Drug Saf (2013) 36:1007–1016
DOI 10.1007/s40264-013-0045-7

ORIGINAL RESEARCH ARTICLE

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

Carmen Ferrajolo · Katia M. C. Verhamme · Gianluca Trifirò · Geert W. 't Jong · Carlo Giaquinto · Gino Picelli · Alessandro Oteri · Sandra de Bie · Vera E. Valkhoff · Martijn J. Schuemie · Giampiero Mazzaglia · Claudio Cricelli · Francesco Rossi · Annalisa Capuano · Miriam C. J. M. Sturkenboom

Published online: 17 April 2013

Abstract
Background Acute liver failure is idiopathic and drug-related in, respectively, around 50 and 15 % of children. Population-based, epidemiologic 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 person-years (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).

C. Ferrajolo (✉) · F. Rossi · A. Capuano
Campania Regional Center of Pharmacovigilance and Pharmacoepidemiology, Department of Experimental Medicine, Pharmacology Section, Second University of Naples, Via L. De Crecchio, 7, 80138 Naples, Italy
e-mail: c.ferrajolo@erasmusmc.nl

C. Ferrajolo · K. M. C. Verhamme · G. Trifirò · G. W. 't Jong · A. Oteri · S. de Bie · V. E. Valkhoff · M. J. Schuemie · M. C. J. M. Sturkenboom
Department of Medical Informatics, Erasmus University Medical Center, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands

G. Trifirò
Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

G. W. 't Jong
Department of Paediatrics, SickKids Hospital, Toronto, ON, Canada

C. Giaquinto
Department of Paediatrics, University Hospital of Padua, Padua, Italy

G. Picelli
Pedianet-Società Servizi Telematici SRL, Padua, Italy

G. Mazzaglia · C. Cricelli
Società Italiana di Medicina Generale, Florence, Italy

M. C. J. M. Sturkenboom
Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands

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.

1 Introduction

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 [1]. Published data on the incidence and characteristics of less severe acute liver injury (ALI) are scarce because initially the disease is asymptomatic and, thus, difficult to recognize [2]. So far, information on clinical features of ALI are only available from disease registries [3–5].

The increasing availability of electronic healthcare record (EHR) and claims databases allows for the conduct of population-based epidemiologic studies in larger populations, including children and adolescents [6]. 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 the underlying potential cause of apparently idiopathic ALI by applying data mining techniques on the pooled data from the three databases.

2 Patients and Methods

2.1 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 Health Search/CSD Longitudinal Patient Database (HSD; from 2002 to 2008) in Italy and the Integrated Primary Care Information database (IPCI; from 2001 to 2007) in The Netherlands [7]. In The Netherlands, general practitioners (GPs) serve as gatekeepers to medical care for all patients, including children and adolescents. In Italy, children receive medical care by family paediatricians (FPs) up to the age of 14 years, and thereafter by GPs. All these databases have been proven valid data sources for pharmacoepidemiological studies [8–13]. Pedianet was set up in the year 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 (International Classification of Primary Care) in IPCI and ICD-9-CM (International Classification of Disease, 9th revision, Clinical Modification) 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 Anatomical Therapeutic Chemical classification system [14].

2.2 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 (31 December 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.

2.3 Outcome Definition and Case Ascertainment

According to the CIOMS (Council for International Organizations of Medical Sciences) criteria and previous evidence [15–20], 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 $\leq$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 [HAV], Hepatitis B virus [HBV], Hepatitis C virus [HCV], Cytomegalovirus [CMV], and Epstein-Barr virus [EBV]); (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 [18, 21].

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*, with 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 [15] and to Benichou [16], with respect to biochemical hepatic function, the pattern of liver injury was defined as (a) *hepatocellular* (or *cytolytic*), 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 the ULN), *moderate* (more than three times the ULN), *severe* (more than five times the ULN), and *most severe* (more than eight times the ULN) [22].

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.

2.4 Drug Exposure

Drug exposure was evaluated based on prescription data from healthcare databases. As drug prescriptions are locally coded using the national product codes, which differ among countries, we defined drug exposure based on the Anatomical Therapeutic Chemical (ATC) classification system of the WHO. The ATC code was used as the drug code in the input files for Jerboa©. Cases were considered as exposed to a 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 according to the legend duration (if dosing regimen is available), or is otherwise based on the defined daily dose (DDD) [23].

