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ORIGINAL REPORT

Risk of acute liver injury associated with use of antibiotics. Comparative cohort and nested case–control studies using two primary care databases in Europe

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ABSTRACT

Purpose To assess the impact of varying study designs, exposure and outcome definitions on the risk of acute liver injury (ALI) associated with antibiotic use.

Methods The source population comprised of patients registered in two primary care databases, in the UK and in Spain. We identified a cohort consisting of new users of antibiotics during the study period (2004–2009) and non-users during the study period or in the previous year. Cases with ALI were identified within this cohort and classified as definite or probable, based on recorded medical information. The relative risk (RR) of ALI associated with antibiotic use was computed using Poisson regression. For the nested case–control analyses, up to five controls were matched to each case by age, sex, date and practice (in CPRD) and odds ratios (OR) were computed with conditional logistic regression.

Results The age, sex and year adjusted RRs of *definite* ALI in the current antibiotic use periods was 10.04 (95% CI: 6.97–14.47) in CPRD and 5.76 (95% CI: 3.46–9.59) in BIFAP. In the case–control analyses adjusting for life-style, comorbidities and use of medications, the OR of ALI for current users of antibiotics was and 5.7 (95% CI: 3.46–9.36) in CPRD and 2.6 (95% CI: 1.26–5.37) in BIFAP.

Conclusion Guided by a common protocol, both cohort and case–control study designs found an increased risk of ALI associated with the use of antibiotics in both databases, independent of the exposure and case definitions used. However, the magnitude of the risk was higher in CPRD compared to BIFAP. Copyright © 2016 John Wiley & Sons, Ltd.

KEY WORDS—primary care databases; incidence rate; case–control study; acute liver injury, antibiotics; BIFAP; CPRD; pharmacoepidemiology

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INTRODUCTION

Drugs account for 13% to 17% of cases of acute liver injury (ALI), and hepatotoxicity remains the most frequent reason for withdrawal of medications from the market.[1–3] Several studies have found antibiotic agents to be the largest class of agents to be associated with drug-induced liver injury.[4–7] Drug-induced liver injury is not always diagnosed when patients present with clinical symptoms, complicating measurements of the incidence of liver injury using electronic health record data.[7–10] The estimated incidence of antibiotic

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induced ALI varies widely, depending on the case definition and source population used.[11,12] UK based estimates of incidence rates of antibiotic induced liver injury range from 2.5 to 8.6 per 100 000 users.[13] Most types of antibiotics have been associated with drug-induced liver injury.[5,14,15]

We aimed to assess the association between antibiotic use and a recorded diagnosis of ALI without a suspected cause, applying a common protocol and methodology using different study designs (cohort and nested case–control) across primary care databases in two European countries (UK and Spain) and to evaluate the impact of these differences on the results of the studied association. We further assessed the impact of different exposure and outcome definitions.

This study was performed within the framework of the IMI-PROTECT project (PROTECT—Pharmacoepidemiological Research on Outcomes of Therapeutics by a European ConsorTium).[16]

METHODS

Data sources

The study population was selected from patients registered in two primary care databases: the UK Clinical Practice Research Datalink (CPRD)[17] and the 'Base de datos para la Investigación Farmacoepidemiologica en Atencion Primaria' (BIFAP)—a Spanish computerised database of medical records.[18] CPRD and BIFAP collect and archive nationwide primary care data provided by general practitioners (GPs) and cover around 8% of the UK population and 9% of the Spanish population respectively. Details of both databases have been described elsewhere.[19,20] The protocol was registered in the electronic register of studies of The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP).[21]

Source population and period of valid data collection

The study period started in January 2004 and ended in December 2009. The source population was comprised of patients of all ages with an active registration status during the study period with at least one year of registry with the GP and one year of recorded history, the earliest of which was considered the patient entry date. From the source population, patients who received at least one antibiotic prescription during the study period were identified and the date of the first prescription of an antibiotic was used as the start date of follow-up. In BIFAP more than one million patients were identified who received at least one antibiotic prescription during the study period, compared to more than 3 million patients in the CPRD. Remaining patients from the source population, for whom we could not identify a prescription for an antibiotic during the study period, were considered non-users. For the non-users a random date was generated during the study period and this date was used as the start date of follow-up. Close to one million patients did not receive an antibiotic prescription in BIFAP compared to two million patients in CPRD.

