IMI PROTECT – Work Package 6 / subpackage antibiotics and liver injury

STUDY REPORT

The risk of liver injury associated with the use of antibiotics

A study using a US database with linkage with hospital data

Version 1: 17 april 2014

Authors: Stéphanie Tcherny-Lessenot, Yunxun Wang, Juhaeri Juhaeri (Sanofi)

Index

Tables

1. Context of PROTECT Work package 6 studies

The study described in this report was performed within the framework of PROTECT (Pharmacoepidemiological Research on Outcomes of Therapeutics by a European ConsorTium) Work package 6 “validation on methods involving an extended audience”. This workpackage aims to test the transferability/feasibility of methods developed in WP2 to 5 (in particular WP2 and WP5) in a range of data sources owned or managed by Consortium Partners or members of the Extended Audience.

As defined in the WP6 research plan, the aims of this study were:

- to evaluate the external validity of the study protocol on the risk of acute liver injury associated with the use of antibiotics by replicating the study protocol in another database,
- to validate the outcome of interest through in hospital data review.

The study protocol was validated on 2 February 2012 (Authors: Stéphanie Tcherny-Lessenot, Sanofi), after adaptation on the initial protocol published in November 2011. Then the initial protocol “PROTECT_WP2 Final Protocol Antibiotics-LiverInjury_29Nov2011” was amended on 10 May 2012 (Amendment 1) and on 20 July 2012 (Amendment 2). The analysis performed before and after the amendments on initial protocol will be presented in this report.

2. Objectives

We proposed to assess the association between antibiotics use and idiopathic acute liver injury by replication of the case-control design in a US claims database (Clinformatics datamart).

The study objectives of the replication were:

- To estimate the risk of acute liver injury associated with antibiotics exposure (users and non-users)
- To estimate the risk of acute liver injury associated with various antibiotics classes

The secondary objectives were:

- To validate cases of liver injury using information from patients’ hospital records

3. Methods

3.1 Data Source

The proposed study was conducted in the US Clinformatics Data Mart database (Optum Insight® formerly Ingenix®). This database was linked to Premier's Perspective™ Comparative Database (PCD) (Premier®) to answer secondary objective.

The main characteristics of these databases are summarised below:

Clinformatics Data Mart (US)

Clinformatics datamart is a large US health insurance database covering United HealthCare® insurance plans including Medicaid. The records are organized into a medical file, containing claims from providers and facilities, a pharmacy file with outpatient pharmacy dispensing records, a lab test file with outpatient lab tests records and an enrolment file that provides demographic data and dates of insurance eligibility for persons in the database. All of these files are linked at the individual level by an encrypted identifier.

Diagnoses on claims are recorded using International Classification of Diseases, Ninth Revision (ICD-9) diagnosis codes. Procedures are identified using Common Procedural Terminology (CPT), ICD, and Health Care Financing Agency (HCFA) Common Procedure Coding System (HCPCS) codes. Drugs are identified by National Drug Codes (NDC) and Hierarchical Ingredient Code List (HICL) codes.

Premier's Perspective™(US)

The Premier's Perspective™ Comparative Database (PCD) is a repository of hospital administrative data that includes approximately one sixth of all hospitalizations in the United States. Annually, more than five million hospital discharges (among 500 acute care hospitals) are processed and recorded in the Premier PCD. Once received, portions of the data are mapped to Premier standards in order to allow direct comparisons between facilities. These mappings occur at both the patient level and the individual charge code level. All data received passes through a rigorous multi-tiered validation process.

Detailed transactional data include information specific to the patient's visit, demographic data including ethnic background, date-stamped logs of all billed items including procedures, medications, laboratory, diagnostic and therapeutic services at individual patient level, ICD9 Primary/ Secondary Indicator and ICD9 Primary/Secondary Description, APR Severity level score, discharge status and length of hospital stay and therapy.

3.2 Period of valid data collection

The period of valid data collection was defined as the period from the left censoring date up to the right censoring date. The left censoring date was the

date that a patient enrolled into the database. The right censoring date was the earliest of the following: the date a patient died, the end of the database's data collection, or the date that the patient left the database.

3.3 Study period

The study period started in January 2004 and ended in December 2009. Information on the use of antibiotics and occurrence of acute liver injury was obtained from claims data where prescription and diagnosis data were recorded.

3.4 Source population

The population was comprised of patients of all ages with an active or died registration status during the study period of January 1st, 2004 to December 31st, 2009 in the Clinformatics Data Mart. Patients had to attained one year of enrolment in the database at the beginning of the study period.

3.5 Study population

From the aforementioned source population, two study cohorts were selected:

- the first cohort included all patients who received at least one antibiotic prescription during the study period. For this cohort, the date of first prescription of an antibiotic after meeting the eligibility criteria (entry date) defined the start of follow-up (start date), for the exposed cohort.
- the second cohort was composed of all members belonging to the same source population and who have not received an antibiotic prescription during their contribution to the follow-up study period and in the year before the entry date (date when the patient meet all the eligibility criteria and enter in the study contribution). For these patients we assigned a random date during the study period. For this cohort of non-users the random date generated after meeting the eligibility criteria was used as the start of follow-up (start date).

All subjects from the study population with one of the codes listed in table 1 (outcome definition) or one of the diagnoses included in table 2 (exclusion criteria: liver cancer and liver metastasis, gallbladder disease, pancreatic disease, and other chronic liver diseases not included in outcome definition) prior to start date were excluded.

For the purpose of a population-based case-control study, all subjects from the aforementioned primary source population were considered as secondary study population.

3.6 Outcome ascertainment

All patients from the study population were followed from the start date (date of first antibiotic prescription or assigned start date in the comparison cohort) until the earliest occurrence of one of the following endpoints: a code from table 1 (outcome), death or end of the study period. Patients were censored when a code for one of the exclusion criteria was recorded during the follow up (liver cancer and liver metastasis, gallbladder disease, pancreatic disease, and other chronic liver diseases not included in outcome definition).

We ascertained patients with the first recorded occurrence of idiopathic acute liver injury (outcome) and the date of diagnosis was their index date (outcome date). For these patients, patient profiles from the claims/electronic data were collected.