2.5 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 [23–25]. Briefly, Jerboa© elaborates input files with the common data model (including patient unique identifier and demographics, follow-up time, exposure and outcome information) from different databases and produces as the output several parameters [e.g. incidence rates (IRs), relative risks (RRs), prescription rates (Rx/PYs)], stratified by

age, sex and calendar years [6, 23]. Age was categorized into four categories: <2 years, 2 to ≤5 years, 6 to ≤11 years and 12 to <18 years.

To calculate the incidence rates, we considered the number of first event of ALI after a 1-year run-in period as the numerator and the number of cumulated person-years of the study population at risk of developing the first event of ALI, as the 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 pooled age- and sex-adjusted rate ratios (RRs, with 95 % CI), for each drug associated with at least two event occurring its use (ATC V level) using the non-use of the drug of interest as comparator [6, 23].

3 Results

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

After exclusion of all clear causes of liver injury and of chronic hepatitis through case-by-case validation (Fig. 1), we identified 1326 cases of idiopathic ALI of which the majority originated from Italy (1237 cases, 93.3 %) (Table 1). In the two Italian databases, 731 cases (59.1 % of 1237) 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.

3.1 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 = 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.

3.2 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) per 100,000 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) [Table 2]. Age distribution was similar in the two countries (mean age: 12.1 ± 4.6 in Italy 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 < 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) [Table 2]. 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).