Patients with recorded codes indicating liver injury, chronic liver disease, hepatitis, cancer, alcohol-abuse, gallbladder- or pancreatic disease prior to start date were excluded from the study population. Remaining patients in each database were followed from start date until the earliest occurrence of one of the following endpoints: a recorded code for liver injury, patient left the practice, death or end of the study period. Patients were censored when a code for exclusion criteria (liver related diseases, alcohol abuse and cancer) was recorded during the follow-up.

Lists of codes to ascertain outcome and exposure, as well as operational algorithms to define liver injury were described previously[22] and are presented as supplementary material (Supplementary Tables S1–S3 online).

Identification of patients with acute liver injury

Cases with a first recorded occurrence of liver injury were ascertained using a three-step process as described in a previous publication from our group.[22] Briefly, medical files were searched for specific codes suggestive of liver disease, with abnormal laboratory liver enzyme test results (more than twice the upper limit). In addition, cases had to be referred to a specialist or hospital around the time of the recorded diagnosis of liver injury to be included. The liver injury was considered regardless of severity and defined as acute if there was not further related record after 6 months from the date of initial event. We created two operational definitions of ALI, based on standard predefined criteria, one including cases that were considered *definite* ALI cases and a broad definition including *probable* cases, when some of the proposed criteria for a definite case were not met (Supplementary Table S3 online).[22] In the BIFAP database information on diagnoses is often recorded in free text comments annotated by the GP. Therefore, manual review was needed to confirm potential cases detected with the computer algorithm. In BIFAP, only manually confirmed cases were included. In CPRD, all cases identified using the comprehensive READ dictionary and the computer algorithm, as described

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before, were included. The validity of the CPRD algorithm was checked in a small sample of cases by manual review.[22]

Identification of antibiotic exposure

Exposure was defined as a recorded prescription for a systemic antibiotic agent listed in chapter 5 of the British National Formulary (BNF) in CPRD or therapeutic subgroup J01 of the Anatomical Therapeutic Chemical (ATC) Classification System in BIFAP (Supplementary Table S4). Individuals with prescriptions for topical antibiotics were not considered as exposed. The expected duration of each prescription was estimated using the prescribed quantity and duration. The median duration of exposure to all antibiotic agents was imputed when information was missing (2%). Antibiotic agents were grouped in seven mutually exclusive categories. We first identified patients who exclusively used agents from a single antibiotic category. Patients who used agents from multiple antibiotic categories were then assigned to an antibiotic combination category.

For the cohort analyses, the follow-up time was divided into periods of non-use, current and past use of antibiotics with patients among the antibiotic-users cohort moving between these periods. A period of *current use* extended until 14 days after the estimated end of supply. *Past use* period started at the end of a current use period until a maximum of 366 days thereafter. All remaining person-time after the end of past use was classed as *non-use* unless a new antibiotic was prescribed resulting in the start of a new period of current use.

For the case–control analyses patients were considered current users of antibiotics if a prescription overlapped the index date, lasted until the index date or ended within 14 days prior to the index date. Recent and past use was defined as use of antibiotics between 15 and 30 days or 31–365 days before the index date respectively. Non-users were those patients with no prescription for antibiotics in the year before the index date.

STATISTICAL ANALYSES

Cohort analyses

The incidence rate (IR) of ALI was estimated separately in the each study population. More than 4.5 million patients in CPRD were followed up for an average of 2.7 years during the study period, compared to close to 2 million patients in BIFAP with a mean follow-up of 1.8 years. We calculated the risk ratio (IRR) and 95% confidence intervals of ALI associated with current and past use vs. non-use periods in both study populations, using a Poisson regression model adjusted for age, sex and calendar year.

Next, we estimated the hazard ratio (HR) of ALI associated with use of antibiotics at baseline adjusting for other variables using a multivariable Cox regression model. All covariates were measured at baseline. The full multivariable model included age (in 10-year categories), sex, most recent record for Body Mass Index (BMI: ($<$20, 20–25, 25–30 and $>$30)), smoking status (never smoker, current smoker and ex-smoker), and we also counted the number of visits to the General Practitioner in the previous year. In the full model we also adjusted for prior history of morbidities (heart failure, rheumatoid arthritis, haemochromatosis, alpha 1-antitrypsin deficiency and diabetes) and use of other drugs in the previous year (non-steroidal anti-inflammatory drugs; other analgesics and antipyretics, statins, antidepressants, oral contraceptives, oral preparation for acne, disease-modifying anti-rheumatic drugs, oral corticosteroids and antidiabetic drugs). Co-morbidities were ascertained based on codes recorded in the patient's medical history before baseline (e.g. Read dictionaries in CPRD and ICPC codes in BIFAP). Missing values of the variables were grouped under the category 'unknown'.