3.7 Outcome definition

The outcome for this study was the first recorded occurrence of idiopathic acute liver injury. To initially identify cases, we used a list of codes (tables 1a, 1b) some of them are specific of liver disease or symptoms (e.g. Hepatitis, Acute Hepatic failure, Icterus) and others are not specific (e.g.: Liver Function Test Abnormal, Increased Transaminases). Patients were classified as definite and probable cases:

1. - Definite case (narrow-specific definition): the information recorded in the patients' medical record met all the criteria to be classified as idiopathic acute liver injury (see above) and the patient presents at least with one of the following conditions (A+B or A+C):

A - A diagnosis of liver injury (specific codes for liver disease, or codes referring to symptoms of liver injury from the list of codes in table 1A, 1B) with a referral to a specialist or hospital related to liver disease within 2 weeks of recorded diagnosis.

Together with

B - An increase of more than two times the upper limit of the normal range in alanine aminotransferase (ALT)

or

C - A combined increase in aspartate aminotransferase (AST), alkaline phosphatase (AP) and total bilirubin provided one of them is twice the upper limit of the respective normal range.

Laboratory results indicating liver injury need to be recorded in the patient's medical record within two months of being diagnosed with a code listed in table 1A, 1B.

2.- Probable case (broad definition): The information recorded in the patients' medical file was compatible with idiopathic acute liver injury, but not fulfilling all conditions and criteria to be defined as definite case.

This case category could include different scenarios/approaches in which some of the conditions for definitive case are missing, applying different algorithms as follows:

Probable case 1: Patients identified with a specific code of liver disease listed in table 1A and with complete laboratory criteria for liver injury (B or C) but without a related referral to a hospital/specialist

Probable case 2: Patients identified with a non-specific code of liver disease listed in table 1B with complete laboratory criteria for acute liver injury B or C, and a related visit to a specialist or hospitalization.

We have manually review available information of subgroups of probable cases, in order to confirm their final status.

3. - Non-case: Any potential or probable case that was excluded in one of the previous steps and those with insufficient data to determine their case status. Patients

presenting normal liver function tests (LFTs), alcohol related problems, gallbladder disease, pancreatic disease, or other liver diseases with clear aetiology such as viral, alcoholic or autoimmune, or presence of other well defined pathology known to cause acute liver injury were considered non-cases. We have not considered cases, those patients with an incidental laboratory finding (e.g. patients with abnormal laboratory tests, without specific code for liver disease or without symptomatology related to liver disease).

When possible and after manual review of the computerised patient profiles, the following classification scheme was used for a case of idiopathic acute liver injury:

- hepatocellular; when there was an increase more than twice the upper limit of the normal range in ALT alone or $R \geq 5$, where R is the ratio of serum activity of ALT over serum activity of AP
- cholestatic; when there was an increase of over twice the upper limit of the normal range in AP alone or $R \leq 2$.
- mixed; when $2 < R < 5$.

The liver injury was considered acute if the clinical or laboratory signs have completely disappeared within 6 months from the date of onset or if the patient died within 6 months of onset date.

3.8 Covariates and confounders

- Potential confounders were measured at baseline (start of study period) for the cohort analysis, and at index date (date of onset of liver injury for both cases and their matched) in the case control analysis.
- The crude estimates of risk were adjusted for age and sex in a first step. A further adjusted analysis was performed including, age sex, calendar year, consultation rate, concurrent medications and diagnoses of any underlying disease that may act as possible confounders (establish/independent risk factors for the adverse event). In addition, body mass index (BMI), smoking, alcohol were also considered as potential confounders.
- Specific possible confounders included prescription drugs associated with raised aminotransferase levels, such as statins, NSAIDS, paracetamol, and antidepressant including bupropion. Underlying diagnoses that may act as confounding factors include hemodynamic abnormalities, such as cardiovascular shock or heart failure, autoimmune disease, and genetic or metabolic disorders such as hemochromatosis or alpha1-antitrypsin deficiency. Co-morbidities were identified by the recording of ICD code in the patient's clinical or referral files, as will data on treatment with any concomitant medication.

4. Study Design

4.1 Case-control study

4.1.1 Cases and controls

All cases, detected within both study cohorts (exposed and non-exposed to antibiotics) with a first recorded occurrence of acute liver injury, identified by the algorithm described above (see case definition) during the follow-up period from January 2004 until December 2009 were identified. The date of diagnosis (acute liver injury) was considered the index date of the case. Separate analyses were done using only definite cases in a first step and definitive and probable cases in a second step.

Controls were sampled from the patients at risk at the time of occurrence of the case (incident or density sampling). Thus, for a given case, potential controls were all non-cases at the time of the occurrence of the event, including future cases. Controls can therefore be cases later on. Controls were matched to cases by age (within one year), sex, and calendar date (month & year). We selected up to five controls per case.

4.1.2 Exposure definition for the case-control analysis

For the main analysis, we defined patients as current users if a prescription for antibiotics lasts until the index date or ends within 14 days prior to the index date (i.e. date of onset of liver injury for both cases and their matched controls). We used a window of 30 days as a secondary definition of current use.

We defined patients as past users if the prescription ends between 14 and 365 days before the index date, and non-users, if there was no prescription in the year before the index date.

The association between the use of antibiotics and the experience of acute liver injury was estimated by comparing the odds of past and current users with the odds of non-users. Non-use of antibiotics was used as reference. We studied the effect of dose and duration of treatment among current users.

Duration of use was defined by the treatment period covering consecutive prescriptions. Prescriptions were considered consecutive when less than 14 days elapse between them.

We estimated the risk associated with different antibiotics drug class categorized in the following groups: Tetracyclines, penicillins, cephalosporins & betalactams, macrolides, quinolones and other antibiotics.

For sensitivity analysis, we defined patients as current users if a prescription for antibiotics last until the index date or ends within 30 days prior to the index date, as past users if the prescription ends between 30 and 365 days before the index date, and non-users, if there was no prescription in the year before the index date.