3.3 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 proportion differed

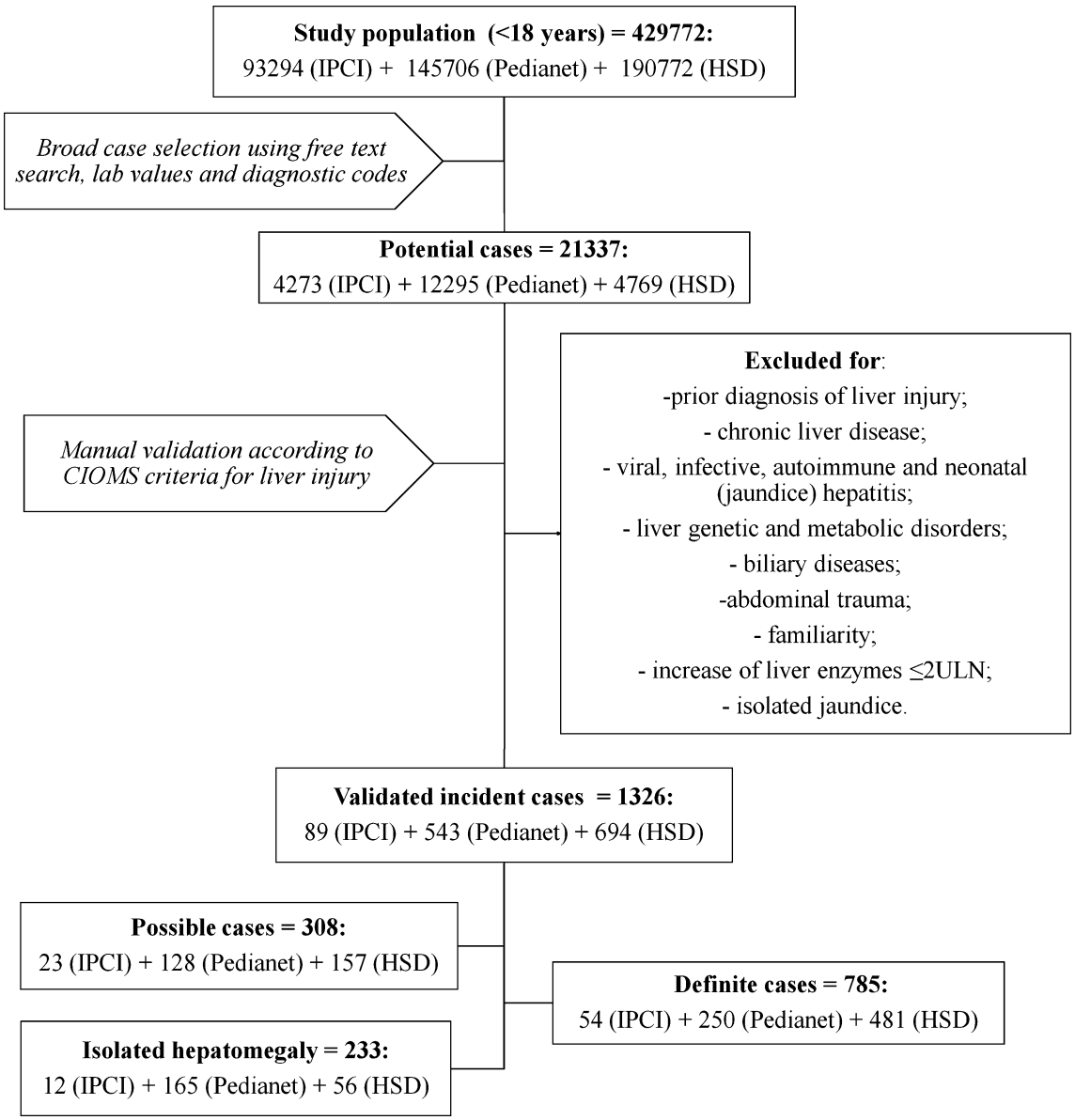

Fig. 1 Flow chart of cohort and cases selection. *CIOMS* Council for International Organizations of Medical Sciences, *HSD* Health Search/CSD Longitudinal Patient Database, *IPCI* Integrated Primary Care Information database, *ULN* upper limit of normal

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 (paracetamol), rokitamycin, sulfamethoxazole/trimethoprim, phenobarbital, ketoprofen, carbamazepine and valproic acid, despite a very low number of exposed cases (n < 3; Table 3).

4 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 PYs. There are no paediatric studies to which we can directly compare our data because of different age-range setting and/or

Table 1 Features of idiopathic acute liver injury by countries

	Italy (HSD + Pedianet)			Netherlands (IPCI)		
	Total cases = 1237			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 test[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 ≤3 ULN)	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)*	–

GP general practitioner, *FP* family paediatrician, *HSD* Health Search/CSD Longitudinal Patient Database, *IPCI* Integrated Primary Care Information database, *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

methodology. The most similar is a study conducted in the UK, which was restricted, however, to the population aged over 15 years. Using healthcare records as the data source, Duh et al. [18] 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 [17]. However, this lower rate could be attributed to the differences in study population (patients aged 4–65 years) and study outcome (acute and clinically relevant drug-induced liver injury) [17]. In 2003, moreover, the prospective observational study, Drug-Induced Liver Injury Network (DILIN) was established to create a registry and biosample repository for clinical and mechanistic studies of DILI among patients >2 years old [5]. However, these data, as well as the most published studies concerning clinical features of DILI, as described by Bell and Chalasani [26], 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 [1, 3, 4, 27–29]. In our study, we identified all cases of acute 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

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

	Definite cases			Total cases[b]	
	N (%)	PYs[a]	IR (95 % CI)	N (%)	IR (95 % CI)
Italy (HSD + Pedianet	**731**	**1,000,720**	**73.0 (67.8–78.4)**	**1237**	**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)
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)**

HSD Health Search/CSD Longitudinal Patient Database, *IPCI* Integrated Primary Care Information database, *IR* incidence rate, *PYs* person-years, *SD* standard deviation, *SIR ref. NL* standardized incidence rate on the population from The Netherlands

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

[b] Including definite and possible cases and hepatomegaly

Table 3 Drugs[a] associated with acute liver injury among definite idiopathic cases

