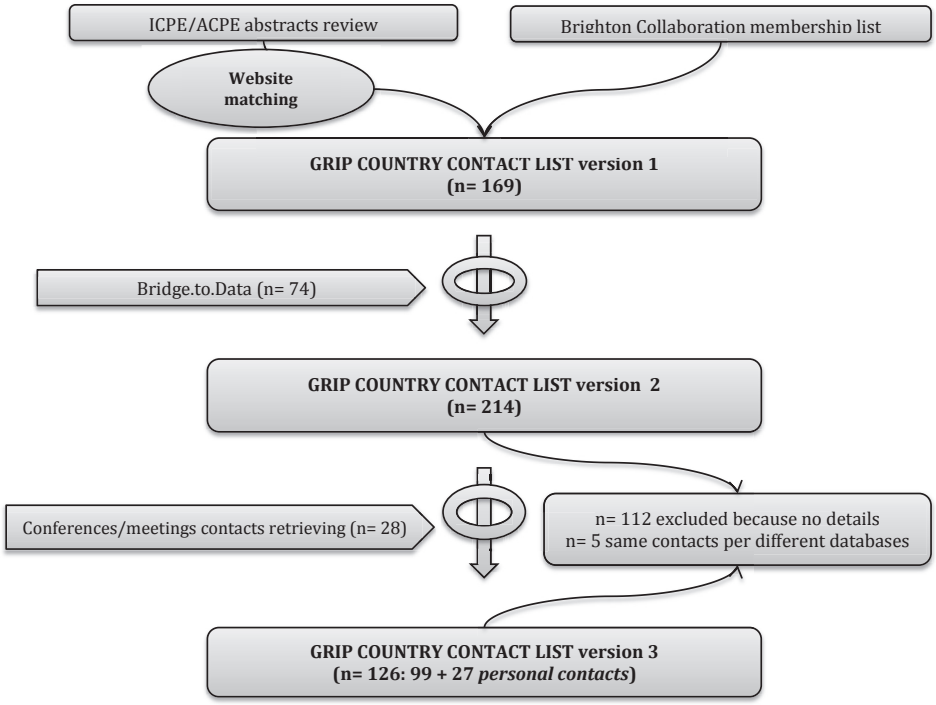

Figure 1. Flowchart on the procedure for selection of the database contacts from different sources.
Abbreviation: ICPE/ACPE= International/Asian Conference of Pharmacoepidemiology

a) Retrieving data from published ICPE abstracts

A systematic review of published abstracts presented at the 25th and 26th International Conferences on Pharmacoepidemiology and Therapeutic Risk Management (ICPE) during the years 2009-2010 and the ICPE abstract books of the *Asian* meetings (ACPE) abstracts were reviewed. All duplicates were excluded and the following information was retrieved: abstract number, conference year, country, name of automated healthcare database. Subsequently, by consulting the corresponding websites, further data on contact details, start-years and type of database (e.g., claims, GPs, pharmacy database, *etcetera*), and covering age range were collected, whenever available. A final list namely "*Abstract database contacts*" included 169 database contacts from all continents.

b) Procedure for identification of the immunization databases

The contact list for the immunization databases was compiled by the Brighton Collaboration Foundation according to the following approach:

Step 1: The Brighton Collaboration member list was screened for potential contacts in each country with emphasis on contacts affiliated with public health authorities.

Step 2: In countries where no contacts with public health background were available, professionals from regulatory authorities or academia or clinical care agencies were approached for recommendation of suitable contacts in their countries.

Step 3: Professionals referred to us based on Step 1 and 2 correspondence were contacted.

Step 4: Other networks or activities such as the International Paediatric Association, INDEPTH, the Global H1N1 vaccine safety case series, were utilized to identify additional contacts.

c) Retrieving form Bridge to Data and meetings/conferences

"B.R.I.D.G.E. to data" is a non-profit organization that provides online reference to different population-based healthcare databases worldwide that can be used in epidemiologic and health outcomes researches (http://www.bridgetodata.org). The centralized "B.R.I.D.G.E. to data" compendium contained at the period of inquiry over 170 standardized database profiles (with 75 defined data fields) representing 24 countries. It is structured in such a way that there can be efficient side-by-side analysis of databases as well as providing extensive database details (with the permission of the database managers). It is being continuously updated. "B.R.I.D.G.E. to_data" includes longitudinal EHR, claims databases, drug or disease specific cohorts registries, national surveys, national surveillance systems and SRS databases. Access is provided upon paying a license fee. For the purpose of this task however, only longitudinal EHR databases have been considered.

The results obtained from "B.R.I.D.G.E. to data" were finally compared with the information that was already available for each country in the list of databases being compiled.

Other sources

The various lists were matched to delete duplicates. In parallel, some members of the GRiP network established direct contacts with the database owners met at the conferences concerning "Vaccine and drug safety in paediatrics" of ECDC and a meeting at the Public Health Agency Canada. An additional inventory was set up including 28 database contacts. This latter inventory and the updated list were matched to provide the final database contact list to be invited to participate to the survey (**Figure 1**).

Creation of the survey

In order to conduct the survey, a questionnaire to capture key characteristics of the databases was developed. Given the objectives of GRiP, the items included in the questionnaire concerning the nature of the databases, the type of data collected and the possibility for the database to contribute data for future GRiP studies.

Two previously tested questionnaires, used in surveys describing existing databases in the European context, were used as reference: a) the questionnaire developed by the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) to collect information on databases with pharmacological data in EU (www.encepp.eu); b) the questionnaire used by the Task-force in Europe for Drug Development for the Young (TEDDY) for a survey on databases for paediatric medicine research[287]. The survey from the TEDDY project, which was specifically designed to describe paediatric databases, provided the guide for most of the paediatric specific questions. Specific additions comprised a complete section on information available on paediatric vaccinations.

The final questionnaire comprised of 14 main sections with a total of 55 questions (**Appendix**). The main sections included:

- Contact information for database and responsible person (name and address) – sections 1, 14;
- Information on nature of database (possible linkage of drugs prescriptions and/or clinical data with population) – sections 2-3;
- Years, population and geographic areas covered by database – sections 4-5;
- Information on data collected: type of demographic and clinical data (including data on referrals), type of data on drugs and vaccines – sections 6-9;
- Possibility of collaboration in future studies: regulations to access the data stored, additional information that could be collected if needed, intent on future collaborations – sections 10-12;
- Previous publications on data collected (with focus on paediatric) – section 13.

Complex questions were broken down in several simple questions and whenever. A user's guide including instructions on most questions was developed together with the questionnaire to be delivered with it prior to the survey.

RESULTS

Databases invited to participate to the survey

A total of 238 automated population-based healthcare databases were identified through manual revision of the ICPE/ACPE abstracts, B.R.I.D.G.E.to.Data software and by personal contact of the members of GRiP network. By continent,

we collected 90 databases from European countries, of which 37 were exclusively extracted by abstract conferences' revision, 22 from "B.R.I.D.G.E. to data", 17 were matched between "B.R.I.D.G.E. to data" and "Abstract database contact list" and 14 were retrieved through networking at the meetings. Similarly, 74 databases were identified from northern America countries: 39 came exclusively from the abstract conference revision, 17 from "B.R.I.D.G.E. to data", 11 were matched between these two inventories and 7 through networking at the meetings. Among Asian-Pacific countries we identified 46 databases: 36 contacts exclusively from abstract review, 6 exclusively from "B.R.I.D.G.E. to data", 1 matched, and 3 through networking at the meetings. Twenty-two databases from African countries and 6 from southern American countries were retrieved only by abstract revision; however, we failed to found enough contact information.

After screening of the email address details and exclusion of all duplicates (n=3) and the records with not sufficient details to be contacted (n=112), 125 databases were identified globally and 99 out of them were invited to participate to the on-line survey. A reminder was sent up to non-responders, and repeated when no reply.

The remaining 26 databases, contacted through direct networking at meetings/ conferences, were personally invited by the leader members of the GRiP network to fill the questionnaires, either on-line or not. The process will be continued during the project life time.

Response rate of survey

To date, 125 surveys have been sent out and we received 64 responses, corresponding to a 51% response rate. In total, 53% of the respondents (n= 34) accepted to collaborate to the GRiP network for future pharmacoepidemiology studies. Five users did not approve or disagreed; one of them expressing concerns about the clarity of the information provided on the involvement in the project. Fourteen other users answered only partially to the survey.

Assessment of the survey responses

Only the data sources of which the responders agreed to collaborate to the GRiP network so far (n= 34) were included in these analysis.

These databases are located in 15 different countries around the world, except for the MediGuard database that is available in more countries. A density world map illustrates the global distribution of paediatric population data provided by these databases in each country (**Figure 2**).

Figure 2. Density map of paediatric population provided by the databases included in the GRiP network.
The number of children provided by each country into GRiP platform is clarified in the legend.
*Data are not fully available for the Countries as follows: US, Canada, EU (Germany, Slovakia, Spain).
#MEDIGUARD (around 5,000 kids) is not included in the map.

Nature and characteristics of the databases

Among 34 databases, 26 provided the total number of paediatric patients, accounting for around a population of 40 millions of children/adolescents from the birth to 17 years of age. Data sources included 14 primary care databases from GPs or FPs, 11 insurance claims, 2 both GP/claims data, 2 hospital and 1 disease registries (RedMIVA). Information about the type of data source for the remaining 4 databases were not assessable from the survey.

Concerning drug information, 30 databases provided drug/vaccine exposure (i.e. 8 databases provide prescribing/dispensing drug data, 4 immunisation data and 18 both) whilst 3 databases capture only clinical data, without exposure information. Among the 30 exposure databases, 26 include also clinical outcome data and 10 specify the indication of medicine use.

Concerning the study-population, 18 databases capture outpatient records, 10 both inpatients and outpatients and 1 only inpatient data. Patient-level linkage between drug exposure and clinical outcome is feasible for all databases (data not shown).

Based on the survey information, literature, and available information on the website, 12 databases were detailed with respect to their potential suitability for use in paediatric drug utilization and drug safety studies. Information collected has been categorized as demographics, drug exposure, clinical outcomes and data access (**Table 1**).

Table 1. Main characteristics of databases involved in GRiP network

Country (n. of databases)	Database	Cumulative number (0-17 years)	Type of data source	Drug prescriptions (exposure)	Indication	Prescription characteristics^	Clinical outcome (disease)	Immunisation data*
Germany (n= 2)	GePaRD		claims	Outpatients	No	yes (no frequency and duration)	In- and outpatients	Yes (brand name n.a.)
	IMS Disease Analyzer	900,000	GP	Outpatients	ICD-10	Yes	Outpatients	Yes (elective immunization n.a.)
UK (n= 3)	THIN	1,808,407	GP	In- and outpatients	Read code -lab data	Yes	In- and outpatients	Yes (brand name n.a.)
	CPRD, Clinical Practice Research Datalink	12,500,000	GP	In- and outpatients	Read code	Yes	In- and outpatients	Yes (elective immunization n.a.)
	PCCIUR (Scotland)	219,270	GP	outpatients	No	yes	outpatients	yes (brand name n.a.)
Denmark (n= 1)	Aarhus University Research Database	1,800,000	claims	In- and outpatients	No	yes (only formulation, units, route)	In- and outpatients	Yes (brand name n.a)
Italy (n= 6)	ASL Cremona	97,400	claims	Outpatients	No	yes (only formulation and route)	Inpatients	Yes
	PEDIANET	180,000	FP	Outpatients	ICD-9 - free text	yes	outpatients	no
	Emilia-Romagna	500,000	GP	Outpatients	No	yes (no dosage frequency and duration)	Inpatients	no
	ARS Toscana	930,000	claims	Outpatients	No	yes (no dosage frequency and duration)	Inpatients	No
	Lombardy Region Healthcare administrative database	2,600,000	claims	Outpatients	No	yes (only formulation and route)	Inpatients	no
	FRIULI VENEZIA GIULIA REGION	300,000	claims - GP	Outpatients	No	yes (no dosage frequency and duration)	Inpatients	yes (vaccine code and brand name, elective immunization n.a.)