4.1.3 Statistical Analyses

We computed odds ratios (OR) and 95% confidence intervals of first occurrence of idiopathic acute liver injury (see outcome definition) associated with current use of antibiotics (as a group and different classes and individual drugs when possible) as compared to non-use with conditional logistic regression. Age, sex, calendar year, and other variables (see Covariates) will be introduced in the model to control for potential confounding. Also, dose and duration-relationships was examined. Separate analyses were done using only definite cases in a first step and definite and probable cases as a second step. Several strategies to select confounders were compared to assess the impact of the selection method on the results. For the analysis, the effect of core variables other than those used to match cases and controls was examined by constructing a series of bivariate models. Likelihood Ratio tests was used to compare models. We also fitted a full model, including all of the potential confounders.

We constructed different regression models, first including general confounders (age, sex, BMI, alcohol, smoking). And in a second step we also fitted a full model including all other potential confounders (comorbidities and drugs) and mean number of visits in the past year.

The statistical analysis plan was:

Descriptive statistics and distribution of antibiotics used were evaluated.

Total person-time of the observation period of the cohort was calculated and was used as the denominator for incidence rate

Incidence rates of acute liver injury was calculated per antibiotic class as mentioned above

Conditional logistic regression was used to estimate the odds ratio of ALI for different antibiotic class as mentioned above: for current user vs. non-user, past user vs. non-user.

4.2 Validation of cases

All cases, detected within the study population with a first recorded occurrence of acute liver injury, identified by the algorithm described above (see case definition) during the follow-up period from January 2004 until December 2009 were identified.

Among these cases, the ones from the study population for which a linkage was available between Clinformatics Data Mart and Premier' perspective were selected.

For each selected case, review of hospital records from premier's perspective allowed for ascertainment of definite and probable non-cases. The case status based on electronic hospital records from premier's perspective was used as the gold standard to evaluate the reliability/validity of the cases.

4.3 Sensitivity analysis

For the sensitivity analysis,

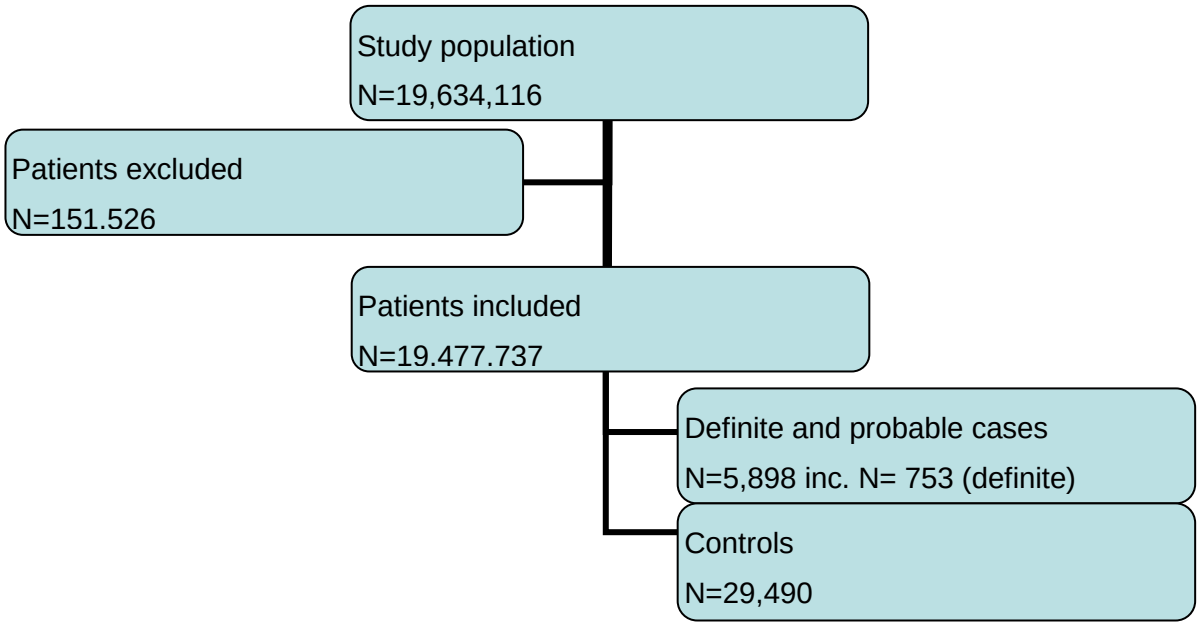
- the definition of current exposure 0-30 days was used
- in addition to the exclusion criteria used in the main analysis (liver cancer and liver metastasis, gallbladder disease, pancreatic disease, and other chronic liver diseases excluding chronic non-alcoholic liver disease) patients with alcoholism and alcohol related problems were also excluded from the analysis.

5. Results

5.1 Study population and antibiotics used

5.1.1 Patient flow

Figure 1: Patient flow



5.1.2 Characteristics of study participants

Table 1 shows baseline characteristics of the study participants. The proportion of the elderly aged 65 or older was 5.2%, which was lower than the proportion of the elderly in the general US population because. The reason for a lower proportion was because Clinformatics datamart was an employment-based insurance database with disproportionately higher proportion of employment-aged members.

Table 1: Baseline characteristics of the study participants (N = 19,477,737), Clinformatics data mart Database, (2004/01 - 2009/12)

Gender (%)	Men	49.7
Age (years) (%)	<18	24.6
	18-29	17.0
	30-49	34.8
	50-64	18.4
	>65	5.2
Comorbidities (%)	Heart failure	0.1
	Rheumatoid arthritis	0.3
	Hemochromatosis	0.0
	Alpha 1-antitrypsin deficiency	0.0
	Diabetes	3.8
Treatment (%)	NSAIDs	7.6
	Other analgesics/antipyretics	0.6
	Statins	6.4
	Antidepressants	8.6
	Oral contraceptives	5.8
	Oral preparation for acne	0.1
	DMARD	0.0
	Oral corticosteroids	4.3
	Antidiabetic drugs	3.3

5.1.3 Antibiotics used in study participants

The proportion of penicillins users was 15.0%, higher than any other single class, followed by macrolides at 10.6% (Table 2). A total 35.1% of participants used any antibiotics.