Case–control analyses

For the case–control analyses, all cases with a first recorded occurrence of ALI identified in the study population were retained as cases, and a set of five controls per case, were selected among the remaining patients from the same database. The date of diagnosis was considered the index date of every case. Controls were matched to cases by age (within one-year), sex, index date (month and year) and, in CPRD, only also by practice.

We computed odds ratios (OR) and 95% confidence intervals (CI) of first recorded occurrence of ALI associated with current, recent and past use of antibiotics compared to non-use using conditional logistic regression. Potential confounders were measured at the index date. The crude estimates accounted for the matching variables (age, gender and date). A further fully adjusted model included all variables that were included in the fully adjusted cohort analyses, but ascertained prior to the index date in this analysis. Prior exposure to covariate drugs (other than antibiotics) was classified as current use if a prescription overlapped the index date, lasted until the index date or ended within 30 days prior to the index date. Recent/past use was defined as use of the other drugs between 31 and 365 days before the index date. Non-

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users (the reference category for the analyses) were those patients with no prescription in the year before the index date. Co-morbidities were ascertained any time before index date.

Using the case–control approach, analyses were stratified to allow for estimations of risk within the seven antibiotic type groups. Within the antibiotic categories, the individual effect of amoxicillin alone and in combination with clavulanic acid was studied.

Secondary analyses

First, secondary cohort and case–control analyses were performed using a broader case definition (including definite and probable cases) to estimate the impact of using a less stringent case algorithm to identify patients with ALI.[22]

An additional secondary case–control analysis was performed to investigate the effect of a broader exposure window on the effect estimates. In this analysis 'current' use of antibiotics was defined as having received a prescription for antibiotics that lasted until the index date or ended within 30 days (instead of 14 days) prior to the index date. A broad risk window was chosen to align it to commonly used windows for drug treatments and to allow for a potential delayed onset of ALI after antibiotic use.

All statistical analyses were conducted using Stata software, version 11 (StataCorp, College Station, TX). A blinding procedure was maintained until final results were available from the two databases, in the coordinator centre at the Utrecht University (the Netherlands).

RESULTS

Descriptive of study population

Study patients in the CPRD were younger than individuals in BIFAP. This difference was observed both in patients using antibiotics during study period (mean age: 31.8 years in CPRD vs. 38.1 years in BIFAP) and non-using patients (30.6 vs. 40 years respectively).

In both databases, antibiotic users were more likely to be female and had a higher prevalence of obesity. They consulted their GP more frequently and had a greater prevalence of co-morbidities and co-medications, compared to non-users of antibiotics during the study period (Supplementary Table S5).

Results from the cohort analyses

The incidence rates (IR) of *definite* ALI were higher among study group patients from BIFAP than from CPRD (Table 1). In BIFAP, among patients who received at least one antibiotic prescription during the study period, 96 definite ALI cases were identified (IR: 3.89 per 100 000 person years). Among patients not using antibiotics, only 28 definite ALI cases were identified (IR: 2.48 per 100 000 person years). In CPRD lower IRs were observed in both group of patients; for users (IR: 2.58 per 100 000 person years) and for non-users of antibiotics (IR: 0.76 per 100 000 person years)

There was an increased risk of *definite* ALI during current antibiotic use periods compared to non-use periods in both databases, after adjusting for age, sex, and calendar year (BIFAP: RR 5.76, 95% CI 3.46–9.59; CPRD: RR 10.04, 95% CI 6.97–14.47). There was also an increased risk of ALI associated with past use of antibiotics as compared to non-use in both databases (see Table 1).

In the full multivariable Cox regression model the estimated HR of *definite* ALI among individuals taking antibiotics at baseline was 2.51 in CPRD (95% CI: 1.67–3.75) and slightly lower in BIFAP (1.56; 95%CI: 0.98–2.49) (Table 4).

Results from nested case–control analyses

In CPRD all but two cases ($n = 263$) with *definite* ALI could be matched to up to five controls. In total, 1284 controls were identified. In BIFAP, the 124 confirmed cases were matched to 620 controls.

Characteristics of cases and controls matched for age and gender are presented in Table 2. There were some differences between lifestyle factors acting as potential confounders between CPRD and BIFAP. In BIFAP, information on lifestyle variables was less complete. In CPRD, cases were more often diagnosed with heart failure, rheumatoid arthritis and diabetes compared to controls and more often prescribed concurrent medications on the index date (see Table 2). In BIFAP, the only substantial difference between cases and controls was that cases used more analgesics and antipyretics than controls.