Table 1 (continued)

Country (n. of databases)	Database	Cumulative number (0-17 years)	Type of data source	Drug prescriptions (exposure)	Indication	Prescription characteristics^	Clinical outcome (disease)	Immunisation data*
	IADB.nl	64,645	GP	Outpatients	No	yes	na	Yes (routine and elective immunization n.a.)
Netherlands (n= 3)	Agis Health Database		claims	Outpatients	No	yes	In- and outpatients	No
	IPCI	300,000	GP	Outpatients	ICPC-code	yes (no formulation)	In- and outpatients	yes (linkage)
	RedMIVA		n.a				In- and outpatients	no
	SIV (RVN)		n.a					yes
Spain (n= 5)	CMBD		hospital				Inpatients	no
	ABUCASIS (SIA-GAIA)	1,025,639	GP	outpatients	Yes	yes	outpatients	no
	SIDIAP pediatrics	1,200,000	claims - GP	outpatients	No	yes (no frequency)	Out- and Inpatients	yes (brand name and elective immunization n.a.
Slovakia (n= 1)	EPIS		claims	no			Out- and Inpatients	Yes (routine and elective immunization n.a.)
Iceland (n= 1)	Tölfræðigrunnur Sjúkratrygginga Íslands		claims	outpatients	No	yes (no duration, formulation and route)	no	no
USA- Minnesota (n= 1)	Rochester Epidemiology Project (REP)	39,400	GP	outpatients	No	yes (no duration)	In- and out-patients	Yes (brand name and routine immunization n.a.)

Table 1 (continued)

Country (n. of databases)	Database	Cumulative number (0-17 years)	Type of data source	Drug prescriptions (exposure)	Indication	Prescription characteristics^	Clinical outcome (disease)	Immunisation data*
	Vaccine and Immunization Surveillance in Ontario (VISION)	5,000,000	claims	Outpatients	No	yes	In- and out-patients	Yes (no brand name)
Canada (n= 4)	Immunization Record Information System (IRIS)	1,600,000	n.a.	Outpatients	No	no		Yes (brand name and elective immunization n.a.)
	IMPACT		n.a.	no			inpatients	yes (brand name and elective immunization n.a.)
	Quebec Pregnancy Registry	299,562	claims	In- and outpatients	ICD-9; ICD-10	yes	In- and outpatients	no
	National Immunisation Register (NIR)	540,000	GP	in- and outpatients	ICD-10	n.a.	no	yes
New Zealand (n= 4)	Sentinel Primary Health Care Database	1,000,000	GP	in- and outpatients	n.a.	yes (no duration, strenght and route)	in- and outpatients	yes
	New Zealand National Collections - Health Outcomes	1,000,000	GP	in- and outpatients	ICD-10	yes (no duration and formulation)	in- and outpatients	yes (elective immunization n.a.)
China (n= 1)	Hospital discarge database	200,000	claims	inpatients	free text	yes	In- and outpatients	no
Taiwan (n= 1)	Taiwan Centers for Disease Control (TCDC)	7,500,000	claims	in-out-patients	ICD-9	yes	In- and outpatients	yes
Australia (n= 1)	SAEFVIC	4,000	GP	no			In- and outpatients	yes
Multi-country	MediGuard.org	5,000		In- and outpatients	No	no	In- and outpatients	no

^prescription characteristics included n. of units, dosage frequency, duration, formulation, route and strength, except when is differently noted.

*immunisation data include vaccine code and brand name, date, routine paediatric and elective childhood immunisation except when is differently note.

Six databases from 3 countries (in UK: The health Improvement Network, THIN, and Clinical Practice Research Datalink, CPRD; in Italy: PEDIANET and Emilia-Romagna; the Netherlands: IADB.nl and IPCI) are longitudinal, population-based databases using EHR from GPs and FPs. PEDIANET comprises also claims data when collected by the FPs. These databases were developed in countries where physicians and/or paediatricians (in Italy) are gatekeepers for medical care and information. All of these EHR databases contain anonymous data on patient demographics, reasons for visits, diagnoses from GPs/FPs and specialists, hospitalizations, drug prescriptions, laboratory and other diagnostic findings for the paediatric population.

Five databases (German Pharmacoepidemiological Research Database - GePaRD, ASL Cremona, ARS Toscana, Agis Health Database, Aarhus) are drug dispensing claims databases processing all prescriptions covered by reimbursement. Patient-level linkage between drug exposure and clinical outcome and patient population file is feasible for all of them. GePaRD provides demographic data as well as information on hospital admissions, outpatient physician visits and outpatient prescriptions from Statutory Health Insurances (SHI).

MediGuard is not a GP neither claim database but is a free medication monitoring service designed specifically for patients by professionals with decades of experience in healthcare market research, clinical drug development, and drug safety (www.mediguard.org). No more info were found concerning the collection of data.

Drug exposure

All databases that participated in the survey collect information on prescription-drugs and the units dispensed or prescribed, the formulation, and most of them also record the dosage regimen, which is particularly important for the paediatric population. Medicines are coded according to the ATC classification system in the majority of the databases. Some of them use also other drug codes such as z-index (IADB.nl and IPCI), DPICS (Agis), AIC (PEDIANET), Multilex coding system (CPRD) and British National Formulary, BNF (THIN). The indication of use is recorded only in CPRD, PEDIANET and IPCI, using Read code, ICD-9th CM code, plus free text, and ICPC-code, respectively. In GePaRD, prescription data contain the prescribed drugs characterized by the central pharmaceutical number (PZN), the dates of prescription and dispensation, and information on the prescribing physician. They are available for all outpatient prescriptions that are reimbursed by the SHIs. Prescription data are linked to a pharmaceutical reference database that adds information on the defined daily dose (DDD), the ATC code, strength, packaging size, and the generic and brand names.

Vaccine exposure
Immunization data are captured in six databases (GePaRD, THIN, CPRD, Aarhus, ASL Cremona, IADB.nl), they all include vaccine code and date of vaccination for routine paediatric immunisation; three databases (CPRD, ASL Cremona, IADB,nl) include also substance name and four (GeParD, THIN, Aarhus, and ASL Cremona) also contain data on elective childhood immunisation.

Clinical outcome
Past and current medical diagnoses are recorded using READ codes (a thesaurus of coded medical terms maintained and distributed by the UK Terminology Centre) in THIN, ICD-10 Germany Modification in GeParD, Aarhus, IADB.nl, and both code systems in CPRD. Symptoms and medical diagnoses are either registered as free text or coded using ICPC in IPCI and ICD-9-CM in Pedianet and all the remaining Italian databases. Hospital data are reported in the majority of the databases and include the dates of admission and discharge with their corresponding diagnoses, and information on in-hospital diagnoses and procedures. Claims databases regarding outpatient physician visits contain diagnoses, ambulatory diagnostic procedures and non-drug treatments.

Accessibility and costs of databases
All these analysed databases allow access data for paediatric researches. The majority of the providers requires a written policy governing and a committee evaluation. Six of the databases may be accessed free of charge, although most of them provide special conditions if data are used for academic research or purposes.

DISCUSSION

Combining and sharing data from different databases and countries is important to increase sample sizes and to perform long-term studies in paediatrics. To date, combining the population of the databases that participated in this survey results in a paediatric population of more than 40 million providing a good potential for paediatric pharmacoepidemiological studies. Creating an inventory of existing health care databases and their willingness to participate in future projects is crucial step as large databases are needed for paediatric pharmacoepidemiology research in terms of power and long term follow-up

Previous projects such as EU-ADR, and others have shown that data from different databases can be combined to conduct international observational studies[208]. The majority of health care databases are created not primarily to conduct

research but are simply a collection of electronic patient's records accessible for the healthcare staff to monitor patient's care. The organisation of health care is country-specific which in part explains the heterogeneity among the databases in terms of disease and drug coding. The development of automatic tools such as disease and drug mapping will further facilitate the combination of data from different healthcare databases according to a common study protocol.

From the survey, we learned that the databases collect information on age, drug dosing, mother-child linkage, immunisation status, *etc*. Other important information such as height and weight are however, not systematically collected. Although we appreciate that the healthcare databases do not have research as primary aim, it would be an asset if databases would start to collect crucial information that has been proven important for paediatric research.

So far, the observed response rate and collaborative opportunities are developed mostly in Europe and in North-America. No databases from South-America and Africa responded to the survey. The majority of non-responders from Asian-Pacific countries is mainly due to the scarce knowledge about the GRIP project and the reluctance to share data. The absence of databases from these regions may be due to the diversity of healthcare systems and our common challenge is to identify the methods and technical requirements to facilitate bridging the different structures. In the coming weeks, we will continue contacting the non-responding databases which will enrich our inventory of paediatric databases. In the end, we would create an up to data inventory of all existing paediatric databases which should allow conduction worldwide paediatric observational research.

CONCLUSION

Several population-based databases on paediatric data are available on a global level. The majority of respondents to the survey is willing to share data and participate in future studies in children. These studies will show the potential of pooling data in term of increase of sample size and information in order to provide knowledge on the use and the safety of medicine in children.