Table 2: Distribution of antibiotics used in study participants (N=19,477,737)

Antibiotic class	%
Tetracyclines	2.6
Penicillins	15.0
Cephalosporins	6.1
Quinolones	1.0
Macrolides	10.6
Others	0.1
Any antibiotics	35.1

5.1.4 Incidence of acute liver injury in study participants

The total person-time of the period of the observation of the cohort was 33209993.24 person-years. Among antibiotics users, the incidence rate of acute liver injury was 0.031 cases per 1000 person-years and 0.017 in non-antibiotic users.

Table 3: Incidence rate of acute liver injury (definite cases) in study participants, Clinformatics Datamart Database, (2004/01 – 2009/12)

	Antibiotics users	Non antibiotic users
Number of cases	505	256
Follow-up (years)	16,531,610	14,668,993
Incidence rate (per 1,000 PY)	0.031(0.028,0.023)	0.017(0.015,0.020)

5.2 Case-control analyses

5.2.1 Characteristics of cases and controls: Definite cases definition

Table 4: Characteristics of cases (N=753) and controls (n=3765), Clinformatics Datamart Database, (2004/01 – 2009/12)

		Cases N=753	Controls N=3765
Gender (%)	Men	51.5	51.5
Age at cohort entry date	mean (sd)	48.3 (13.4)	48.2 (13.3)
Number of visits			
Primary care physician	Mean (sd)	3.8 (5.3)	2.6 (3.4)
Specialist	Mean (sd)	7.0 (12.2)	3.5 (6.6)
Comorbidities (%)	Heart failure	2.3	0.3
	Rheumatoid arthritis	0.7	0.6
	Hemochromatosis	0.0	0.0
	Alpha1 anti trypsin deficiency	0.0	0.0
	Diabetes	13.4	7.8
Treatment (%)	NSAIDs	13.3	11.3
	Other analgesics/antipyretics	1.6	0.8
	Statins	10.9	15.9
	Antidepressant	22.0	14.9
	Oral contraceptives	2.7	4.0
	Oral preparation for acne	0.4	0.2

		Cases N=753	Controls N=3765
	DMARD Oral corticosteroids Antidiabetic drugs	0.0 7.2 12.7	0.1 6.9 6.8
Any antibiotics (%)	Current user (≤ 15) past user ([365,15) non user (>365)	16.6 49.0 42.1	7.3 38.9 56.9
Tetracyclines (%)	Current user (≤ 15) past user ([365,15) non user (>365)	0.9 4.6 94.6	0.8 4.2 95.2
Penicillins (%)	Current user (≤ 15) past user ([365,15) non user (>365)	6.8 22.3 73.3	2.4 16.4 82.1
Cephalosporins (%)	Current user (≤ 15) past user ([365,15) non user (>365)	3.7 13.8 82.9	1.1 8.9 90.3
Quinolones (%)	Current user (≤ 15) past user ([365,15) non user (>365)	0.9 2.3 96.8	0.5 1.1 98.4
Macrolides (%)	Current user (≤ 15) past user ([365,15) non user (>365)	4.9 21.2 75.3	2.7 16.7 81.4
Others (%)	Current user (≤ 15) past user ([365,15) non user (>365)	0.5 0.8 98.9	0.0 0.1 99.9

According to the definite definition, 753 cases were identified in the US Clinformatics Data Mart database between January 2004 and December 2009, and 3765 controls were selected and matched on age at the index date and sex.

The mean age at index date was 49.7 years (sd 13.4), and 48.5% were women. The characteristics of the definite cases and controls and the antibiotics they used are summarized in table 4.

5.2.2 Characteristics of cases and controls: all cases definition

According to the broad definition, 5519 cases were identified in the Clinformatics Database between January 2004 and December 2009, and 27 595 controls were selected and matched on age at index date and sex. The mean age at index date was 45.1 years (st 14.8), and 42.1% were women. The characteristics of the broad cases and controls and the antibiotics they used are summarized in table 5. For any antibiotics, we counted 9.8% currents users among the cases and 4.1% among the controls. The most antibiotic drug class used was penicillin.

Table 5: Characteristics of cases (N=5,898) and controls (n=29,490), Clinformatics Datamart Database, (2004/01 – 2009/12)

		Cases N=5,898	Controls N=29,490
Gender (%)	Men	57.9	57.9
Age at cohort entry date	mean (sd)	43.8 ± 14.8	43.8 ± 14.8
Comorbidities (%)	Heart failure	1.0	0.2
	Rheumatoid arthritis	1.3	0.6
	Hemochromatosis	0.0	0.0
	Alpha1 anti trypsin deficiency	0.0	0.0
	Diabetes	11.2	6.2
Treatment (%)	NSAIDs	13.8	10.2
	Other analgesics/antipyretics	1.2	0.6
	Statins	12.0	12.6
	Antidepressant	16.9	12.1
	Oral contraceptives	4.0	4.3
	Oral preparation for acne	0.2	0.1
	DMARD	0.2	0.1
	Oral corticosteroids	7.3	6.1
	Antidiabetic drugs	10.3	5.6
Any antibiotics (%)	Current user (≤ 15)	9.8	4.1
	past user ([365,15)	53.1	41.8
	non user (>365)	41.9	56.0
Tetracyclines (%)	Current user (≤ 15)	0.9	0.5
	past user ([365,15)	7.0	5.0
	non user (>365)	92.3	94.8
Penicillins (%)	Current user (≤ 15)	3.6	1.4
	past user ([365,15)	23.3	18.6

		Cases N=5,898	Controls N=29,490
	non user (>365)	74.2	80.5
Cephalosporins (%)	Current user (≤ 15) past user ([365,15) non user (>365)	1.9 13.2 85.3	0.7 9.8 89.7
Quinolones (%)	Current user (≤ 15) past user ([365,15) non user (>365)	0.6 2.1 97.3	0.1 1.4 98.6
Macrolides (%)	Current user (≤ 15) past user ([365,15) non user (>365)	3.2 23.2 74.7	1.5 17.3 81.6
Others (%)	Current user (≤ 15) past user ([365,15) non user (>365)	0.1 0.4 99.5	0.0 0.2 99.8

5.2.3 Adjusted Odds ratios per antibiotic class: main analysis on definite cases

Table 6 shows the ORs of liver injury (definite definition) any and for specific types of antibiotics. Compared to non-use as the reference, current and past use of any antibiotics was associated with liver injury with adjusted ORs of 4.43 (95% CI 3.20-6.14) and 1.41 (95% CI 1.18-1.69), respectively. Current and past use of penicilins, cephalosporins and macrolides were also associated with liver injury. Quinolones use also tended to be associated with liver injury, although the ORs were not significant and with wide confidence intervals.