Drugs	ATC code	Exposure time (in days)	No. of events	Crude IR/100,000 person-days (95 % CI)	Adjusted RR[b] (95 % CI)	*p-value*
Clarithromycin	J01FA09	156763	9	5.7 (2.8–10.5)	25.9 (13.4–50.0)	<0.001
Amoxicillin and clavulanic acid	J01CR02	437708	16	3.6 (2.2–5.8)	18.6 (11.3–30.6)	<0.001
Amoxicillin	J01CA04	759972	6	0.8 (0.3–1.7)	7.5 (3.4–16.8)	<0.001
Other drugs with only 2 events						
Rifampicin and isoniazid	J04AM02	114	2	1754.4 (349.9–5623.5)	4858.2 (1214.0–19442.4)	<0.001
Acetaminophen combination	N02BE51	9740	2	20.5 (4.1–65.8)	94.2 (23.4–378.3)	<0.001
Rokitamycin	J01FA12	13329	2	15.0 (1.9–54.2)	52.3 (13.0–209.6)	<0.001
Sulfamethoxazole and trimethoprim	J01EE01	42325	2	4.7 (0.9–15.1)	28.6 (7.1–114.7)	0.002
Phenobarbital	N03AA02	23337	2	8.6 (1.7–27.5)	25.8 (6.4–103.4)	0.003
Ketoprofen	M01AE03	49585	2	4.0 (0.8–12.9)	11.1 (2.8–44.5)	0.015
Carbamazepine	N03AF01	76355	2	2.6 (0.3–9.5)	9.5 (2.4–38.2)	0.019
Valproic acid	N03AG01	134725	2	1.5 (0.3–4.8)	6.9 (1.7–27.6)	0.035

ATC Anatomical Therapeutic Chemical Classification, *IR* incidence rate, *PY* person-years, *RR* rate ratio

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

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

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, most of the liver enzyme elevations were asymptomatic with a mild/moderate degree of severity, whereas in The Netherlands were reported in combination with other typical signs/symptoms of hepatotoxicity. This discrepant pattern may be explained by differences in healthcare systems and healthcare-seeking behavior 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 [30]. 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 [12].

In line with recent evidence [5], 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 [31]. 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 cytochrome P450 (CYP) iso-enzymes [32]. 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 in early life. On the other hand, a determinant for the slightly high rates in the early stage of 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 pediatricians, 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 the potential underlying cause. In our study, antibiotics are associated with the highest risk of ALI in children and adolescents. Amoxicillin (with or without clavulanic acid) has already been described in the literature as an hepatotoxic drug in paediatrics [5, 31, 33, 34], whereas we failed to find evidence of clarithromycin-induced liver injury in children. Nevertheless, paediatrics cases of liver injury have also been reported for other macrolides, such as erythromycin, azythromycin and roxithromycin [5, 33], 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 [17, 35]. 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 pediatrics [31, 36]. A recent study confirmed that susceptibility to amoxicillin/clavulanate-induced liver injury is influenced by genetic multiple variability [37]. We do believe that the hepatotoxicity related to the combination of amoxicillin and 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 [33] and its wide use in paediatric patients [38]. 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 the 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 [12]. 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 healthcare databases was necessary. Other initiatives such as OMOP (Observational Medical Outcomes Partnership) and EU-ADR (Exploring and Understanding Adverse Drug Reactions) may also be explored for signal detection and validation in children and adolescents [6, 39].

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 [5]. 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.

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 [40–43]. Part of this potential misclassification has been tackled by excluding children with enzyme values less than twice the 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.

5 Conclusions

The incidence rate of idiopathic ALI is relatively low in children and adolescents and comparable with 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 the 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.

Acknowledgments We would like to thank Prof. John Van den Anker and Dr. Giuseppe Indolfi for their revision of the final version of the manuscript, and the Erasmus Trustfonds for research supporting.

Financial disclosure Nothing to disclose.

Conflict of interest Katia MC Verhamme has been involved as project leader in analyses contracted by various pharmaceutical companies and received unconditional research grants from Pfizer, Yamanouchi, and Boehringer Ingelheim, none of which are related to the subject of this study. Vera E Valkhoff has conducted research for AstraZeneca as an employee of the Erasmus Medical Center. Miriam CJM Sturkenboom is scientific coordinator of the IPCI group which is partially funded through unconditional research grants from the pharmaceutical industry, namely Pfizer, Merck, AstraZeneca, Eli Lilly, GSK and Altana. She has been consultant to Pfizer, Novartis Consumer Health, Servier, Celgene and Lundbeck. Carmen Ferrajolo, Gianluca Trifiró, Geert W 't Jong, Carlo Giaquinto, Gino Picelli, Alessandro Oteri, Sandra de Bie, Martijn J Schuemie, Giampiero Mazzaglia, Claudio Cricelli, Francesco Rossi, and Annalisa Capuano declare that they have no conflicts of interest.