Appendix 1. GRiP survey

GRIP Survey on electronic health care databases		
01 - Main Info		
S001: Name of the database		
S002: Database URL		
S003: Contact persons Administrative Contact person Title		
Name		
Address City, Postcode, Country, Phone number (incl. country code), Alternative phone number, Fax num.		
Email address for administrative contact		
Scientific Contact person Title		
Name		
Address City, Postcode, Country, Phone number (incl. country code), Alternative phone number, Fax num.		
Email address for scientific contact		
S004: Brief Description		
02 – Nature of the database		
S005: Does the database capture drug prescriptions?	Yes	No
If yes: does it capture drug prescriptions for Outpatients?	Yes	No
- through Insurance claims	Yes	No
- through Medical records	Yes	No
Does the database capture drug prescriptions for Inpatients?	Yes	No
- through Insurance claims	Yes	No
- through Medical records	Yes	No
S006: Does the database capture clinical data?	Yes	No
If yes: Outpatient clinical data	Yes	No
Inpatient clinical data	Yes	No
S007: Linkage with population data		
Is patient-based linkage of clinical data to follow-up time (population file) possible?	Yes	No
If Yes:		
- Probabilistic linkage	Yes	No
- Deterministic linkage (with unique identifier)	Yes	No
S008: Is patient-based linkage between drug prescriptions and clinical data possible?	Yes	No
If Yes		
- Probabilistic linkage	Yes	No
- Deterministic linkage (with unique identifier)	Yes	No
03 - General Characteristics		
S009: Start date of data collection		
S010: Is the database updated:		
Continuously	Yes	No
At intervals	Yes	No
If Yes, please specify the interval		

S011: Total Cumulative number of registered subjects, including adults		
S012: Total Cumulative number of registered children (0-18 years of age)		
S013: Number of active (registered) children (0-18 years of age) in 2010		
04 - Geographical Coverage		
S014: Are the patients in the database representative for national population? (according to age and gender distribution)	Yes	No
S015: Names of covered regions or provinces		
05 - Collected Data – Demographics		
S016: Exact date of birth available as ○ Date, Month, Year ○ Month, Year ○ Year ○ None		
S017: Gender	Yes	No
S018: Height	Yes	No
S019: Weight	Yes	No
S020: Mother-child linkage	Yes	No
06 - Collected Data - Clinical Data		
S021: Reason for accessing care	Yes	No
S022: Diagnosis If Yes, how is diagnosis collected?: ○ as text ○ as code: ○ ICD-10 ○ Read code ○ ICPC code ○ ICD-9 code ○ Others (please specify).....	Yes	No
S023: Measurements (laboratory/diagnostics)	Yes	No
07 - Collected Data – Drugs		
S024: Name of drugs prescribed	Yes	No
S025: Identification code for each drug If Yes, which codes are used?: ○ The Anatomical Therapeutic Chemical (ATC) Classification System ○ The Drug Products Information Coding System (DPICS) ○ The Multilex Coding System ○ The National Drug Code (NDC) System ○ Others (please specify)	Yes	No
S026: Indication for prescription If Yes, how is indication collected?: ○ as text ○ as code: ○ ICD-10 ○ Read code ○ ICPC code ○ ICD-9 code	Yes	No
S027: Total number of prescribed units (tablets/ml, suppositories etc) for each drug	Yes	No

S028: Prescribed dosage frequency for each drug	Yes	No
S029: Prescribed duration of treatment for each drug	Yes	No
S030: Drug Formulation	Yes	No
S031: Drug Strength (of each unit)	Yes	No
S032: Route of administration	Yes	No
08 - Collected Data – Vaccines		
S033: immunizations	Yes	No
If Yes:		
S034: Routine paediatric immunization	Yes	No
if Yes: select from the list (all possible):		
BCG		
Cholera		
Diphteria		
Haemophilus influenzae		
Hepatitis A		
Hepatitis B		
HPV		
Influenza		
Japanese encephalitis		
Measles		
Meningococci		
Mumps		
Pertussis		
Pneumococci		
Poliomyelitis		
Rabies		
Rotavirus		
Rubella		
Tetanus		
Tick born encephalitis		
Typhoid		
Varicella		
Yellow fever		
S035: Additional (elective) childhood immunisation	Yes	No
if Yes: select from the list: (as above)		
S036: Date of vaccination	Yes	No
S037: Brand name of vaccination	Yes	No
09 - Collected Data – Referrals		
S038: Referral to specialist	Yes	No
S039: Results of referral visits	Yes	No
S040: Emergency room admission	Yes	No
S041: Results of emergency room admission	Yes	No
S042: Hospital admission	Yes	No
S043: Hospital discharge diagnosis	Yes	No

If Yes:

S044: How is the diagnosis collected:

- ○ as text
- ○ as code:
  - ○ ICD-10
  - ○ Read code
    - ○ ICPC code
    - ○ ICD-9 code
  - ○ Others (please specify) .....

10 – Would it in principle be possible to obtain the following additional information on the patient?

S045: Clinical information from treating physician?	Yes	No
S046: Data from questionnaires completed by the patient?	Yes	No
S047: Genetic information or samples?	Yes	No

11 – Data access

S048: Is there a written policy governing data access?	Yes	No
S049: Do you have a committee (governance/ethics) to evaluate requests for data access?	Yes	No
S050: Is a charge made for data access	Yes	No
S051: Are you allowed to provide data / do industry sponsored studies	Yes	No

S052: Would you allow for auditing of the data/studies by external parties?

- ○ yes to regulators
- ○ yes to companies for whom studies are done
- ○ No

12 - Please list the 5 most relevant publications using your data for the last five calendar years (please focus on paediatrics). If there is a publication explicitly reporting on assessment of data quality, please include first

13 - Comments (please add comments or questions on this survey, or additional information on your database)

14 - Survey completed by: .....

on: DD/MM/YY

CHAPTER 7

General Discussion

Spontaneous reporting systems (SRS) and Electronic Healthcare Records (EHR) are crucial systems to learn about the safety of drugs post-licensure.

The field of pharmacovigilance is evolving and new methods are implemented to facilitate the systematic and timely detection, assessment and understanding of potential safety signals (**Chapter 1**). Very few researchers are focusing on paediatrics specifically, whereas a lot is to be learned about the effects and safety of drugs in children, which are not just small adults. The research as described in this thesis therefore focuses on children. It first describes information on all adverse events/drug associations in SRS, as well as EHR databases in paediatrics and subsequently focuses on hepatic toxicity in paediatric patients using both SRS and EHR as data source.

MAIN FINDINGS

Spontaneous Reporting System

There are different international databases collecting spontaneous reports of adverse drug events (ADEs) from multiple countries. First of all there is the VigiBase database managed by the WHO Uppsala Monitoring Centre (WHO-UMC). Initiated in 1960 after the Thalidomide disaster, VigiBase is the largest database worldwide with > 4 million individual case safety reports covering more than 40 years. The reports are sent to WHO-UMC by the national centres participating to the WHO Drug Monitoring Programme[102, 160].

In the US, the FDA Adverse Event Reporting System (FAERS) is a database that contains information on adverse event and medication error reports of drugs licensed in the US[138].

In Europe, spontaneous reports of ADEs are collected nationally and, subsequently, transferred as individual case safety reports into both VigiBase and Eudravigilance (that includes also reports coming from non-European countries)[108, 288].

As an example of a national database, in Italy, ADE reports are collected through the Italian Pharmacovigilance Network (*Rete Nazionale di Farmacovigilanza*, RNF) established by the Italian Medicines Agency (AIFA) in 2001. AIFA submits reports to Eudravigilance[110].

The characteristics of the reports in children and adolescents, including number and type of ADEs, their outcome and the most frequently implicated drugs have been described both in the Italian RNF database (**Chapter 2.1**) and the US FAERS database (**Chapter 2.2**).

In both the AIFA and FAERS database, the largest proportion of ADEs were reported for boys until the age of 11 after which it reversed to girls. Several differ-

ences were observed between the Italian and US reports. First of all the type of reporters differed with more than two thirds of the ADEs reported by physicians (including paediatricians, specialist and general practitioners) in RNF and only a third in FAERS; moreover, consumer reports made up 24.9% of the FAERS reports while this was less than 1% within the Italian RNF. In the US, patients have been allowed to report ADEs directly to FAERS, which is different in Europe. More recently, there has been an increase in the number of countries who encourage patients to report ADEs[289]. In Europe, the new EU legislation on Pharmacovigilance, as applied from July 2012 onwards, allows direct patient reporting to the competent authorities in all EU Member States. Each member State had to implement the provisions of the Directive into their national laws by October 2013. Systems for direct reporting of ADEs by patients (or consumers) already operate in some EU Member States, notably the UK, Denmark and the Netherlands, with positive results (www.monitoringmedicines.org). Several studies have shown that patient reports are as valuable as reports by healthcare professionals[290]. Patients report different types of reactions than health professionals and they perceive the impact and severity of reactions differently (www.eu-patient.eu/Events). There are still many countries that do not yet have schemes for direct ADE reporting by patients[152]. In Italy, local initiatives have been launched to disseminate awareness to patient/consumer communities and to inform them about their role to improve medicines safety. As a consequence, RNF shows an increase of direct ADE reporting by consumers/patients since 2013[112, 291].

In our exploration of paediatric reports in the FAERS and RNF databases, we observed important differences in reported drug groups and events. Reports concerning anti-infective agents were more often submitted to RNF than to FAERS (44.9% *vs*. 12.1%), while neurological drugs were most frequently reported in FAERS than in RNF (28.8% *vs.* 15.6%, respectively). This finding reflects the differences in drug utilisation between the US and Europe, with e.g. high rates of prescriptions of methylphenidate in US adolescents in recent years[154] and the high prescription of antibiotics in Italy[122]. Similarly, the differences in terms of reported events between two Countries reflect the different drug use between these countries. Indeed, allergic reactions, the most frequently ADRs reported among Italian children, are well-known to be induced by antibiotics use[124], the most commonly prescribed drug in children[122].

These findings need to be interpreted considering that special characteristics related to the different stage of growth and development could explain the differences in terms of drugs and adverse events observed across age.

Signal detection of hepatic injury in SRS

Drug-induced hepatic injury is one of the most important reasons for drug withdrawal after launch[158], but little is known about drug-induced hepatic injury in children. Most of the evidence comes from small case series[159], but, except for specific drugs well-known to be hepatotoxic in both adults and children, evidence about the hepatotoxicity of old/new products if used in children is scarce. In post-marketing drug safety surveillance, the analysis of spontaneously reported ADEs is the first approach for new safety signal detection.

In **Chapter 3** we described a case/non case approach using data from VigiBase to identify which drugs have been associated with hepatic injury in children and adolescents.

The main findings are described below. First, only a small proportion of reports in children and adolescents (1% of total reports) concerned hepatic injury. This means that either drug-induced hepatic injury is rare in children or that it is not recognized/reported. Since there is no denominator data on drug exposure in the spontaneous reporting databases, reporting odds ratio (ROR) were used to identify potential associations[169]. When investigating individual drugs, in general, the ROR decreased with age, whilst the absolute number of reports increased. Contrary to expectations, the ROR for acetaminophen and liver injury was higher in younger children which usually have less metabolic capacity to produce the toxic metabolite[158, 171, 172]. An explanation for this finding could be that, among toddlers, intoxication from acetaminophen is mainly due to unintentional therapeutic error by inappropriate dosing, unintentional multiple overdosing, ingestion of acetaminophen along with another hepatotoxic drug or use of adult rather than paediatric formulations[173]. The ROR for valproic acid, ciclosporin and vincristine decreased with age which is consistent with published data[81, 171]. Indeed, the isoenzyme CYP 3A4, which plays a fundamental role in the metabolism of these drugs, is more expressed in adolescents than in newborns and infants, leading to a larger capacity in older children to eliminate these drugs.

Signal detection on VigiBase demonstrated that most of the drugs that are potentially associated with hepatotoxicity in children have already showed to be hepatotoxic in adults, except for one drug, namely basiliximab. This association could be considered as a new signal of hepatotoxicity in children.

Analysis of SRS is a cheap and rather sensitive approach to detect signals of well-recognized ADEs after marketing . Since these systems only capture case reports and no denominators, risks need to be verified and quantified through pharmacoepidemiological studies.

Electronic Healthcare Records databases

EHR databases are, at the state of the art, a source for verification and quantification of potential drug safety signals. Due to the safety issues around rofecoxib and rosiglitazone, both widely used drugs that were withdrawn years after their initial licensure, discussions started on the ability of SRSs to detect safety signals around common events (e.g. myocardial infarctions). Several initiatives were started both in the US as well as in EU to explore whether EHR databases could be used for signal detection as well.