Table 6: Results of case-control main analysis for definite cases, Clinformatics Datamart Database, (2004/01 – 2009/12)

Antibiotic class	Exposure prior to index date	Crude OR	Adjusted* OR
Any antibiotics	current user (]0,15[) past user ([365,15) non user (>365)	2.84 (2.20-3.66) 1.49 (1.26-1.77) 1	4.43 (3.20-6.14) 1.41 (1.18-1.69) 1
Tetracyclines	current user (]0,15[) past user ([365,15) non user (>365)	1.10 (0.48-2.55) 1.10 (0.75-1.61) 1	1.41 (0.48-4.11) 0.97 (0.66-1.43) 1
Penicillins	current user (]0,15[) past user ([365,15)	2.72 (1.89-3.90) 1.39 (1.14-1.69)	4.93 (3.02-8.06) 1.32 (1.07-1.62)

Antibiotic class	Exposure prior to index date	Crude OR	Adjusted* OR
	non user (>365)	1	1
Cephalosporins	current user (]0,15[)	3.64 (2.18-6.07)	3.60 (1.83-7.07)
	past user ([365,15)	1.65 (1.30-2.11)	1.51 (1.18-1.94)
	non user (>365)	1	1
Quinolones	current user (]0,15[)	2.11 (0.81–5.50)	3.03 (0.90-10.19)
	past user ([365,15)	2.33 (1.25-4.35)	1.83 (0.97-3.44)
	non user (>365)	1	1
Macrolides	current user (]0,15[)	1.87 (1.25-2.80)	3.06 (1.82-5.12)
	past user ([365,15)	1.33 (1.09-1.63)	1.24 (1.01-1.52)
	non user (>365)	1	1
Other	current user (]0,15[)	11.24 (1.10-114.94)	NA
	past user ([365,15)	10.61 (1.99-56.45)	6.22 (1.32-29.32)
	non user (>365)	1	1

* adjusted by co-morbidities: Heart failure, Rheumatoid arthritis, Diabetes; AND co-medications: Non-steroidal anti-inflammatory drug (NSAIDs), Other analgesics /antipyretics, Statins, Antidepressants , Oral contraceptives, Oral preparation for acne, Oral corticosteroids, Antidiabetic drugs, Number of visit to primary care, Number of visit to specialist care

5.2.4 Adjusted Odds ratios per antibiotic class: analysis on all cases (broad definition)

As in the case of definite definition, liver injury using broad definition was also associated with current and past use of any antibiotics, penicillins, cephalosporins and macrolides, using non-use as the reference (Table 7). It was also associated with penicilins, cephalosporins, macrolides, tetracyclines, quinolones and “other”. Different from definite case, the associations with liver injury broad definition were significant (except for current use of “other”), most likely because of a larger number of cases resulting in narrower confidence intervals.

Table 7: Results of case-control main analysis for all cases (broad definition, Clinformatics Datamart Database, (2004/01 – 2009/12)

Antibiotic class	Exposure prior to index date	Crude OR	Adjusted* OR
Any antibiotics	current user (]0,15[)	2.66 (2.38-2.98)	2.57 (2.30-2.89)
	past user ([365,15)	1.59 (1.50-1.69)	1.52 (1.43-1.61)
	non user (>365)	1	1

Antibiotic class	Exposure prior to index date	Crude OR	Adjusted* OR
Tetracyclines	current user (]0,15[)	1.67 (1.21-2.30)	1.59 (1.15-2.20)
	past user ([365,15)	1.39 (1.24-1.56)	1.34 (1.19-1.50)
	non user (>365)	1	1
Penicillins	current user (]0,15[)	2.60 (2.18-3.09)	2.49 (2.09-2.97)
	past user ([365,15)	1.32 (1.23-1.41)	1.26 (1.17-1.35)
	non user (>365)	1	1
Cephalosporins	current user (]0,15[)	2.74 (2.17-3.46)	2.62 (2.06-3.32)
	past user ([365,15)	1.39 (1.28-1.52)	1.29 (1.18-1.41)
	non user (>365)	1	1
Quinolones	current user (]0,15[)	6.95 (4.11-11.77)	6.86 (4.03-11.70)
	past user ([365,15)	1.67 (1.33-2.10)	1.58 (1.25-2.00)
	non user (>365)	1	1
Macrolides	current user (]0,15[)	2.11 (1.77-2.53)	2.06 (1.72-2.47)
	past user ([365,15)	1.43 (1.33-1.53)	1.37 (1.27-1.47)
	non user (>365)	1	1
Other	current user (]0,15[)	4.40 (1.08-17.92)	3.70 (0.88-15.62)
	past user ([365,15)	2.80 (1.72-4.54)	2.76 (1.68-4.54)
	non user (>365)	1	1

* adjusted by co-morbidities: Heart failure, Rheumatoid arthritis, Diabetes; AND co-medications: Non-steroidal anti-inflammatory drug (NSAIDs), Other analgesics /antipyretics, Statins, Antidepressants , Oral contraceptives, Oral preparation for acne, Oral corticosteroids, Antidiabetic drugs

5.3 Sensitivity analysis

5.3.1 Adjusted Odds ratios per antibiotic class with the 30 days definition of exposure

Tables 8 and 9 show the ORs of liver injury for antibiotics using a different window of exposure, 0 – 30 days for current use, for definite and broad definitions, respectively. Except for tetracyclines, use of any antibiotics or specific classes of antibiotics were associated with liver injury, definite definition, although for current use of quinolones the association was not significant.