References

1. Narkewicz MR, Dell Olio D, Karpen SJ, et al. Pattern of diagnostic evaluation for the causes of pediatric acute liver failure: an opportunity for quality improvement. J Pediatr. 2009;155:801–6.e1.
2. Fontana RJ, Seeff LB, Andrade RJ, et al. Standardization of nomenclature and causality assessment in drug-induced liver injury: summary of a clinical research workshop. Hepatology (Baltimore). 2010;52:730–42.
3. De Valle MB, Av Klinteberg V, Alem N, et al. Drug-induced liver injury in a Swedish University hospital out-patient hepatology clinic. Aliment Pharmacol Ther. 2006;24:1187–95.
4. Andrade RJ, Lucena MI, Fernandez MC, et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology. 2005;129:512–21.
5. Molleston JP, Fontana RJ, Lopez MJ, et al. Characteristics of idiosyncratic drug-induced liver injury in children: results from the DILIN prospective study. J Pediatr Gastroenterol Nutr. 2011;53:182–9.
6. Trifiro G, Pariente A, Coloma PM, et al. Data mining on electronic health record databases for signal detection in pharmacovigilance: which events to monitor? Pharmacoepidemiol Drug Saf. 2009;18:1176–84.
7. Schwimmer JB, Deutsch R, Kahen T, et al. Prevalence of fatty liver in children and adolescents. Pediatrics. 2006;118:1388–93.
8. Cricelli C, Mazzaglia G, Samani F, et al. Prevalence estimates for chronic diseases in Italy: exploring the differences between self-report and primary care databases. J Public Health Med. 2003;25:254–7.
9. Mazzaglia G, Mantovani LG, Sturkenboom MC, et al. Patterns of persistence with antihypertensive medications in newly diagnosed hypertensive patients in Italy: a retrospective cohort study in primary care. J Hypertens. 2005;23:2093–100.
10. Neubert A, Verhamme K, Murray ML, et al. The prescribing of analgesics and non-steroidal anti-inflammatory drugs in paediatric primary care in the UK, Italy and The Netherlands. Pharmacol Res. 2010;62:243–8.
11. Sen EF, Verhamme KM, Neubert A, et al. Assessment of pediatric asthma drug use in three European countries; a TEDDY study. Eur J Pediatr. 2011;170:81–92.
12. Sturkenboom MC, Verhamme KM, Nicolosi A, et al. Drug use in children: cohort study in three European countries. BMJ (Clin Res Edn.) 2008;337:a2245.
13. van Soest EM, Valkhoff VE, Mazzaglia G, et al. Suboptimal gastroprotective coverage of NSAID use and the risk of upper gastrointestinal bleeding and ulcers: an observational study using three European databases. Gut. 2011;60(12):1650–9.
14. Charlton M, Angulo P, Chalasani N, et al. Low circulating levels of dehydroepiandrosterone in histologically advanced nonalcoholic fatty liver disease. Hepatology (Baltimore). 2008;47:484–92.