One of these initiatives was the "*Exploring and Understanding Adverse Drug Reactions by Integrative Mining of Clinical Records and Biomedical Knowledge*", the EU-ADR projects which aimed to develop an innovative computerized system to detect adverse drug reactions (ADRs)[92]. The EU-ADR project exploited clinical data from EHRs of over 30 million patients from several European countries (The Netherlands, Denmark, United Kingdom, and Italy) (www.euadr-project.org)[187, 286, 292, 293]. First of all in **Chapter 4**, we describe the statistical power of the EU-ADR network to perform paediatric safety signal detection. The paediatric population of the EU-ADR network comprised 4.8 million children and adolescents contributing 25.6 million PY of follow-up. An important first finding was that use of medicines in children was rare and was limited to only a few medicines; only 18 medicines represented 50% of the total drug exposure time in paediatrics. Second, events that were thought to be most relevant for safety monitoring in adults showed very low incidence rates in the paediatric population. Of the original 13 events that were prioritized in EU-ADR because of their serious nature, only 10 events had an IR of >1/100,000 PY in paediatrics, including acute liver injury, acute renal failure, anaphylactic shock, bullous eruptions, cardiac valve fibrosis, hip fractures, neutropenia, acute pancreatitis, pancytopenia, and upper gastrointestinal bleeding (UGIB). This combination of rare events and low exposure time to medicines resulted in a low number of medicines with enough exposure to allow safety monitoring for these events. For a rare but serious event such as anaphylactic shock there were no drugs with enough exposure to study a weak association (RR≥2) and only 20 drugs to study a strong association (RR≥6). For a more frequent event such as UGIB there were 5 medicines with enough exposure to study a weak association (RR≥2) and 79 drugs for which an association with a RR≥6, if present, can be investigated. The statistical power will be much higher for events more frequently occurring in the paediatric population. Therefore, future initiatives on drug surveillance systems for the paediatric population using EHRs, should focus on age-appropriate prioritization of events.

Signal detection for drug-induced liver injury on EHRs

In **Chapter 5.1** we describe signals for acute liver injury (ALI) in paediatrics from the EU-ADR databases. Acute liver injury was identified by using codes (non-validated) for this rapid identification and was associated with use of antibacterial agents, peptic ulcer drugs and gastro-oesophageal reflux disease, all drugs that are well-known to be hepatotoxic[98]. According to the definition of safety signals by Hauben and Aronson[218], not all statistically significant associations should be regarded as potential new signals. To check whether the identified signals were indeed new, we performed some triage by manually reviewing the Summary of Product Characteristics (SmPCs) and other main drug information sources. To the best of our knowledge, hepatotoxicity in association with domperidone, flunisolide and insulin (human) was not yet described in the literature, both for adults and children, and also not labelled in the SPCs. In addition, other two drug-ALI associations (citalopram, cetirizine) were not yet described in children although they had been described in adults. While a biological/pharmacological explanation could suggest a potential mechanism of liver injury for some of these potential signals, other associations, such as insulin and ALI, could be partly explained by potential confounding factors or bias. Subsequently, we investigated the same outcome but in this instance we manually validated the outcomes in data from 3 databases (IPCI, PEDIANET, HSD) in the Netherlands and Italy (all three part of EU-ADR) (**Chapter 5.2**)[99]. The overall incidence of idiopathic acute liver injury in children and adolescents was found to be relatively low (IR 62.4/100,000 person-years). When we stratified by country, the incidence rate was much higher in Italian than in Dutch children. The cases we identified were not such severe cases of acute liver injury. Indeed, most of our cases were characterized by mild or moderate elevations of liver enzymes which were mainly asymptomatic in Italy, while symptomatic in the Netherlands. These differences observed between Italy and the Netherlands are more likely due to the variability in the healthcare system and differences in drug prescribing rather than regional variation in susceptibility to liver injury. The incidence of ALI increased with age. Noteworthy, a slightly higher incidence of idiopathic ALI has been seen in the first year of age, suggesting a varying activity of CYP 450 in early life[99]. This high incidence in young children either reflects a change in the enzymatic maturation of the liver as metabolizing organ or reflects a higher or different intake of hepatotoxic drugs during childhood compared to adolescence. Although we excluded all competing well-known aetiologies for ALI, differential diagnosis of idiopathic liver injury versus liver injury triggered by drug use is extremely complicated. Accordingly, among all included "idiopathic" cases, we did identify several drugs, mainly antibiotics, that are known to be associated with liver injury. All potentially new detected signals

as described in **Chapters 5.1** and **5.2** require further evaluation in hypothesis testing studies (e.g. formal pharmacoepidemiological studies) to better account for bias and confounding.

Signal verification for drug-induced liver injury on EHRs

Chapter 5.3 of this thesis describes the further analysis and validation of antibiotic-induced hepatotoxicity in the same databases as we used in Chapter 5.2, but now with proper control for confounding and adequate risk windows. Because antibiotic-induced hepatotoxicity mimics other liver diseases, diagnosis is necessarily one of elimination and is usually based on a high degree of clinical suspicion following exclusion of competing aetiologies, such as viral hepatitis or biliary disease. Confounding by indication is a main concern when studying this association. To control for this confounder, we conducted several sensitivity analyses. Upon these analyses, current exposure of ceftriaxone, co-trimoxazole and some macrolides (i.e. clarithromycin and rokitamycin) were and remained associated with liver injury after several analyses. These associations and timing of event in relation with exposure can well be explained by the following mechanisms. Ceftriaxone-induced hepatotoxicity is explained by the direct toxic effect associated with calcium precipitation and development of gallbladder sludge after 9-11 days of treatment, while co-trimoxazole hepatotoxicity results in fever and rush followed by jaundice and increase of liver enzymes within few days or weeks[268, 294]. The hepatotoxic profile of macrolides is characterized by transient and asymptomatic elevation in serum aminotransferases which occurs in 1-2% of patients after short-term period and in higher proportions after long-term period[268].

Need for sharing data

More research is needed to guarantee safe and effective use of drugs in children. This requires development of infrastructure, capacity building and funding. Spontaneous reporting databases as well as electronic healthcare records are important sources and have the potential to improve post-marketing safety research especially for patients generally not included in RCTs (children, elderly, women). So far, EHR are mainly used in isolation. In recent years, regulators encourage international collaboration and pooling of data to study drug safety. In line with US consortia such as Mini-Sentinel and Vaccine Safety Datalink (VSD), EU initiatives are expanding and establishing new networks and in addition EU funding is provided for research and training[286].

One of these EU projects is GRiP which focuses on paediatric clinical pharmacology and the effects of drugs and vaccines in children and adolescents. This FP7-funded consortium aims to implement an infrastructure facilitating the

development and safe use of medicines in children on a global level. Currently, GRiP is mobilizing 21 partners from Europe, North America and Japan, as well as the WHO[286]. Combining and sharing data from different databases and countries is important to increase sample size and to perform long-term studies in paediatrics. To realize the objectives of GRiP, the first step of the project was to identify and involve all existing databases with paediatric data. **Chapter 6** describes the procedure that GRIP used to identify these databases. To date, the cumulative population of the databases that participated in this survey results in more than 40 million of children/adolescents. This provides a good potential for paediatric pharmacoepidemiological studies, in terms of power, heterogeneity and long term follow-up.

METHODOLOGICAL CONSIDERATIONS

Spontaneous reporting databases

In **Chapter 2** we analysed two SRSs, either national or international, and in **Chapter 3** we used data from the worldwide spontaneous reporting database, VigiBase.

These SRSs reflect both real-life events and real-life prescribing, and therefore comprise drug use patterns that for ethical reasons, cannot be studied in clinical trials, such as overdoses and inappropriate co-medication[128]. Moreover, the system is sensitive and capable of detecting side effects quickly after market launch[128], and, mainly for VigiBase, covers all drugs and patients from most countries worldwide. On the other hand, the reliance of SRSs on voluntary reporting makes the system susceptible to various limitations, including underreporting and reporting bias, lack of information on the user prevalence and lack of details on patterns of drug use. This lack of denominator data does not allow to quantify the risk of drug-event combinations, implying that this system can only be used for hypothesis generation studies.

Electronic Healthcare Record databases

The rise in safety warnings and market withdrawals of widely used products in the first decade of the new millennium has fuelled efforts to explore other data sources and develop new methodologies[187, 292, 293]. In **Chapters 4** and **5** we used data from EHR databases, an important resource for both active surveillance and validation studies. These databases include detailed clinical information such as patients' symptoms, findings from physical examination, specialist care referrals and discharge letters, diagnostic tests, prescribed medications and other interventions.

Data from EHRs reflect actual clinical practice and as such have been employed to characterize healthcare utilization patterns, monitor patient outcomes and carry out formal drug safety studies. As all data have already been collected as part of standard care, use of these databases is efficient in terms of time management, man-power and financial costs[286]. Active surveillance within these databases should result in earlier detection, and hence earlier management of potential safety issues[293]. On the other hand, data mining on a large scale may generate more signals than effectively can be evaluated with currently available resources[292]. To minimize the potential of spurious signals, several data mining techniques within EU-ADR, as already described by Schuemie *et al*[214, 215]. Within our research we applied, apart from the standard case-control study, self-controlled case series (SCCS), Longitudinal Gamma Poisson Srinkage (LGPS) and Leopard, as described below.

LGPS estimates the age- and sex-adjusted incidence rate ratios, during the exposure of interest against all other follow-up time (on other drugs and off drugs) as while additionally applying Bayesian shrinkage (RR_{LGPS})[214, 215]. To reduce the risk of confounding factors, we used SCCS methods to control for time-fixed confounders such as genetic factors, socio-economic status and underlying chronic disease[214]. SCCSs investigate the association between acute outcomes and exposures, whereby cases are used as their own control. In essence, the SCCS is a Poisson regression conditioned on the patient[214, 215].

In addition, we applied a tool called 'LEOPARD' (Longitudinal Evaluation of Observational Profiles of Adverse Events related to drugs) to optimally control for protopathic bias[214, 217]. Protopathic bias occurs when a drug is prescribed for an early manifestation of a disease that has not yet been diagnosed. LEOPARD compares the number of prescriptions of a specific drug in a defined window prior to the outcome to the number of prescriptions of that same drug starting in the same window after the event. An increase in the number of prescriptions after the event date, relative to the number before the event, is considered an indication that the drug is used to treat the event or precursor of the event, rather than to cause it[214].

Finally, we used harmonised database-specific disease codes and free text search to automatically identify cases of liver injury from the EU-ADR database network. Manual case validation was not conducted for the signal detection study. A previous study using a US database network demonstrated that outcome misclassification does not influence the results concerning signal detection[232]. Despite using filtering criteria for significance and sensitivity analyses, the likelihood to obtain false positive results cannot be excluded and further validation of the newly identified associations needs to be carried out[233].

After signal detection analysis, a verification study has been performed to quantify the association between antibiotic use in children and hepatotoxicity by means of a case-control study. Data from three databases were combined to profit from the heterogeneity of antibiotic prescribing patterns across countries. In contrast to SRS, the use of these data allowed us to calculate incidence rates of liver toxicity and to quantify the association between use of antibiotics and hepatotoxicity.