The results of the fully adjusted analyses showed evidence of an increased risk of ALI up to 14 days after the receipt of antibiotic agent in CPRD (OR 5.7, 95% confidence interval [CI] 3.46–9.36) and BIFAP (OR 2.6, 95% CI 1.26–5.37). There was no clear evidence of an association between recent and past use of antibiotics and an increase in the risk of ALI (see Table 3). These estimates of risk of ALI adjusting for several confounding variables in the case–control analyses were slightly higher but comparable with results of the fully adjusted Cox regression model (as shown in Table 4).

The risk associated with current use of antibiotics was further investigated by stratifying current users by antibiotic class. Because of small numbers, confidence intervals of stratified estimates were wide. In CPRD, there was evidence of an association between ALI and the use of penicillin, cephalosporin and any combination of antibiotics (see Table 3). In BIFAP the largest association was found for users of quinolones, followed by macrolides.

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Table 1. Cohort results: incidence rate and risk of ALI in study population (using and not using antibiotics during the study period) in both databases (BIFAP and CPRD)

	BIFAP				CPRD			
	Cases	Person-years	IR per 100 000 p-y (95%CI)	Adjusted RR* (95% CI)	Cases	Person-years	IR per 100 000 p-y (95%CI)	Adjusted RR* (95% CI)
Definite		Overall IR = 3.45 (2.87–4.11)				Overall IR = 2.03 (180–2.29)		
Never use and non-use (>365)*	47	2 004 151	2.35 (1.72 – 3.12)	1	87	7 620 943	1.14 (0.93–1.41)	1
Current (0–14)	23	184 496	12.47 (7.9 – 18.71)	5.76 (3.46 – 9.59)	46	359 149	12.81 (9.59–17.1)	10.04 (6.97–14.47)
Past (14–365)	54	1 410 343	3.83 (2.88 – 5)	1.72 (1.16 – 2.57)	132	5 059 485	2.61 (2.2–3.09)	2.13 (1.61–2.82)
Definite + probable		Overall IR = 12.11 (11–13.31)				Overall IR = 7.65 (7.19–8.14)		
Never use and non-use (>365)	176	2 004 151	8.78 (7.53–10.18)	1	329	7 620 943	4.32 (3.87–4.81)	1
Current (0–14)	69	184 496	37.4 (29.1–47.33)	5. 11 (3.84–6.79)	144	359 149	40.09 (34.05–47.21)	8.26 (6.76–10.09)
Past (14–365)	191	1 410 343	13.54 (11.69–15.61)	1.75 (1.42–2.16)	524	5 059 485	10.36 (9.51–11.28)	2.22 (1.92–2.56)

*Reference category included non-use periods after past periods among the cohort of patients with at least 1 RX antibiotics during study period, and never use period from the group of patients not using antibiotics during the study period.
IR = incidence rate; RR = relative risk; CI = confidence interval × adjusted RR for age, sex, calendar year and antibiotic drug use by Poisson regression analyses.

The results of the CPRD analysis investigating the association between amoxicillin and the risk of ALI showed that the OR for current use of amoxicillin alone was 3.6 (95% CI 1.43–8.83) and 6.5 (95% CI 1.93–21.5) in combination with clavulanic acid, compared to those not using antibiotics. In BIFAP, the OR associated with the use of amoxicillin in combination with clavulanic acid was 4.53 (95% CI 1.47–13.9). There were no amoxicillin only users among the cases in BIFAP (data not shown).

Results of secondary analyses

Using a broader case definition (by including definite and probable cases), we identified 989 cases in CPRD and 436 cases in BIFAP. As expected, in the cohort analyses higher incidence rates were observed for *definite and probable* ALI in both data sources, and the estimates of risk associated with periods of current use of antibiotics were slightly lower both in BIFAP (IRR 5.11, 95% CI 3.84–6.79) and CPRD (IRR 8.26, 95% CI 6.76–10.09) compared to non-use periods (Table 1).

In the case–control analyses the adjusted OR for *definite and probable* ALI in current users of any type of antibiotic was 3.08 (95% CI 2.05–4.62) in BIFAP and 3.59 (95% CI 2.79–4.61) in CPRD. These adjusted risk estimates when using this broad outcome definition were lower than for *definite* ALI shown in Table 3 and were quite similar in magnitude in both databases (Table 4).