Table 8: Results of case-control sensitivity analysis with 30 days definition of exposure for definite cases, Clinformatics Datamart Database, (2004/01 – 2009/12)

Antibiotic class	Exposure prior to index date	Crude OR	Adjusted* OR
Any antibiotics	current user (]0,30[)	2.84 (2.20-3.66)	2.83 (2.18-3.69)
	past user ([365,30)	1.49 (1.26-1.77)	1.48 (1.24-1.76)
	non user (>365)	1	1
Tetracyclines	current user (]0,30[)	1.10 (0.48-2.55)	1.05 (0.44-2.53)
	past user ([365,30)	1.10 (0.75-1.61)	0.99 (0.67-1.47)
	non user (>365)	1	1
Penicillins	current user (]0,30[)	2.72 (1.89-3.90)	2.73 (1.88-3.97)
	past user ([365,30)	1.39 (1.14-1.69)	1.36 (1.11-1.68)
	non user (>365)	1	1
Cephalosporins	current user (]0,30[)	3.64 (2.18-6.07)	3.61 (2.13-6.12)
	past user ([365,30)	1.65 (1.30-2.11)	1.55 (1.20-1.99)
	non user (>365)	1	1
Quinolones	current user (]0,30[)	2.11 (0.81-5.50)	2.38 (0.90-6.29)
	past user ([365,30)	2.33 (1.25-4.35)	2.37 (1.24-4.52)
	non user (>365)	1	1
Macrolides	current user (]0,30[)	1.87 (1.25-2.80)	1.81 (1.20-2.75)
	past user ([365,30)	1.33 (1.09-1.63)	1.33 (1.08-1.63)
	non user (>365)	1	1
Other	current user (]0,30[)	11.24 (1.10-114.94)	16.71 (1.55-179.80)
	past user ([365,30)	10.61 (1.99-56.45)	11.02 (1.98-61.45)
	non user (>365)	1	1

* adjusted by co-morbidities: Heart failure, Rheumatoid arthritis, Diabetes; AND co-medications: Non-steroidal anti-inflammatory drug (NSAIDs),Other analgesics

/antipyretics, Statins, Antidepressants , Oral contraceptives, Oral preparation for acne, Oral corticosteroids, Antidiabetic drugs

Table 9: Results of case-control sensitivity analysis with 30 days definition of exposure for all cases (broad definition, Clinformatics Datamart Database, (2004/01 – 2009/12)

Antibiotic class	Exposure prior to index date	Crude OR	Adjusted* OR
Any antibiotics	current user (]0,30[)	2.33 (2.13-2.55)	2.25 (2.06-2.47)
	past user ([365,30)	1.51 (1.42-1.60)	1.44 (1.35-1.53)
	non user (>365)	1	1
Tetracyclines	current user (]0,30[)	1.31 (1.02-1.68)	1.27 (0.99-1.64)
	past user ([365,30)	1.41 (1.25-1.59)	1.35 (1.20-1.53)
	non user (>365)	1	1
Penicillins	current user (]0,30[)	2.12 (1.86-2.42)	2.03 (1.78-2.32)
	past user ([365,30)	1.27 (1.18-1.36)	1.21 (1.13-1.30)
	non user (>365)	1	1
Cephalosporins	current user (]0,15[)	2.37 (1.98-2.84)	2.25 (1.87-2.70)
	past user ([365,15)	1.34 (1.22-1.46)	1.24 (1.13-1.36)
	non user (>365)	1	1
Quinolones	current user (]0,30[)	3.98 (2.62-6.05)	3.78 (2.47-5.78)
	past user ([365,30)	1.74 (1.37-2.20)	1.65 (1.30-2.10)
	non user (>365)	1	1
Macrolides	current user (]0,30[)	2.05 (1.79-2.36)	1.97 (1.71-2.27)
	past user ([365,30)	1.37 (1.28-1.47)	1.31 (1.22-1.41)
	non user (>365)	1	1
Other	current user (]0,30[)	3.68 (1.41-9.60)	3.52 (1.33-9.31)
	past user ([365,30)	2.49 (1.48-4.18)	2.47 (1.46-4.20)
	non user (>365)	1	1

* adjusted by co-morbidities: Heart failure, Rheumatoid arthritis, Diabetes; AND co-medications: Non-steroidal anti-inflammatory drug (NSAIDs), Other analgesics /antipyretics, Statins, Antidepressants , Oral contraceptives, Oral preparation for acne, Oral corticosteroids, Antidiabetic drugs

5.3.2 Sensitivity analysis with different exclusion criteria

Table 10: Exclusion criteria

	Main analysis	Sensitivity analysis
Before exclusion	19 634 116	19 634 116
Exclusion criteria :		
History of liver injury (as outcome defined)	519	519
Liver cancer and liver metastasis	9 256	9 265
Gallbladder diseases/ Cholelithiasis/ cholecystitis/ Cholangitis	303	303
Diseases of pancreas / pancreatitis/ other diseases of pancreas	56	56
Other chronic liver disease (including autoimmune hepatitis)	147 378	148 503
Alcoholism/alcohol abuse/alcohol related disease	0	10 566
After exclusion	19 477 737	19 471 470

Table 11: Results of case-control sensitivity analysis on exclusion criteria for definite cases, Clinformatics Datamart Database, (2004/01 – 2009/12)

Antibiotic class	Exposure prior to index date	Crude OR	Adjusted* OR
Any antibiotics	current user (]0,15[)	4.42 (3.24-6.02)	4.49 (3.27-6.17)
	past user ([365,15)	1.54 (1.30-1.82)	1.51 (1.26-1.80)
	non user (>365)	1	1
Tetracyclines	current user (]0,15[)	1.85 (0.72 -4.74)	1.50 (0.56-4.03)
	past user ([365,15)	1.06 (0.73-1.53)	0.97 (0.66-1.42)
	non user (>365)	1	1