Studying the relationship between antibiotic use and new onset of liver injury in EHR databases is extremely challenging due to a variety of potential biases and confounders. As for all observational studies, various types of bias, e.g. selection bias, protopathic bias, information bias and confounding by indication, may influence the validity of our findings, as briefly discussed below. First, the potential of selection bias was minimal as all data were obtained from prospectively collected medical records that are kept and maintained for patient care purposes.

Information bias may result from misclassification of either exposure or outcome. To minimize outcome misclassification, a two-step case validation process was undertaken. First, potential cases were identified through broad searches of disease codes and narratives in the electronic medical record of the study patients. Second, medical records of all potential cases were reviewed and manually validated by at least two experts and trained medical doctors, who were blinded to the exposure. Misclassification of the exposure may have occurred because we used outpatient prescription data and no information on actual filling or drug administration was available. However, such a bias would be likely non-differential between case and controls, thus underestimating the actual risk. The increased risk for penicillin and macrolides when we restricted to current use only suggests that in the initial analysis non-exposed time was misclassified as exposed. If indeed there is a carryover effect, this is probably not strong enough to cause liver injury.

Confounding by indication is a concern in the association between use of antibiotics and the risk of ALI. Confounding by indication is a term used when a variable is a risk factor for a disease among non-exposed persons and is associated with the exposure of interest in the population from which the cases derive, without being an intermediate step in the causal pathway between the exposure and the disease[295]. The indication of antibiotic use, i.e. infection, is itself a confounder because it correlates with the intervention and by itself is a risk factor for liver injury. To reduce the potential of confounding by indication, current use of amoxicillin was used as reference. This allowed us to compare the risk of all other antibiotics to a drug with similar indication of use but considered less hepatotoxic. Despite all corrective measures, residual confounding due to unmeasured severity of disease cannot be excluded.

Data network advantages

Multinational collaboration and pooling of data may yield information more rapidly and thus may reduce the time needed to obtain the desired sample size. Hence, networks can facilitate research on rare events, such as drug-induced liver injury and accelerate investigation of drug safety issues. As previously demonstrated in EUADR project, pooling data provide heterogeneity of drug exposure across countries allowing the study of the effect of individual drugs. In addition, multinational studies may provide additional knowledge on regional differences in drug safety issues, on the consistency of information and on the impact of biases on estimates (i.e. drug prescription, regulatory aspects, role of health care system)[286, 293].

FUTURE PERSPECTIVES

This thesis demonstrated the potential of the use of existing data sources to learn about the effects of drugs in children. Due to the need to focus we only explored DILI, but clearly many safety outcomes could be assessed in a similar way. We recommend to do this systematically for events of interest in paediatrics and for old drugs (that will not undergo PIP or PUMA processes anymore) that are frequently used in paediatrics. We also showed that paediatric specific signal detection is important, due to the different pattern of drug use and events in adults, paediatric signals may be masked. Very little is done to develop/test performance of methods specifically in paediatrics. The GRiP project will focus on methods development, and much is expected from this research. As for epidemiological studies, the area of paediatric pharmacoepidemiology is still small. Very few research groups conduct studies in paediatrics. More attention should be drawn to this area since specific problems exists such as polypharmacy and unlicensed use of critically ill neonates. Also we showed that pooling of fragmented data in different databases is necessary to reach enough power for hypothesis testing studies. The GRiP project recognized this need and is now identifying databases and trying to pool them on a global level. GRiP aims to pool data across many countries and databases, not only increasing the scale but also the heterogeneity in exposure, allowing for more drugs to be studied. As demonstrated in this thesis, DILI is rare in children and only its association with antibiotics, drugs widely prescribed in children, has been investigated, other drug groups: anti-convulsants and anti-asthmatics should be further studied but require more power. GRiP could be used to conduct a proof of concept study on DILI, this study would allow to test the feasibility and the power of this network as well as test new methodologies in the field of pharmaco-

epidemiological research in children, which is desperately needed to improve the knowledge about effects of drugs in children It is unlikely that any one database will be large enough to adequately study safety of drugs in children, therefore a sustainable collaboration should be established.

Finally, the number of reports is low in paediatrics, especially for the youngest children, more consumer reporting may be necessary, especially in Europe. It should be explored whether parent reporting of ADEs, makes a difference in paediatrics.

CHAPTER 8

Summary & Samenvatting

8.1. SUMMARY

This thesis was inspired by the increasing concerns about drug safety in children . Within this research, we focused on drug-induced liver injury (DILI) in children and applied several pharmacovigilance and pharmacoepidemiology methods.

SPONTANEOUS REPORTING SYSTEMS

Spontaneous Reporting Systems (SRS) represent the main data source for post-authorisation drug safety signal detection both in children and in adults. The analysis of spontaneously reported adverse drug reaction (ADR) reports has as main advantage that it is relatively easy, cheap and allows for safety monitoring of medicines during their whole life-cycle (**CHAPTER 2**). The exploratory studies of the Italian Pharmacovigilance Network (*Rete Nazionale di Farmacovigilanza*, RNF), established by the Italian Medicines Agency (AIFA) (**CHAPTER 2.1**), and of the Adverse Event Reporting System (FAERS), maintained by US FDA (**CHAPTER 2.2**), contributed to the understanding of the characteristics of both systems. This knowledge is important to guarantee optimal use of the SRS in paediatric hypothesis generation research.

As described in **CHAPTER 2.1**, the number of spontaneously reported paediatric ADRs in RNF, over 11-years of observation, linearly increased with age and was higher in boys (52%) than in girls, consistently with results from other EU SRS and VigiBase. The majority of ADRs occurring in paediatrics were not serious (70%) and children fully recovered (47%) or improved (23%). ADR reports issued by paediatricians only accounted for a small proportion (8%), while hospital physicians were more active in spontaneous reporting of ADRs (62%). '*Anti-infectives for systemic use*' (44.9%), '*nervous system agents*' (15.6%) and '*anti-inflammatory drugs*' (10.2%) were the most frequently reported drug classes among children. '*Skin disorders*' (52.2%), followed by '*gastrointestinal reactions*' (17.3%) and '*nervous system disorders*' (12.3%) were the most frequently reported ADRs. Adverse events related to the skin or gastro-intestinal tract were mainly reported upon use of antibiotics, acetaminophen, anti-inflammatory drugs and antineoplastic agents. This was consistent across all four paediatric age-categories.

As reported in **CHAPTER 2.2**, only a third of paediatric ADRs in FAERS were reported by physicians, while a notable number of ADRs were reported by consumer/parents. The most commonly reported drug classes were '*neurological*'

(58%), '*antineoplastic*' (32%) and '*anti-infectives*' (25%), whilst the most frequently reported system organ classes were '*general disorders*' (13%), '*nervous system*' (12%) and '*psychiatric reactions*' (11%). With respect to the time-to-event, '*systemical hormonal preparations*', '*alimentary drugs*' and '*antineoplastic/immunomodulating agents*' were prominently reported after long-term treatment (> 6 months), while '*anti-infective medicines*', '*anti-inflammatory drugs*' and '*sensory organ drugs*' were reported mostly with short term use.

Knowledge about the distribution of the classes of medicines and events within SRSs of both countries, either US or Italy, is a key first step to develop paediatric specific methods for drug safety surveillance. In light of the observed differences in the frequency and the type of reports between children and adults, our results stress the need to use specific age-group settings when performing signal detection or signal verification studies on children.

SIGNAL DETECTION OF DILI USING SPONTANEOUS REPORTING SYSTEMS

In **Chapter 3**, we describe new potential signals of hepatic toxicity in children, using VigiBase as data source. Among 624,673 ADRs were reported in children and adolescents, and only 1% concerned hepatic injury. Most of the reported hepatic reactions occurred in adolescents. Drugs that were most frequently associated with hepatic injury were acetaminophen, valproic acid, carbamazepine, methotrexate, minocycline, zidovudine, pemoline, ceftriaxone, bosentan, ciclosporin, atomoxetine, olanzapine, basiliximab, erythromycin, and voriconazole. All of these drugs, apart from basiliximab (a monoclonal antibody used in transplant patients), have already been associated with hepatotoxicity. The potential association between basiliximab and hepatic injury represents a new signal and requires further investigation.

ELECTRONIC HEALTHCARE RECORD DATABASES

The increasing availability of electronic healthcare record (EHR) and claims databases allow for the conduct of signal detection in these databases. These databases are not hampered by publication bias and underreporting in contrast to SRS. In Europe, as part of the EU-ADR project, a multi-database network of 8 EHR databases from 4 EU countries has been developed to produce a computerized integrated system for the early detection of drug safety signals.

In **Chapter 4.1**, the potential role of such networks in paediatric drug safety surveillance was explored. Although the EU-ADR network comprised a large paediatric sample size of around 5 million children contributing 25,575,132 person years (PYs) of follow-up, only 18 out of the total of 2,170 individual prescribed drugs made up half of the total drug exposure time. For a relatively frequent event such as '*upper gastrointestinal bleeding*' (IR=14.4/100,000 PYs), there were 79 drugs (80% of total exposure) for which a strong association (RR≥6) could be investigate, 39 drugs (66% of total exposure) to study a moderate association (RR≥4) and 5 drugs (26% of total exposure) to study a weak association (RR≥2). For rare events such as '*anaphylactic shock*' (IR=3.2/100,000 PYs) there were no drugs with enough exposure to study a weak association (RR≥2), 8 drugs to study a moderate association (RR≥4), and 20 drugs to study a strong association (RR≥6). Accordingly, mining within EHR databases seems especially favourable for events with a high background incidence and for drugs with a large amount of exposure. These results demonstrate that worldwide collaboration is crucial to achieve enough statistical power for paediatric drug safety detection.

SIGNAL DETECTION AND VERIFICATION OF DILI USING ELECTRONIC HEALTHCARE RECORD DATABASES

Chapter 5 describes the use of existing EHR data sources to identify new associations between drugs and liver injury in outpatient children and adolescents using the EU-ADR network (**Chapter 5.1**) and using a case-validated dataset (**Chapter 5.2**) and to confirm detected associations (**Chapter 5.3**).

In the EU-ADR network (**Chapter 5.1**), comprising around 5 million children, 1,015 potential cases of acute liver injury (ALI) were identified. By applying several methods of signal detection, 20 positive drug-ALI associations were detected. The associations between ALI and domperidone, flunisolide, and human insulin were never published before, and thus could be considered as new signals in paediatrics as well as in adults. Citalopram and cetirizine have been previously described as hepatotoxic in adults but were new for children, while all remaining associations were already known in both adults and children.

Chapter 5.2 describes a similar analytic approach using a smaller and 'case-validated' set from three longitudinal population-based databases of two European countries (Italy and the Netherlands). First the incidence of idiopathic ALI in outpatient children and adolescents was assessed followed by the investigation of the role of drugs as a potential cause of idiopathic ALI. Among 785 definite cases of idiopathic ALI, the pooled IR was 62.4/100,000 PYs (95 % CI 58.1–66.8). The

incidence and clinical presentation of ALI differed between the two countries. The country-specific IR was higher in Italy than in the Netherlands (73 vs. 21 per 100,000 person years). ALI was mostly characterized by isolated elevations of liver enzymes in Italy while by a combination of signs and symptoms in the Netherlands. Use of antibiotics, such as clarithromycin (RR 25.9), amoxicillin/clavulanic acid (RR 18.6) and amoxicillin (RR 7.5), compared to no use, showed the highest risk of ALI.