Using a longer exposure window (up 30 days prior to the index date) to define 'current' use of antibiotics resulted in similar estimates, but slightly lower than the results of the main analyses (Table 3). There was evidence that current use of antibiotics was associated with an increased risk of ALI, both in CPRD (adjusted OR 4.37, 95% CI 2.85–6.69) and BIFAP (adjusted OR 2.47, 95% 1.25–4.88) (Table 4).

DISCUSSION

This cohort study with a nested case–control analysis performed in two databases using a common protocol confirms the increased risk of ALI associated with the use of antibiotics in line with previous studies.[2,3,6,13,23,24] This established drug/outcome association served to address methodological aspects of performing studies using electronic health care databases in different countries.

Impact of using multiple sources of information: CPRD and BIFAP

Using multiple databases permits the study of rare events associated with drug exposure, but the

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Table 2. General covariates and life style factors assessed as risk factors for *definite* ALI among cases and controls from both study cohorts in BIFAP and CPRD databases

		BIFAP		CPRD	
		Controls (*N* = 620)	Cases (*N* = 124)	Controls (*N* = 1284)	Cases (*N* = 263)
Sex, *n* (%)	Male	310 (50)	62 (50)	638 (50)	130 (50)
Age, *mean* (*SD*)		46.6 (21.3)	46.6 (21.4)	57.2 (20.7)	57.8 (20.9)
Smoking, *n* (%)	Non-smoker	206 (33.2)	44 (35.5)	633 (49.3)	119 (45.2)
	Smoker	109 (17.6)	23 (18.5)	201 (15.7)	48 (18.3)
	Ex-smoker	17 (2.7)	5 (4.0)	389 (30.3)	95 (36.1)
	Unknown	288 (46.5)	52 (41.9)	61 (4.8)	1 (0.4)
Body mass index (kg/m^2), *n* (%)	<19	23 (3.7)	5 (4.0)	27 (2.1)	8 (3)
	19–24.9	117 (18.9)	22 (17.7)	432 (33.6)	89 (33.8)
	25–29.9	131 (21.1)	33 (26.6)	369 (28.7)	83 (31.6)
	≥30	84 (13.5)	20 (16.1)	255 (19.9)	56 (21.3)
	Unknown	265 (42.7)	44 (35.5)	201 (15.7)	27 (10.3)
Visits to GP in previous year, *n* (%)	0	81 (13.1)	5 (4.0)	81 (6.3)	0 (0)
	1–3	104 (16.8)	14 (11.3)	206 (16.0)	10 (3.8)
	4–10	193 (31.1)	40 (32.3)	229 (17.8)	40 (15.2)
	11+	242 (39.0)	65 (52.4)	768 (59.8)	213 (81)
Co-morbidities, *n* (%)	Heart failure	12 (1.9)	2 (1.6)	35 (2.7)	13 (4.9)
	Diabetes	61 (9.8)	9 (7.3)	97 (7.6)	30 (11.4)
	Rheumatoid arthritis	4 (0.65)	1 (0.81)	27 (2.1)	9 (3.4)
	Hemochromatosis	—	—	—	1 (0.4)
	Alpha-antitrypsin-deficiency	—	—	1 (0.1)	—
Treatment*, *n of users* (%)	NSAIDs	65 (10.5)	25 (20.2)	83 (6.5)	25 (9.5)
	Other analgesics/antipyretics	73 (11.8)	25 (20.2)	291 (22.7)	80 (30.4)
	Statins	67 (10.8)	13 (10.5)	213 (16.6)	53 (20.2)
	Antidepressants	37 (6.0)	5 (4.0)	97 (7.6)	36 (13.7)
	Oral contraceptives	—	—	39 (6.0)	8 (6.0)
	Oral preparation for acne	—	—	3 (0.2)	1 (0.4)
	DMARD	5 (0.8)	0	10 (0.8)	5 (1.9)
	Oral corticosteroids	9 (1.5)	5 (4.0)	19 (1.5)	13 (4.9)
	Antidiabetic drugs	33 (5.3)	6 (4.8)	54 (4.2)	21 (8.0)
	Other hepatotoxic drugs (list FDA)	31 (5.0)	2 (1.6)	45 (3.5)	28 (10.6)

*Treatment as current use: use of the drug at the index date or within 30 days.

differences inherent to each population, country, health care system and the characteristics of the dataset need to be taken into consideration, as well as the difference in drug use and prescription patterns in each country.[25,26] A previous study by our group found minimal variability in the prevalence of antibiotic use across the UK, Spain, Denmark and Germany.[27] However, we found some differences in age and comorbidity characteristics between the two source populations from which the study groups were selected. We controlled for measured confounders in the fully adjusted analyses and explored the role of potential exposure and outcome misclassification by performing several sensitivity analyses.