Antibiotic class	Exposure prior to index date	Crude OR	Adjusted* OR
Penicillins	current user (]0,15[)	5.09 (3.21-8.05)	5.19 (3.23-8.36)
	past user ([365,15)	1.37 (1.13-1.67)	1.35 (1.10-1.65)
	non user (>365)	1	1
Cephalosporins	current user (]0,15[)	3.57 (1.90-6.71)	3.57 (1.86-6.86)
	past user ([365,15)	1.79 (1.42-2.26)	1.68 (1.32-2.14)
	non user (>365)	1	1
Quinolones	current user (]0,15[)	2.37 (0.73-7.75)	2.71 (0.81-9.07)
	past user ([365,15)	2.10 (1.18-3.76)	2.15 (1.18-3.91)
	non user (>365)	1	1
Macrolides	current user (]0,15[)	2.83 (1.73-4.63)	2.88 (1.74-4.78)
	past user ([365,15)	1.33 (1.10-1.62)	1.31 (1.07-1.60)
	non user (>365)	1	1
Other	current user (]0,15[)	NA	NA
	past user ([365,15)	8.28 (1.98-34.65)	8.85 (2.01-38.91)
	non user (>365)	1	1

* adjusted by co-morbidities: Heart failure, Rheumatoid arthritis, Diabetes; AND co-medications: Non-steroidal anti-inflammatory drug (NSAIDs) ,Other analgesics /antipyretics, Statins, Antidepressants , Oral contraceptives, Oral preparation for acne, Oral corticosteroids, Antidiabetic drugs

Table 10 shows number of patients with alcoholism/alcohol abuse/alcohol related disease excluded in the sensitivity analysis. Table 11 shows the ORs after excluding these patients. In this sensitivity analysis excluding patients with known history of alcohol abuse, the results of adjusted odds ratios are very close to the odds ratios obtained in the main analysis.

5.4 Validation of cases

5.4.1 Definite cases definition

Among the definite cases, 222 cases were present both in Clinformatics Datamart and Premier's perspective. Among these cases, 197 were considered as cases according to the premier's database, and 25 patients were considered as non-cases.

The predictive positive value was 88.72%.

Table 12: Validation table for definite cases

		Premier (2005-2009)		
		yes	no	total
Clinformatics datamart (2004-2009)	yes	197	25	222
	no	3698	191 312	195 010
	total	3895	191 337	195 232

5.3.2 All cases definition

Among all cases, 460 cases were present both in Clinformatics Datamart and Premier's perspective. Among these cases, 215 were confirmed as cases according to the premier's database and 245 were considered as non cases. The positive predictive value was 46.74%.

Table 13: Validation table for all cases (broad definition)

		Premier (2005-2009)		
		yes	no	total
Clinformatics datamart (2004-2009)	yes	215	245	460
	no	3645	190 883	194 528
	total	3860	191 128	194 988

7. Possible limitations

Liver disorders are unlikely to be recorded in a systematic way, and we have to rely on diagnoses entered and codes used that could vary between physicians, and therefore could lead to potential misdiagnosis.

We also have to take into account that the outcome we are studying (acute idiopathic liver injury) is not a diagnosis made by physicians, in comparison to, for instance, cancer or hip fracture. It is an entity when certain conditions, symptoms and test results are present. And these criteria are variable depending on the type of studies or the researchers' operational definition. Results from the feasibility counts gave estimates of "incidence rates of codes of unspecified liver disease" close to 100 times (196- 400 per 100000 p-y) higher than the reported for acute liver injury in clinical and observational studies (using a detailed, laborious and specific case ascertainment). This suggests that a more specific case ascertainment strategy for the outcome of acute liver injury is needed. It should be acknowledged that none of the database would be able to assess with 100% sensitivity/specificity the diagnosis of liver injury. Information on hospitalization and/or referrals may be not complete in the database and the linkage to hospital data available in a sample of patients may help to improve validity of identified cases in the database.

Patients with acute liver injury are defined as patients with clinical and/or laboratory signs that have completely disappeared within 6 months from the date of onset. As it may be difficult to distinguish between a) on-going disease and b) unrecorded resolution of disease, we may not be able to classify all cases accordingly. Review of hospital records will be used to help distinguish between cases with acute versus chronic liver injury.

Patients with an indication for antibiotic agents may have a different underlying risk profile for idiopathic acute liver injury that could wrongfully lead to attributing a changed risk of idiopathic acute liver injury to the use of antibiotics. When the patients taking antibiotics are not as healthy as those not receiving antibiotics (other than suffering from a bacterial infection), this could lead to an overestimation of risk. We will adjust for some of the underlying difference in risk (see covariates). Residual and uncontrolled confounding may still occur.

Over-the counter antibiotic use is not expected to be a major source of misclassification, since medical prescription is required for antibiotics. But we must consider the lack of systematic recording of concomitant use of over-the-counter medicine, such as acetaminophen, as this may be another source of misclassification, wrongfully leading to attributing a changed risk of idiopathic acute liver injury to the use of antibiotic agents.

We must also considered that prescription made outside the outpatient practice setting could be missed, as well as prescription not collected and finally not used by the patient. This is less probable for medications used for non-chronic medical conditions, like antibiotics.

An important limitation in studies using existing databases is that some information may be incomplete or not available. Data on life style factors (weight, height,

smoking or alcohol consumption) as well as data on socioeconomic leve and not captured in the database used in this study

8. Conclusion

The main result observed in this study showed an association between antibiotics use and acute liver injury. The replication of the nested case-control design in the US claims database Clinformatics datamart provided the estimation of the risk of acute liver injury associated with antibiotics exposure. Through use of different definitions of current exposure, these results showed that a larger window in the definition of exposure lead to a decrease in the magnitude of the association. The magnitude of the association betweenacute liver injury with various antibiotics classes was different exposure definition of 15 and 30 days. For the tetracyclines, penicillins, macrolids, and quinolones the odds ratio was higher when the exposure definition was 15 days, and for the cephalosporins the odds ratio was higher when the exposure definition was 30 days. These results showed that the use of narrow definition of cases compared to broad definition lead to an increase in the association level. The addition of exclusion criteria for patients with known history of alcohol abuse did not have an impact on the results.