As a consequence of the previous results, antibiotic-induced liver injury in children required further investigations. **Chapter 5.3** describes the association between the use of antibiotics and risk of ALI using the dataset as described above. Children exposed to any antibiotic showed a 3-fold increased risk of hepatotoxicity as compared to children being exposed in the past (adjusted OR 3.2; 95% CI 2.6-4.0). The ORs varied between 1.9 (95% CI 1.1-3.2) for amoxicillin to 24.2 (95% CI 11.8-49.5) for co-trimoxazole, 26.7 (95% CI 12.1-59.0) for ceftriaxone, and 31.8 (95% CI 14.7-69.0) for rokitamycin. Sensitivity analyses, by considering current use of amoxicillin as the reference group or by removing the carryover period, confirmed the associations between the use of ceftriaxone, co-trimoxazole, clarithromycin and rokitamycin and the risk of hepatotoxicity in children. These results underline the importance of liver function monitoring in children who are prescribed the above mentioned antibiotics, especially in case of long-term exposure or in patients with additional risk factors for ALI.

NETWORK OF DATA SHARING AND SCIENTIFIC COLLABORATION: GRIP PROJECT

The results of previous studies suggest the need of large sample sizes to study drug safety in children, especially in case of rare adverse events. As part of the the Global Research in Paediatrics Network of Excellence (GRiP), we made an inventory of existing EHR databases with population-based information on drug/vaccine use and outcomes in children (**Chapter 6**). So far, the GRiP network includes 34 databases that agreed to collaborate in future pharmacoepidemiology studies. These collaborating databases are located across the globe namely 22 databases in 8 different European countries, 6 in 4 Asian/Pacific area countries, 4 in Canada and 2 in the US; one database is located in more than one country. Currently, the GRiP network includes different type of data sources (i.e. General Practitioner/Family paediatrician databases, insurance claims, hospital registries) accounting for a total of more than 40 million children (<18 years). The magnitude and the heterogeneity of this network will allow to investigate different aspects of

paediatric health care not only focusing on drug safety studies but also investigating drug use, health economics and comparative effectiveness.

CONCLUSION

Our findings highlight the potential of existing spontaneous reporting and electronic healthcare databases, complementary to traditional SRS, for drug safety signal detection and validation in a paediatric setting. Definitely, pooling data from additional longitudinal healthcare and paediatric-specific databases is necessary to gain sufficient statistical power to investigate a large range of drugs specifically used in children and adolescents.

8.2. SAMENVATTING

Dit proefschrift is voortgekomen uit de toenemende bezorgdheid omtrent de veiligheid van medicijnen bij kinderen. Dit proefschrift heeft als doel om de potentiële rol en de mogelijkheden van bestaande datasources (in plaats van nieuwe klinische studies) voor het onderzoek naar de geneesmiddelen-veiligheid bij kinderen, te evalueren. Voor het onderzoek in dit proefschrift hebben we ons vooral gericht op de relatie tussen geneesmiddelen gebruik en leverschade bij kinderen onderzocht. Voor dit onderzoek hebben we diverse methodes en studie designs, binnen het domein van farmacoepidemiologie en farmacovigilantie, toegepast.

DATABASES MET SPONTANE MELDINGEN VAN BIJWERKINGEN

Databases met spontane meldingen zijn een belangrijke bron voor onderzoek naar bijwerkingen (na registratie) bij zowel kinderen als volwassenen. Ondanks de beperkingen van die databases zoals onderrapportage of publicatie bias, heeft de analyse van die spontane meldingen als voordeel dat het relatief makkelijk, snel en goedkoop is. Bovendien is het mogelijk om de veiligheid van de geneesmiddelen te evalueren gedurende de totale periode na registratie (**HOOFDSTUK 2**).

Als eerste deel van het onderzoek werden 2 databases met spontane meldingen beschreven. Allereerst werd de Italiaanse database met spontane meldingen namelijk het Italiaanse Farmacovigilantie Netwerk (*Rete Nazionale di Farmacovigilanza*, RNF), ontwikkeld door het Italiaanse Agentschap voor de Geneesmiddelen (AIFA) beschreven (**HOOFDSTUK 2.1**). Ten tweede werden de karakteristieken van de database met spontane meldingen van het Amerikaans Voedsel en Geneesmiddelen agentschap (FDA) in kaart gebracht (**HOOFDSTUK 2.2**). Zoals beschreven in **HOOFDSTUK 2.1**, is er een toename te zien van het aantal gerapporteerde bijwerkingen bij toenemende leeftijd en ziet men iets meer meldingen bij jongens (52%) ten opzichte van meisjes. Deze bevindingen zijn conform met gepubliceerde gegevens binnen VigiBase (de database met spontane meldingen van de Wereldgezondheidsorganisatie, WHO) en van andere nationale databases met spontane meldingen. Het merendeel van de bijwerkingen bij kinderen was niet ernstig (70%) en er trad vaak een volledig (47%) of gedeeltelijk (23%) herstel op. Bijwerkingen werden voornamelijk door specialisten gerapporteerd (62%) terwijl de bijdrage van rapportage door eerstelijns artsen veel lager was (8%). Mel-

dingen werden voornamelijk gerapporteerd bij het gebruik van geneesmiddelen ter behandeling van infecties (44.9%), middelen voor het zenuwstelsel (15,6%) en anti-inflammatoire middelen (10,2%). Bijwerkingen die het meest genoemd werden bij kinderen waren huidziekten (52,2%), maag- en darmziekten (17,3%) en aandoeningen/klachten van het centraal zenuwstelsel (12,.3%). Binnen de bijwerkingen die het vaakst werden gemeld (maag- en darmstelsel of de huid), betrof het voornamelijk antibiotica, anti-inflammatoire en antineoplastische middelen. Deze bevindingen waren consistent over de verschillende leeftijdscategorieën.

Zoals beschreven in **Hoofdstuk 2.2**, is slechts een derde van alle bijwerkingen bij kinderen in FAERS door een arts gemeld, terwijl de overige twee derde gemeld is door een ouder of gebruiker. De meest voorkomende medicijnen die gemeld zijn in FAERS zijn de neurologische (58%), antineoplastische (32%) en de geneesmiddelen ter behandeling van een infectie(25%). De orgaansystemen die het vaakst als betrokken orgaan werden gemeld waren "algemene ziekten" (13%), "het zenuwstelsel" (12%) en psychiatrische ziekten (11%). Binnen de bijwerkingen die gemeld werden na tenminste 6 maanden scoorden systemische hormoonpreparaten, medicijnen voor het maag- en darmstelsel en anti-neoplastische/immuunmodulerende middelen het hoogst. Bij kortdurend gebruik zag men vooral geneesmiddelen ter behandeling van infecties, anti-inflammatoire geneesmiddelen en geneesmiddelen medicijnen voor de tastorganen.

Inzicht in de karakteristieken van databases met spontane meldingen is belangrijk voor de verdere ontwikkeling van geneesmiddelenonderzoek bij kinderen. Uit ons onderzoek blijkt dat het type meldingen en het gebruik van geneesmiddelen bij kinderen afhankelijk is van de leeftijd. Het is dus van groot belang om specifieke leeftijds-karakteristieken te gebruiken bij het onderzoeken van signaal detectie of signaal validatie bij kinderen.

SIGNAALDETECTIE VAN MEDICIJN-GEÏNDUCEERDE LEVERSCHADE IN VIGIBASE

In **Hoofdstuk 3**, deel 2 van dit proefschrift, werden nieuwe potentiële signalen van levertoxiciteit bij kinderen onderzocht. Hierbij werd gebruik gemaakt van VigiBase, de database met spontane meldingen van de Wereldgezondheidsorganisatie. Van alle 624,673 bijwerkingen die zijn gemeld in kinderen en volwassenen, ging het slechts in 1% van de bijwerkingen om leverschade. Het merendeel hiervan kwam voor bij adolescenten. Medicijnen die met leverschade geassocieerd waren zijn paracetamol, valproïnezuur, carbamazepine, methotrexaat, minocycline, zidovudine, pemoline, ceftriaxon, bosentan, ciclosporine, atomoxetine, olanza-

pine, basiliximab, erythromycine en voriconazol. Al deze medicijnen, met uitzondering van basiliximab (een monoklonaal antilichaam gebruikt bij transplantatie patiënten), zijn al eerder in relatie gebracht met levertoxiciteit. De potentiële associatie tussen basiliximab en leverschade is nieuw en dient verder onderzocht te worden.

DATABASES MET ELEKTRONISCHE MEDISCHE DOSSIERS

Databases van elektronische medische dossiers of claims kunnen gebruikt worden voor signaaldetectie en hebben als voordeel dat ze minder onderhevig zijn aan onderrapportage of selectief rapporteren ten opzichte van de databases met spontane meldingen. In Europa is een netwerk van databases ontwikkeld voor het EU-ADR project. Dit netwerk bestaat uit 8 verschillende databases uit 4 Europese landen.

In **Hoofdstuk 4.1**, wordt de potentiële rol van zulke netwerken voor het onderzoek naar medicijnveiligheid bij kinderen, beschreven. Hoewel het EU-ADR netwerk een grote populatie kinderen omvatte van ongeveer 5 miljoen kinderen met 25.575.132 persoons-jaren aan follow-up, bleek dat 18 van de 2.170 voorgeschreven medicijnen bijdroegen aan 50% van de totale medicijngeëxposeerde tijd. Aan de hand van powerberekening bleek dat voor een relatief veelvoorkomende bijwerking, zoals maagbloedingen (Incidentie=14,4/100.000 persoonsjaren) er slechts 39 medicijnen (66% van totale exposure) waren waarbij de duur van gebruik voldoende groot was om een gemiddelde associatie (RR≥4) te kunnen aantonen. Voor een zeldzame ziekte, zoals anafylactische shock (Incidentie=3,2/100.000 PYs), was er onvoldoende exposure om een zwakke associatie (RR≥2) te onderzoeken. Er was echter wel voldoende exposure bij 8 medicijnen om een matige associatie (RR≥4) met anafylactische shock te onderzoeken, en voor 20 medicijnen was er voldoende exposure om een sterke associatie (RR≥6) te onderzoeken. Signaal detectie, gebruik makend van elektronische patiëntendossiers, is met name veelbelovend voor ziektes/uitkomsten die een hoge achtergrond incidentie in kinderen hebben en voor medicijnen die vaak gebruikt worden bij kinderen. Deze resultaten benadrukken dat wereldwijde samenwerking van cruciaal belang is om voldoende statistische power te hebben om medicijnveiligheid bij kinderen te onderzoeken.