We found a higher incidence rate of ALI in the Spanish study population than in the UK. Although both settings showed an increased risk of ALI associated with antibiotic use, higher relative risks were observed in CPRD than in BIFAP. We believe that this difference was mainly caused by the very low incidence rate of ALI reported in UK non-users of antibiotic agents. Overall, our results are in line with those previously published.[13,23,24] For individual antibiotic classes great variation and imprecise estimates were found because of the small number of cases in each group as well as differences in the preference of antibiotic classes in the two countries as shown by a study in this same journal issue.[27] We reported an increased risk of ALI in users of amoxicillin and even greater among users of the combination with clavulanic acid. This finding is in line with previous work by Garcia-Rodriguez et al..[23] Moreover, a recent paper by the EU-ADR network to investigate drug-induced liver injury in children and adolescents found that the most frequent signals of ALI among users of antibacterial agents, came from patients using amoxicillin and amoxicillin/clavulanic acid (*n* = 19).[9]

Impact of case and exposure definition

In the present study we implemented a new user cohort design that has been advocated as the primary design to be considered for studies of drug safety.[28] Under this

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Table 3. The risk of *definite* ALI associated with the use of types of antibiotics in BIFAP and CPRD databases

		BIFAP				CPRD			
Antibiotic use by type	Exposure	Controls ($N = 620$)	Cases ($N = 124$)	Crude OR* (95% CI)	Adjusted OR† (95% CI)	Controls ($N = 1284$)	Cases ($N = 263$)	Crude OR* (95% CI)	Adjusted OR† (95% CI)
Any antibiotics	Non-user	354 (57.1%)	49 (39.5%)	1	1	719 (60.0%)	94 (35.7%)	1	1
	Current user (14 days)	40 (6.5%)	20 (16.1%)	3.97 (2.08—7.58)	2.6 (1.26—5.37)	66 (5.1%)	59 (22.4%)	7.77 (4.98—12.12)	5.70 (3.46—9.36)
	Recent user	94 (15.2%)	21 (16.9%)	1.77 (0.99—3.17)	1.2 (0.63—2.35)	167 (13.0%)	52 (19.8%)	2.58 (1.75—3.81)	1.52 (0.99—2.32)
	Past user	132 (21.3%)	34 (27.4%)	1.90 (1.17—3.11)	1.5 (0.87—2.57)	332 (25.9%)	58 (22.1%)	1.44 (1.01—2.06)	0.94 (0.64—1.39)
Single tetracyclines	Current user	3 (0.5%)	1 (0.8%)	2.71 (0.27—26.85)	2.43 (0.21—27.92)	6 (0.5%)	1 (0.4%)	1.66 (0.19—14.33)	2.26 (0.25—20.56)
Single penicillins	Current user	19 (3.1%)	6 (4.8%)	2.45 (0.90—6.68)	1.73 (0.60—5.01)	32 (2.5%)	26 (9.9%)	6.86 (3.75—12.75)	5.18 (2.63—10.2)
Single other betalactams (cephalosporins)	Current user	3 (0.5%)	2 (1.6%)	5.10 (0.83—31.40)	2.24 (0.32—15.60)	5 (0.4%)	10 (3.8%)	18.13 (5.84—56.27)	16.8 (4.92—57.37)
Single macrolides	Current user	8 (1.3%)	6 (4.8%)	6.02 (1.86—19.46)	4.08 (1.12—14.93)	10 (0.8%)	3 (1.1%)	2.61 (0.69—9.84)	2.02 (0.51—8.1)
Single quinolones	Current user	2 (0.3%)	3 (2.4%)	10.31 (1.68—63.09)	9.21 (1.28—66.45)	3 (0.2%)	3 (1.1%)	8.02 (1.53—42.15)	5.46 (0.95—31.57)
Single other antibiotics/ combinations	Current user	3 (0.5%)	2 (1.6%)	4.53 (0.74—27.94)	3.50 (0.49—25.25)	5 (0.4%)	7 (2.7%)	11.54 (3.45—38.66)	6.6 (1.78—24.44)

*Crude OR, taking into account the matching variables age, sex and date

†Adjusted odds ratio (OR) by general confounders (BMI, smoking and visits to GP in previous year), comorbidity variables (heart failure, diabetes and rheumatoid arthritis) and co-medications (NSAIDs, other analgesics, statins, oral preparations for acne, DMARDs, oral corticosteroids, anti-diabetics, antidepressants and other hepatotoxic drugs).