The validation of cases of liver injury using information from patient's hospital records showed that the predictive positive value of cases with narrow-specific definition was lower than that of cases with the broad definition.

In conclusion, within the framework of PROTECT Work package 6 "validation on methods involving an extended audience" we tested the feasibility of methods developed in WP2.

9. APPENDICES

Table 1A: Specific ICD9 codes for idiopathic liver injury

DISEASES	
570	Acute and subacute necrosis of liver Acute hepatic failure Acute or subacute hepatitis, not specified as infective Necrosis of liver (acute) (diffuse) (massive) (subacute) Parenchymatous degeneration of liver Yellow atrophy (liver) (acute) (subacute) Excludes: icterus gravis of newborn (773.0-773.2) serum hepatitis (070.2-070.3) that with: abortion (634-638 with .7, 639.8) ectopic or molar pregnancy (639.8) pregnancy, childbirth, or the puerperium (646.7) viral hepatitis (070.0-070.9)
572.2	Hepatic coma Hepatic encephalopathy Hepatocerebral intoxication Portal-systemic encephalopathy Excludes: Hepatic coma associated with viral hepatitis
572.4	Hepatorenal syndrome Excludes: that following delivery (674.8)
573.3	Hepatitis, unspecified Toxic (non-infectious) hepatitis Use additional E code to identifiy cause
573.9	Unspecified disorder of liver

SYMPTOMS	
782.4	Jaundice, unspecified, not of newborn Cholemia NOS Icterus NOS Excludes: Jaundice in newborn (774.0-774.7) Due to immunization (773.0-773.2, 773.4)
789.1	Hepatomegaly Enlargement of liver
789.59	Other ascites
791.4	Biliuria
PROCEDURES	
50.5	Liver transplant 50.5 Liver transplant 50.51 Auxiliary liver transplant Auxiliary hepatic transplantation leaving patient's own liver in situ 50.59 Other transplant of liver

Table 1B: Non specific ICD9 codes for idiopathic liver injury

790.4	Nonspecific elevation of levels of transaminase or lactic acid dehydrogenase [LDH]
790.5	Other nonspecific abnormal serum enzyme levels Abnormal serum level of: Acid phosphatise Alkaline phosphatise Amylase Lipase Excludes: Deficiency of circulating enzymes (277.6)
794.8	Abnormal results of liver function Abnormal liver scan

50.1	Diagnostic procedures on liver 50.11 Closed (percutaneous) [needle] biopsy of liver Diagnostic aspiration of liver 50.12 Open biopsy of liver Wedge biopsy 50.13 Transjugular liver biopsy Transvenous liver biopsy Excludes: closed (percutaneous) [needle] biopsy of liver (50.11) laparoscopic liver biopsy (50.14) 50.14 Laparoscopic liver biopsy Excludes: closed (percutaneous) [needle] biopsy of liver (50.11) open biopsy of liver (50.12) transjugular liver biopsy (50.13) 50.19 Other diagnostic procedures on liver Excludes: laparoscopic liver biopsy (50.14) liver scan and radioisotope function study (92.02) microscopic examination of specimen from liver (91.01-91.09) transjugular liver biopsy (50.13)
50.91	Percutaneous aspiration of liver Excludes: Percutaneous biopsy (50.11)

Table 2: Specific ICD9 codes for exclusion criteria for outcome definition

Liver cancer and liver metastasis	
155	Malignant neoplasm of liver and intrahepatic bile ducts 155.0 Liver, primary 155.1 Intrahepatic bile ducts 155.2 Liver, not specified as primary or secondary
197.7	Secondary malignant neoplasm of liver, specified as secondary
Gallbladder diseases/ Cholelithiasis/ cholecystitis/ Cholangitis	
574	Cholelithiasis
575	Other diseases of gallbladder
576	Other diseases of biliary tract
Diseases of pancreas / pancreatitis/ other diseases of pancreas	
577	Diseases of pancreas
Other chronic liver disease (including autoimmune hepatitis)	
571.4	Chronic hepatitis 571.40 chronic hepatitis, unspecified 571.41 chronic persistent hepatitis 571.42 autoimmune hepatitis 571.49 other Chronic hepatitis: active aggressive Recurrent hepatitis
070 (070.0-0.70.9)	Viral hepatitis (acute) (chronic)
571.8	Other chronic non-alcoholic liver disease Chronic yellow atrophy (liver) Fatty liver, without mention of alcohol
571.9	Unspecified chronic liver disease without mention of alcohol

Table 3: Specific ICD9 codes for exclusion criteria for sensitivity analysis

Liver cancer and liver metastasis	
155	Malignant neoplasm of liver and intrahepatic bile ducts 155.0 Liver, primary 155.1 Intrahepatic bile ducts 155.2 Liver, not specified as primary or secondary
197.7	Secondary malignant neoplasm of liver, specified as secondary
Alcoholism / alcohol abuse / alcohol related disease	
571	Alcoholic fatty liver 571.1 Acute alcoholic hepatitis Acute alcoholic liver disease 571.2 Alcoholic cirrhosis of liver Florid cirrhosis Laennec's cirrhosis (alcoholic) 571.3 Alcoholic liver damage, unspecified
291	Alcohol induced mental disorders
303	Alcohol dependence syndrome
305	Alcohol abuse
Gallbladder diseases/ Cholelithiasis/ cholecystitis/ Cholangitis	
574	Cholelithiasis
575	Other diseases of gallbladder
576	Other diseases of biliary tract
Diseases of pancreas / pancreatitis/ other diseases of pancreas	
577	Diseases of pancreas
Other chronic liver disease (including autoimmune hepatitis)	
571.4	Chronic hepatitis 571.40 chronic hepatitis, unspecified 571.41 chronic persistent hepatitis 571.42 autoimmune hepatitis 571.49 other, Chronic hepatitis: active, aggressive Recurrent hepatitis
070 (070.0-070.9)	Viral hepatitis (acute) (chronic)
571.9	Unspecified chronic liver disease without mention of

	alcohol