SIGNAALDETECTIE VAN MEDICIJN-GEÏNDUCEERDE LEVERSCHADE GEBRUIK MAKEND VAN ELEKTRONISCHE MEDISCHE DOSSIER DATABASES

In **HOOFDSTUK 5** worden de resultaten van het gebruik van huidige Elektronische Medische Dossier Databases voor het identificeren van nieuwe associaties tussen medicijnen en leverschade bij kinderen en adolescenten beschreven. **HOOFDSTUK 5.1** bespreekt de resultaten op basis van de analyses binnen het EU-ADR netwerk, en **HOOFDSTUK 5.2** beschrijft de resultaten van een gevalideerde dataset. Binnen het EU-ADR netwerk (**HOOFDSTUK 5.1**), werden 1.015 potentiële cases van acute leverschade gevonden. We vonden 20 positieve associaties tussen medicijn blootstelling en acute leverschade met behulp van diverse signaal detectie methodes. Nieuwe signalen werden gevonden voor domperidon, flunisolide en humaan insuline, aangezien deze associaties nog niet eerder gerapporteerd zijn bij kinderen en ook niet bij volwassenen. De relatie tussen het gebruik van citalopram en cetirizine en leverschade is eerder beschreven voor volwassenen maar nog niet bij kinderen. Alle andere gevonden associaties waren al bekend bij zowel kinderen als volwassenen.

HOOFDSTUK 5.2 herhaalt de studie als beschreven in **HOOFDSTUK 5.1**, maar op een kleinere dataset met gevalideerde cases uit drie longitudinale populatie-gebaseerde databases in Italië en Nederland. Het doel van dit onderzoek was enerzijds om de incidentie van idiopathische acute leverschade in poliklinische kinderen en adolescenten te beschrijven maar ook om de rol van medicijnen als oorzaak van idiopathische acute leverschade, te bestuderen. We identificeerden 785 cases van acute idiopathische leverschade resulterend en de incidentie betrof 62,4 per 100.000 PYs (95% betrouwbaarheidsinterval (BI) 58,1-66,8). De incidentie en klinische presentatie van acute leverschade bij kinderen verschilde tussen Italië en Nederland. De incidentie was hoger in Italië dan in Nederland (73 en 21 per 100.000 PYs, respectievelijk). Acute leverschade werd het meest gekarakteriseerd door geïsoleerde leverenzym verhogingen in Italië, en in Nederland door een combinatie van symptomen en parameters. Gebruik van antibiotica zoals clarithromycine (RR 25,9), amoxicilline/clavulaanzuur (RR 18,6) en amoxicilline (RR7,5) ten opzichte van geen gebruik van antibiotica lieten het hoogste risico op acute leverschade zien.

Als vervolg op de hierboven besproken resultaten, werd een vervolgstudie gepland waarbij de relatie tussen het gebruik van antibiotica en risico op leverlijden bij ambulante kinderen verder werd onderzocht (**HOOFDSTUK 5.3**). Aan de hand van een case-controle studie bij 938 kinderen met acuut leverlijden bleek dat kinderen die antibiotica hadden gebruikt een 3-maal verhoogd risico

op hepatotoxiciteit hadden ten opzichte van kinderen die enkel in het verleden antibiotica hadden gebruikt (gecorrigeerde Odds Ratio (OR) 3,2; 95% BI 2,6-4,0). De ORs varieerden van 1,9 (95% BI 1,1-3,2) voor amoxicilline, 24,2 (95% BI 11,8-49,5) voor cotrimoxazol, 26,7 (95% BI 12,1-59,0) voor ceftriaxon tot 31,8 (95% BI 14,7-69,0) voor rokitamycine. Sensitiviteits analyses bevestigden de associaties tussen het gebruik van ceftriaxon, cotrimoxazol, clarithromycine en rokitamycine en heptatoxiciteit in kinderen. Deze resultaten benadrukken het belang van het monitoren van de leverfunctie bij kinderen die één van de bovengenoemde antibiotica gebruiken. Dit laatste is met name belangrijk indien antibiotica langdurig worden gebruikt en in patiënten met extra risicofactoren.

NETWERK VAN DATABASES EN WETENSCHAPPELIJKE SAMENWERKING: HET GRIP PROJECT

De resultaten van onze eerdere studies suggereren dat er grote aantallen nodig zijn om medicijnveiligheid bij kinderen te onderzoeken, met name indien bijwerkingen heel zelden voorkomen. Als onderdeel van het 'Global Research in Paediatrics Network of Excellence (GRiP)' hebben we geprobeerd alle bestaande databases met populatie-gebaseerde informatie over medicijn- en vaccingebruik en ziektes bij kinderen te identificeren (**HOOFDSTUK 6**). Momenteel bevat het GRiP netwerk 34 databases die bereid zijn om mee te werken in toekomstige farmacoepidemiologische studies. Deze databases zijn wereldwijd verspreid over verschillende landen, inclusief 8 verschillende landen in Europa (22 databases), 4 Aziatische landen (6 databases), Canada (4 databases) en de Verenigde Staten van Amerika (2 databases). Het GRiP netwerk bevat verschillende type datasources, zoals huisartsendatabases, familie-kinderartsen databases, en claims en ziekenhuisregistraties. In het totaal is informatie beschikbaar van meer dan 40 miljoen kinderen (< 18 jaar).

CONCLUSIES

Onze bevindingen benadrukken het belang en de mogelijkheden van onderzoek naar de effecten van geneesmiddelen in kinderen, door gebruik te maken van bestaande databases in de gezondheidszorg. Om voldoende power te hebben is het noodzakelijk dat de gegevens van verschillende longitudinale medische en kinderspecifieke databases wordt gepoold.

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ABBREVIATIONS

95% CI	95% Confidence Interval
ADHD	Attention Deficit Hyperactivity Disorder
ADR	Adverse Drug Reaction
A(D)E	Adverse (Drug) Event
AIFA	*Agenzia Italiana del FArmaco*
AP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ALI	Acute Liver Injury
ARS	*Agenzia Regionale di Sanità*
ASL	*Azienda Sanitaria Locale*
AST	ASpartate aminoTransferase
ATC	Anatomical Therapeutic Chemical
CDC	Centres for Disease Control and Prevention
CHMP	Committee for Human Medicinal Products
CIOMS	Council for International Organizations of Medical Sciences
CYP P450	cytochrome P450
DILI	Drug-Induced Live Injury
DDD	Defined Daily Dose
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EHR	Electronic Healthcare Records
EMA	European Medicines Agency
EU	the Europe/the European Union
EU-ADR	Exploring and Understanding Adverse Drug Reactions by Integrative Mining of Clinical Records and Biomedical Knowledge
FAERS	Food and Drug Administration Adverse Event Reporting System
FDA	Food and Drug Administration
FP	Family Paediatrician
GePaRD	German Pharmacoepidemiological Research Database
gGT	gamma-Glutamyl Transferase
GP	General practitioner
GRiP	Global Research in Paediatrics Network of Excellence
HLGT	High-Level Group Terms
HLT	High-Level Terms
HSD	Health Search/CSD
ICD	International Classification of Diseases
ICH	International Conference of Harmonization
ICPC	International Classification of Primary Care

ICSR	Individual Case Safety Report
IPCI	Integrated Primary Care Information
IR	Incidence Rate
LEOPARD	Longitudinal Evaluation of Observational Profiles of Adverse events Related to Drugs
LGPS	Longitudinal Gamma Poisson Shrinker
MedDRA	Medical Dictionary for Regulatory Activities
NALFD	Non-Alcoholic Fatty Liver Disease
OMOP	Observational Medical Outcomes Partnership
OR	Odds ratio
OTC	Over-the-counter
P	P value
PDCO	PaeDiatric COmmittee
PIL	Patient Information Leaflet
PIP	Paediatric Investigation Plan
PT	Preferred term
PUMA	Paediatric Use Marketing Authorisation
PY	Person years
RCT	Randomized Clinical Trial
ROR	Reporting Odds Ratio
RNF	*Rete Nazionale di Farmacovigilanza*
RR	Rate Ratio/Relative Risk
SCCS	Self-Controlled Case Series
SOC	System Organ Class
SmPC	Summary of Product Characteristics
SRS	Spontaneous reporting system
TEDDY	Task force in Europe for Drug Development for the Young
THIN	The Health Improvement Network
UGIB	Upper Gastro-Intestinal Bleeding
UK	United Kingdom
ULN	Upper Limit of the Normal range
UMLS1	Unified Medical Language System1
US	United States
VAERS	Vaccine Adverse Events Reporting System
VSD	Vaccine Safety Datalink
WHO	World Health Organisation
WHO-ART	WHO-Adverse Reaction Terminology
WHO-UMC	WHO-Uppsala Monitoring Centre

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PORTFOLIO

Portfolio

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Erasmus MC Department	Medical Informatics
Research School	Netherlands Institute for Health Sciences
PhD Period	November 2010 – November 2014
Collaboration	Second University of Naples, Naples - Italy
Promotors	Prof.dr. M.C.J.M. Sturkenboom and Prof.dr. F. Rossi
Copromotors	Dr. K.M.C. Verhamme and Dr. A. Capuano

1. PhD training

Research skills
Statistics and Methodology

2010-2013 **PhD in Pharmacology**, with title of *Doctor Europeus*, at the Second University of Naples, Naples, Italy.

2009-2010 **Master of Health Science** in Clinical Epidemiology, at the Netherlands Institute for Health Sciences. Rotterdam (30 ECTs).

Oral communications at international and national conferences

2010 *Drug-induced liver injury in children: data mining on health records.* "XIX SEMINARIO NAZIONALE: LA VALUTAZIONE DELL'USO E DELLA SICUREZZA DEI FARMACI: ESPERIENZE IN ITALIA". *Istituto Superiore di Sanità*, Rome (Italy), December 2010.

2011 *Incidence rate of liver injury in paediatric population: data mining on electronic healthcare databases in Europe*.
27th International Conference on Pharmacoepidemiology & Therapeutic Risk Management (ICPE), International Society of Pharmacoepidemiology (ISPE). Chicago (US Illinois), 16 August 2011.

2012 *Paediatric Acute Liver Injury: Signal Detection Using Multiple Healthcare Databases from EU-ADR Network.*
28th International Conference on Pharmacoepidemiology & Therapeutic Risk Management (ICPE), International Society of Pharmacoepidemiology (ISPE). Barcelona (Spain), 23-26 August 2012.

Hepatic safety of antibiotics in paediatric primary care: a case-control study using electronic healthcare databases.
XXIV National Conference of Italian Society of Preventive and Social Paediatrics – "Bambini di Vetro". Caserta (Italy), 12-15 September 2012. Award best oral communication.
Italian Society of Pharmacology, Conference: "IL RUOLO DELLA FARMACOLOGIA CLINICA IN ETA' PEDIATRICA". Naples (Italy), 14 Decembr 2012.

2013 *Antibiotics and hepatotoxicity in paediatric primary care.*
29th International Conference on Pharmacoepidemiology & Therapeutic Risk Management (ICPE). Montreal (Canada), 25-28 August 2013.
36th National Conference of Italian Society of Pharmacology. Torino, 23-26 October 2013. Award best oral communication.

Suspected Adverse Drug Reactions in Children: a Descriptive Study of the Italian Spontaneous Reporting System.
XIII Annual Meeting of International society of Pharmacovigilance (ISOP). Pisa (Italy), 1-4 October 2013.

2014 *Paediatric Drug Safety Surveillance in Italian Pharmacovigilance Network: An Overview over the Years 2001-2012.*
SIF Conference "La Farmacologia Clinica tra Impegno nella Ricerca e Ruolo nel Servizio Sanitario Nazionale". Naples (Italy), 2-3 october 2014.