15. Standardization of definitions and criteria of causality assessment of adverse drug reactions. Drug-induced liver disorders: report of an international consensus meeting. Int J Clin Pharmacol Ther Toxicol. 1990;28:317–22.
16. Benichou C. Criteria of drug-induced liver disorders. Report of an international consensus meeting. J Hepatol. 1990;11:272–6.
17. de Abajo FJ, Montero D, Madurga M, et al. Acute and clinically relevant drug-induced liver injury: a population based case–control study. Br J Clin Pharmacol. 2004;58:71–80.
18. Duh MS, Walker AM, Kronlund KH Jr. Descriptive epidemiology of acute liver enzyme abnormalities in the general population of central Massachusetts. Pharmacoepidemiol Drug Saf. 1999;8:275–83.
19. Lucena MI, Garcia-Cortes M, Cueto R, et al. Assessment of drug-induced liver injury in clinical practice. Fundam Clin Pharmacol. 2008;22:141–58.
20. Traversa G, Bianchi C, Da Cas R, et al. Cohort study of hepatotoxicity associated with nimesulide and other non-steroidal anti-inflammatory drugs. BMJ (Clin Res Edn). 2003;327:18–22.
21. Suzuki A, Brunt EM, Kleiner DE, et al. The use of liver biopsy evaluation in discrimination of idiopathic autoimmune hepatitis versus drug-induced liver injury. Hepatology (Baltimore). 2011;54:931–9.
22. Navarro VJ, Senior JR. Drug-related hepatotoxicity. N Engl J Med. 2006;354:731–9.
23. Coloma PM, Schuemie MJ, Trifiro G, et al. Combining electronic healthcare databases in Europe to allow for large-scale drug safety monitoring: the EU-ADR Project. Pharmacoepidemiol Drug Saf. 2011;20:1–11.
24. Schuemie MJ. Methods for drug safety signal detection in longitudinal observational databases: LGPS and LEOPARD. Pharmacoepidemiol Drug Saf. 2011;20:292–9.
25. Trifiro G, Patadia V, Schuemie MJ, et al. EU-ADR healthcare database network vs. spontaneous reporting system database: preliminary comparison of signal detection. Stud Health Technol Inform. 2011;166:25–30.
26. Bell LN, Chalasani N. Epidemiology of idiosyncratic drug-induced liver injury. Semin Liver Dis. 2009;29:337–47.
27. Hussaini SH, O'Brien CS, Despott EJ, et al. Antibiotic therapy: a major cause of drug-induced jaundice in southwest England. Eur J Gastroenterol Hepatol. 2007;19:15–20.
28. Ostapowicz G, Fontana RJ, Schiodt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med. 2002;137:947–54.
29. Sgro C, Clinard F, Ouazir K, et al. Incidence of drug-induced hepatic injuries: a French population-based study. Hepatology (Baltimore) 2002;36:451–5.
30. Nicastro E, Ranucci G, Vajro P, et al. Re-evaluation of the diagnostic criteria for Wilson disease in children with mild liver disease. Hepatology (Baltimore) 2010;52:1948–56.
31. Ferrajolo C, Capuano A, Verhamme KM, et al. Drug-induced hepatic injury in children: a case/non-case study of suspected adverse drug reactions in VigiBase. Br J Clin Pharmacol. 2010;70:721–8.
32. Hines RN. The ontogeny of drug metabolism enzymes and implications for adverse drug events. Pharmacol Ther. 2008;118:250–67.
33. Murray KF, Hadzic N, Wirth S, et al. Drug-related hepatotoxicity and acute liver failure. J Pediatr Gastroenterol Nutr. 2008;47:395–405.
34. Pineiro-Carrero VM, Pineiro EO. Liver. Pediatrics. 2004;113:1097–106.
35. Garcia Rodriguez LA, Stricker BH, Zimmerman HJ. Risk of acute liver injury associated with the combination of amoxicillin and clavulanic acid. Arch Intern Med. 1996;156:1327–32.
36. Salvo F, Polimeni G, Moretti U, et al. Adverse drug reactions related to amoxicillin alone and in association with clavulanic acid: data from spontaneous reporting in Italy. J Antimicrob Chemother. 2007;60:121–6.
37. Lucena MI, Molokhia M, Shen Y, et al. Susceptibility to amoxicillin-clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. Gastroenterology. 2011;141:338–47.
38. Cuzzolin L, Antonucci R, Fanos V. Paracetamol (Acetaminophen) Efficacy and Safety in the Newborn. Curr Drug Metabol. 2012;14(2):178–85.
39. Observational Medical Outcomes Partnership (OMOP). Available from URL:http://omop.fnih.org. Accessed 23 Feb 2011.
40. Chan DF, Li AM, Chu WC, et al. Hepatic steatosis in obese Chinese children. Int J Obes Relat Metab Disord. 2004;28:1257–63.
41. Nobili V, Marcellini M, Devito R, et al. NAFLD in children: a prospective clinical–pathological study and effect of lifestyle advice. Hepatology (Baltimore). 2006;44:458–65.
42. Rashid M, Roberts EA. Nonalcoholic steatohepatitis in children. J Pediatr Gastroenterol Nutr. 2000;30:48–53.
43. Schwimmer JB, Behling C, Newbury R, et al. Histopathology of pediatric nonalcoholic fatty liver disease. Hepatology (Baltimore). 2005;42:641–9.