design, special consideration is required when selecting the comparator group and evaluating its appropriateness to assess the effect of drugs.[29] We chose all non-use periods as comparator time, including periods of non-use after past use and the 'never' use time among patients not receiving antibiotics during the study period. Antibiotic prescriptions given outside the primary care setting might have been missed, as well as concomitant use of other over-the-counter (OTC) medicine, but is not thought to have affected the results to a great extent and any potential non-differential misclassification would have pushed the results towards the null. In BIFAP and CPRD, prescriptions are systematically captured at the time of GP recording, which allowed for good adjustments for medications prescribed other than antibiotic agents around the index date. Over-the counter antibiotic use is not recorded, but is not thought to have been a major source of misclassification, because medical prescriptions for antibiotics are required in both the UK and Spain.

As part of the PROTECT project and with a formal testing perspective, we evaluated the effect of alternative definitions of ALI and the impact on the strength of the association with antibiotic use. Using pre-defined algorithms in BIFAP and CPRD, measures of association were similar with both definitions, though slightly lower with the broad definition in both databases. These findings were expected, as the broad case algorithm was more sensitive, but less specific. This may have also contributed to more similar results between BIFAP and CPRD as the non-differential misclassification of cases (not depending on exposure status) may have moved the results towards the null. This highlights the trade-off between validity of the results and the precision with which they are estimated. It has been reported that differences in outcome definition may lead to marked variation in incidence estimates, as observed when comparing ALI detected using US claims databases with other European databases, under the OMOP project.[11,30] We advocate for a more valid and restrictive case definition, as a prior validation study showed a higher specificity with less than 5% of identified patients being false positive cases and higher rates of agreement between computer search and manual review of definite cases compared to the broad definition.[22] However, to enable comparison with studies using a less restrictive case definition, we also presented our results with broad definitions.

The sensitivity analysis, changing the time window from 14 to 30 days, led to relative risk estimates closer to null in CPRD. The 30-day window may have included time when the presence of the drug has already disappeared corresponding to a period of no

Table 4. Comparison of estimates of risk of ALI across designs, using different outcome and exposure definitions in CPRD and BIFAP databases

Design—data base	Age, sex and year adjusted model		Multi-adjusted model**	
	RR	95% CI	RR	95% CI
Cohort study	Periods of current use (14 days) vs. non use periods (Poisson regression)		Use of antibiotics at baseline vs. non use (Cox regression)	
BIFAP definite ALI	5.76	3.46–9.59	1.56	(0.98–2.48)
CPRD definite ALI	10.04	6.97–14.47	2.50	(1.67–3.74)
BIFAP definite and probable ALI	5.11	3.84–6.79	1.48	(1.16–1.88)
CPRD definite and probable ALI	8.26	6.76–10.09	1.89	(1.55–2.29)
Nested case–control study/current users (14 days) vs. non users (conditional logistic regression)				
BIFAP definite ALI	3.9	2.08–7.58	2.60	(1.26–5.37
CPRD definite ALI	7.77	4.98–12.12	5.70	(3.46–9.36)
BIFAP definite and probable ALI	4.45	3.06–6.45	3.08	(2.05–4.62)
CPRD definite and probable ALI	5.88	4.67–7.40	3.59	(2.79–4.61)
Nested case–control study/current users (30 days) vs. non users (conditional logistic regression)				
BIFAP definite ALI	3.82	2.09–6.97	2.47	(1.25–4.88)
CPRD definite ALI	6.42	4.37–9.44	4.37	(2.85–6.69)
BIFAP definite and probable ALI	3.60	2.60–4.97	2.37	(1.66–3.38)
CPRD definite and probable ALI	5.60	4.57–6.86	3.36	(2.69–4.18)

*Adjusted risk by general confounders (age, sex, calendar year, BMI, smoking and visits to GP in previous year), comorbidity variables (heart failure, diabetes and rheumatoid arthritis) and co-medications (NSAIDs, other analgesics, statins, oral preparations for acne, DMARDs, oral corticosteroids, anti-diabetics, antidepressants and other hepatotoxic drugs).

risk. The extension of the exposure window had a minimal impact on the risk estimates in BIFAP.