Association between use of asthma drugs in children and hepatotoxicity. 30th Anniversary of International Conference on Pharmacoepidemiology & Therapeutic Risk Management (ICPE). Taipei (Taiwan), 24-27 October, 2014.

Poster presentation at international and national conferences

2010 *Drug-induced liver injury: data from Italian Pharmacovigilance Network.*
Academy Scientific Days 2010, Naples (Italy).

Active surveillance of adverse drug reactions in paediatric inpatients.
Academy Scientific Days 2010, Naples (Italy).

2011 *Incidence rate of hepatic injury in paediatric population: data mining on electronic healthcare databases in Europe*.
Academy Scientific Days 2011, Naples (Italy).
35th National Conference of Italian Society of Pharmacology. Bologna (Italy), 14-17 September 2011.

Prescribing pattern and adherence to the statins therapy: a population-based study in Italian primary health care.
27th International Conference on Pharmacoepidemiology & Therapeutic Risk Management (ICPE), International Society of Pharmacoepidemiology (ISPE). Chicago (US Illinois), 14-17 August 2011.
35th National Conference of Italian Society of Pharmacology. Bologna (Italy), 14-17 September 2011.

2012 *Idiopathic acute liver injury in paediatric outpatients: incidence and signal detection in two European countries.*
Conference SIF: "IL RUOLO DELLA FARMACOLOGIA CLINICA IN ETA' PEDIATRICA". Naples (Italy), 14 December 2012.

2013 *Prescribing pattern of blood glucose-lowering drugs in new diabetic patients in real practice.*
36th National Conference of Italian Society of Pharmacology. Torino (Italy), 23-26 October 2013.
29th International Conference on Pharmacoepidemiology & Therapeutic Risk Management (ICPE). Montreal (Canada), 25-28 August 2013.

Antibiotics and Liver Injury in Paediatric Primary Care: a Case-Control Study using Healthcare Database Network.
Identifying Potentially Drug-Induced Acute Liver Injury in Children using a Multinational Healthcare Database Network.
XIII Annual Meeting of International society of Pharmacovigilance. Pisa, Italy, 1-4 October 2013.

2014 *Association between use of asthma drugs in children and hepatotoxicity.*
SIF Conference: "La Farmacologia Clinica tra Impegno nella Ricerca e Ruolo nel Servizio Sanitario Nazionale". Naples (Italy), 2-3 October 2014.

Healthcare Databases for Paediatric Studies: A Report from the GRiP Network Global Survey.
Paediatric Drug Safety Surveillance in Italian Pharmacovigilance Network: An Overview over the Years 2001-2012.
30th Anniversary of International Conference on Pharmacoepidemiology & Therapeutic Risk Management (ICPE). Taipei (Taiwan), 24-27 October, 2014.

Courses, seminars, workshops

2008-2013 Research seminars at Department of Medical Informatics, Erasmus MC University, Rotterdam.

2009-2013 Preconference courses on Pharmacoepidemiology and Drug Utilization during the Annual International Conferences on Pharmacoepidemiology and Therapeutic Risk Management of International Society of Pharmacoepidemiology (ISPE).

2010 Biomedical English writing and communication, Erasmus MC University, Rotterdam.

2011-2013 Plenary meetings and Working Group 3 of ENCePP (European Network of Centres for Pharmacoepidemiology and Pharmacovigilance, EMA)

2012 Workshop on "Drug utilization through administrative database ". ENCePP Italian Network.

2012-current Member of Scientific Committee for several Courses and Seminars on Pharmacovigilance and Pharmacoepidemiology.

Others

2007-current	Co-editor of web news-letter "SIF: Farmaci in Evidenza" of Italian Society of Pharmacology.
2008-current	Referee activities for international journals (Pediatrics, Clinical and Experimental Pharmacology, PLOSone)
2010-current	Collaboration to EU-funded project, Global Research in Paediatrics – Network of Excellence (GRiP), workpackage 2.

2. Teaching activities

2010-2013	Tutor of master degree students.
2010-2012	Teaching on drug prescribing at "Updating courses for physicians" at the Local Health Unit of Caserta, Italy.
2014	Teaching on the use data mining techniques for exploring drug safety in children at the annual II level MASTER on "Pharmacovigilance, Pharmacoepidemiology and Regulatory" module III "Pharmacovigilance", Naples, Italy.

LIST OF PUBLICATIONS

MANUSCRIPTS WITHIN THIS THESIS

Chapter 2.1
Ferrajolo C, Capuano A, Trifirò G, Moretti U, Rossi F, Santuccio C. Paediatric Drug Safety Surveillance in Italian Pharmacovigilance Network: an Overview of Adverse Drug Reactions in the years 2001-2012. *Expert Opin Drug Saf* 2014; 13(S1): 9-20.

Chapter 2.2
de Bie S, **Ferrajolo C**, Straus S.M.J.M., Verhamme KMC, Bonhoeffer J, Wong ICK, Sturkenboom MCJM, on behalf of the GRiP network. Paediatric Drug Safety Surveillance in FDA-AERS, a description of Adverse Events: a GRiP Study. *Submitted.*

Chapter 3.1
Ferrajolo C, Capuano A, Verhamme KMC, Schuemie M, Rossi F, Stricker BH, Sturkenboom MCJM. Drug-induced hepatic injury in children: a case/non case study of suspected adverse drug reactions in VigiBase. *Br J Clin Pharmacol* 2010; 70(5):721-8.

Chapter 4.1
de Bie S and **Ferrajolo C**, Coloma PM, Verhamme KMC, Trifirò G, Schuemie MJ, Straus SMJM, Gini R, Herings R, Mazzaglia G, Picelli G, Ghirardi A, Pedersen L, Stricker BHC, van der Lei J, and Sturkenboom MCJM, on behalf of the EU-ADR consortium. The power of electronic health care databases for active drug safety surveillance in children and adolescents. *Submitted.*

Chapter 5.1
Ferrajolo C, Coloma PM, Verhamme KM, Schuemie MJ, de Bie S, Gini R, Herings R, Mazzaglia G, Picelli G, Giaquinto C, Scotti L, Avillach P, Pedersen L, Rossi F, Capuano A, van der Lei J, Trifiró G, Sturkenboom MCJM, on behalf of EU-ADR consortium. Signal detection of potentially drug-induced acute liver injury in children using a multi-country healthcare database network. *Drug Saf* 2014; 37(2):99-108.

Chapter 5.2
Ferrajolo C, Verhamme KMC, Trifirò G, 't Jong GW, Giaquinto C, Picelli G, Oteri A, de Bie S, Valkhoff VE, Schuemie MJ, Mazzaglia G, Cricelli C, Rossi F, Capuano A, Sturkenboom MC. Idiopathic Acute Liver Injury in Paediatric Outpatients: Incidence and Signal Detection in Two European Countries. *Drug Saf* 2013; 36(10):1007-16.

Chapter 5.3
Ferrajolo C, Verhamme KMC, Capuano A, 't Jong GW, Picelli G, Giaquinto C, Mazzaglia G, Stricker BHC, Rossi F, Trifirò G, Sturkenboom MJCM. Antibiotics and hepatotoxicity in paediatrics? A case-control study using primary care databases. *Submitted.*

Chapter 6.1
Ferrajolo C, Osokogu O, Nan C, Brauchli Pernus Y, Weibel D, Verhamme KMC, Gazarian M, Wong ICK, Nakamura H, Bonhoeffer J, Giaquinto C, Sturkenboom MJCM, on behalf of the GRiP network. Healthcare databases for paediatric studies: a report from the GRiP network global survey. *Submitted.*

OTHER PUBLICATIONS

Parretta E, Rafaniello C, Magro L, Coggiola Pittoni A, Sportiello L, **Ferrajolo C**, Mascolo A, Sessa M, Rossi F, Capuano A. Improvement of patient adverse drug reaction reporting through a community pharmacist-based intervention in the Campania region of Italy. *Expert Opin Drug Saf* 2014; 13(S1):21-29.

Ferrajolo C, Arcoraci V, Sullo MG, Rafaniello C, Sportiello L, Ferrara R, Cannata A, Pagliaro C, Tari MG, Caputi AP, Rossi F, Trifirò G, Capuano A. Pattern of statin use in southern italian primary care: can prescription databases be used for monitoring long-term adherence to the treatment? *PLoS One* 2014; 9(7):e102146.

Rafaniello C & Lombardo F, **Ferrajolo C**, Sportiello L, Parretta E, Formica R, Potenza S, Rinaldi B, Irpino A, Raschetti R, Vanacore N, Rossi F, Capuano A. Predictors of Mortality in Atypical Antipsychotics-Treated Community Dwelling Elderly Patients with Behavioural and Psychological Symptoms of Dementia: A Prospective Population-Based Cohort Study from Italy. *Eur J Clin Pharmacol* 2014; 70(2):187-95.

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Carmen Ferrajolo was born on August 29th 1980 in Caserta (Italy) and she obtained the High School diploma at *Liceo Classico* "P. Giannone" in Caserta on 1998, when she started her graduate programs at School of Pharmacy, at University of Naples "Federico II". She obtained her degree in Pharmacy in 2004 and worked as pharmacist in private pharmacies during the following two years. Since 2006, she started a training at the Campania Regional Centre of Pharmacovigilance and Pharmacoepidemiology of the "Second University of Naples", where she also obtained the Postgraduate degree in Clinical Pharmacology in 2010 and international PhD (*Doctor Europeus*) in Clinical Pharmacology in 2013. In 2010 she obtained a Master of Science degree in Clinical Epidemiology at the National Institute of Health Sciences in The Netherlands. Since 2008, she is collaborating with Department of Medical Informatics of the Erasmus University Medical Centre in Rotterdam for the conduct of national and international pharmacoepidemiology and pharmacovigilance studies using different spontaneous adverse drug reaction reporting and healthcare databases.

Currently she is actively involved in the scientific activities of the FP7 EU-funded project Global Research in Paediatric (GRiP) as well as in other activities/project as part of the Pharmacovigilance and Pharmacoepidemiology Centre of Campania Region at several projects: i) updating and management of the pharmacovigilance website (www.farmacovigilanza.unina2.it); ii) analysis of the Italian spontaneous adverse drug reaction reporting database in collaboration with Italian Agency of Medicines (AIFA) and "Italian National Institute of Health"; iii) analysis of claims databases of the Local Health Unit of Caserta. She is teaching at the Master on "Pharmacovigilance, Pharmacoepidemiology and Regulatory" at Second University of Naples. She is actively involved as editor of the online newsletter of the Italian Society of Pharmacology "*SIF: Farmaci in Evidenza*" (http://www.sifweb.org/) and she is member of Scientific Committee for several courses or seminars on pharmacovigilance and pharmacoepidemiology.

She is just married to Gianluca Trifirò on May 2014.

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Once upon a time...

What a strange way to start the acknowledgment of the thesis! Actually, this should be the best way, as this chapter of my life stared like a fairy tale. Usually, the protagonist of a story meets lovely as well as unfriendly people. In this story, instead, I had pleasant and hostile moments, but I was always surrounded by lovely people. And as any story deserves, there were three "little fairies"...three women who push me in the right direction, and according to my ambitions, were able to get the best out of me.

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L'importante non è quello che provi alla fine di una corsa, l'importante è quello che provi mentri corri.