Limitations of the present study deserve some comments. First, there were differences between both databases in recording patterns, coding dictionaries for health conditions and medications, as well as differences in software and structure for data entry. Furthermore, the method of case validation (manual review of all potential cases in BIFAP vs. review of a small sample of cases in CPRD) differed between the two data sources. Although we tried to unify all these aspects in the protocol, they may have still have contributed to any residual differences in results. As the rate of ALI was lower in CPRD than in BIFAP, it is unlikely that not being able to manually ascertain cases in CPRD led to an overestimation of the absolute rates.

A valid comparison between the results of the cohort and case–control study designs could only be achieved in models adjusted for age, sex and calendar time as any other potential confounding variables were captured at different time points in both \designs: fixed at the start date in the cohort analyses and as time-dependent in the case–control analyses. For the cohort study, we also estimated the HR of ALI associated with antibiotic use at baseline, but we acknowledge the limitation of Cox regression analyses when not taking the variation of the variables during follow-up into account as this is likely to underestimate the true risk associated with current use of antibiotics.

Our results may not be directly applied to the general population as patients that were more likely to be diagnosed with ALI (patients with cancer, alcoholism or previous related diseases) were excluded from the study population. However, based on the specificity of the outcome we chose to maximise internal validity to the expense of losing generalizability.

Confounding by indication is always present to some degree in drug association studies. In the present study we applied strict exclusion criteria, to exclude any other suspected cause of ALI. Patients with prior liver disease (infective or not) and related conditions, as well as viral hepatitis, were excluded.

Finally, even though we used large databases, the absolute number of ALI cases was small and estimates of risk associated with the use of different type of antibiotics lacked precision with marked variations in incidence between databases. These variations could reflect a difference in the prevalence of individual antibiotic use by country and differences in recording details between both databases.

CONCLUSION

Using a common protocol and procedures, and maintaining blinded reporting of results in two databases, we have confirmed the previously reported increased risk of ALI associated with the use of antibiotics. Cohort and case–control designs gave qualitatively comparable results independent of the exposure and case definitions used. The extent of risk associated with antibiotics use, though elevated as observed in the two

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databases, was higher in the UK CPRD than in the Spanish BIFAP database. This may reflect variability in database recording, data management or real differences in patterns of use of antibiotic agents and associated risks of ALI. Nonetheless, this comparison demonstrates the feasibility of conducting unified, cross-country, collaborative drug safety studies.

CONFLICT OF INTEREST

The protocol was approved by the Spanish Agency for Medical products (AEMPS) to be performed in the BIFAP database as part of the PROTECT project and was registered in the ENCePP electronic register of studies (http://www.encepp.eu/encepp/studiesDatabase.jsp). CPRD ISAC approval for the study was obtained in October 2011 (11_019A_2). The London School of Hygiene and Tropical Medicine ethics committee approved the study in May 2011 (reference number 5973). We maintained a blinding procedure until the final results from both CPRD and BIFAP were submitted to the coordinating centre at Utrecht University. None of the results presented in the current manuscript were published elsewhere.

KEY POINTS

- This is the first study to compare the incidence of acute liver injury (ALI) associated with antibiotic use in Spain and the United Kingdom using primary care data, applying a common protocol and maintaining blinded reporting of results.
- Qualitatively comparable risks estimates of ALI were found using both a cohort and a nested case-control study in both databases, confirming the previously reported increased risk of ALI associated with the use of antibiotics.
- Whilst the incidence rate of *definite* ALI was lower in CPRD than BIFAP, consistently higher relative risks estimates of ALI associated with antibiotic use were found in CPRD. A broad definition of ALI including *definite and probable* cases resulted in slightly lower relative risk estimates.
- The estimates of risk associated with the use of specific type of antibiotics lacked precision with marked variations in incidence between databases. This may reflect variability in database recording, data managing or real differences in patterns of use of antibiotic agents and associated risks of ALI. Nonetheless, this comparison demonstrates the feasibility of conducting unified, cross-country, collaborative drug safety studies.

ETHICS STATEMENT

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AUTHOR CONTRIBUTIONS

Ruigomez A and Garcia Rodriguez LA designed the research study and wrote the protocol.

Ruigomez A and Brauer R are responsible for database, performed data extraction and computer research and analysed the data.

Huerta C collected and managed the data in BIFAP and reviewed the manuscript.

Brauer R, Downey G and Feudjo Tepie collected and managed the data in CPRD.

Bate A, de Abajo F, de Groot M, Schlienger R, Reynolds R, Klungel O, Smeeth L and Douglas I reviewed the protocol and manuscript.

Brauer R and Ruigomez A wrote the first draft, and all authors contributed with critical comments to the final version.

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