WP2 Framework for pharmacoepidemiological studies
WG1 Databases

Study Protocol

The risk of acute liver injury associated with the use of antibiotics.
A methodological comparison across epidemiological data sources

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WG1 Drug AE group

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Contents

1. Context of the studies

The studies described in this protocol are all performed within the framework of PROTECT (Pharmacoepidemiological Research on Outcomes of Therapeutics by a European ConsorTium) Work Package 2 and Working Group 1. The primary aim of these studies is to develop, test and disseminate methodological standards for the design, conduct and analysis of Pharmacoepidemiological (PE) studies applicable to different safety issues and using different data sources. To achieve this, results from PE studies on 5 key adverse events (AEs) performed in different databases will be evaluated. Therefore, emphasis will be on the methodological aspects of the studies in this protocol and not on the clinical consequences of studying the association under investigation.

2. Background

Acute liver injury is one of the most important safety concerns, being the leading cause for drug withdrawal from the market on safety grounds (1). For most suspected hepatotoxic drugs the only existing information comes from spontaneous reports, lacking appropriate risk quantification through formal epidemiological studies (2). A few population-based studies examining the risk of acute and clinically relevant liver injury among users of various drugs have been published, reporting an elevated risk of liver injury in users of antibiotics (3-6). As acute liver injury is often idiosyncratic and because its diagnostic criteria used in epidemiological studies have been variable, the reported range of incidences of acute liver injury caused by antibiotics is broad. In the UK, case-control studies investigating the effect of antibiotics on acute liver injury have generated odds ratios ranging from 94.8 for the combination of amoxicillin/clavulanic acid to 6.2 for tetracyclines (3). Age, sex, alcohol intake, concomitant medication and comorbidities have been proposed as risk factors for antibiotic induced liver injury and may have influenced the quantification of risk estimates (3, 5, 7). In the present protocol, we propose to further quantify the risk of acute liver injury associated with antibiotics in the general population using different study designs and in different primary care databases, and to compare the results to evaluate the impact of design and population differences on the outcome of the study association.

Acute liver injury or hepatotoxicity in this study implies chemical, drug driven liver damage which can be classified based on clinical presentation and laboratory features ranging from asymptomatic mild biochemical abnormalities to acute liver failure. The most common classification used for drug induced liver injury (DILI) is according to laboratory abnormalities (hepatocellular, cholestatic or mixed) and according to mechanism of toxicity (direct, immune-mediated, idiosyncratic, or mitochondrial toxicity) (8). Being idiosyncratic in most cases, reactions often cannot be reproduced experimentally in laboratory animals (9-10). The relationship between the dose and the occurrence or severity of the reaction is not constant, and the latent period between drug exposure and sensitivity reaction is rather variable. The infrequency of DILI, though with significant impact, and complicated case ascertainment in pharmaco-epidemiological studies has led to wide ranges of reported incidence rates. A recent study, using data from the GPRD database, reported crude incidence rates of liver injury caused by any type of drug ranging from 1 to 18 per 100,000 prescriptions (3). The Drug-Induced Liver Injury Network (DILIN), a US based collaboration between academic and health institutions to study the aetiology and prevention of DILI, found antibiotics to be the largest class of agents to cause drug-induced liver injury (4). UK based estimates of incidence rates of antibiotic induced liver injury range from 2.5 to 8.6 per 100,000 users (3).

Antibiotics are a type of antimicrobial used to treat infections and are amongst the twenty most prescribed drugs in England, with approximately 38.7 million prescriptions dispensed in 2009 (11). The most frequently prescribed type of antibiotics is penicillins, a group of bactericidal antibiotics that interfere with bacterial cell wall synthesis (12). Other bactericidal antibiotics include cephalosporins and aminoglycosides. Antibiotics with bacteriostatic mechanisms of action, inhibiting the growth or proliferation of bacterial cells, include tetracyclines, macrolides, sulphonamides and quinolones (12). Most types of antibiotics have been associated with drug-induced liver injury (13-17).

Liver injury accounts for 10% of all adverse reactions to drugs and is the most frequent reason for withdrawal of medications from the market (18). This study would provide a valuable contribution to our current knowledge as drug induced liver injury is the most common cause of acute liver failure and antibiotics are the largest drug class of agents, with the highest exposure prevalence, to cause acute liver injury.

3. Objectives

We propose to assess the association between antibiotics use and idiopathic acute liver injury with different study designs (descriptive, cohort, nested case-control and case crossover) across different primary care databases (GPRD, BIFAP, THIN) and to compare the results between databases, across designs to evaluate the impact of design/database/population differences on the outcome of the studied association.

Specific aims (in each database):

1. To describe characteristics, clinical features, and risk factors for acute liver injury in patients exposed and unexposed to antibiotics.
2. To estimate the overall risk of acute liver injury associated with antibiotics exposure (users and non-users) in each database
3. To estimate the risk of acute liver injury associated with various antibiotics classes
4. To estimate the risk of acute liver injury associated with specific individual antibiotics
5. To assess the effect of dose and duration of use for specific individual antibiotics.
6. To compare the results of a case-control study with the results of a retrospective cohort study and self-controlled case series study in the different databases (GPRD, BIFAP and THIN).

4. Methods

4.1. Data Source

The proposed studies will be conducted in populations from the databases decribed below. Note that the information from these databases was collected for another purpose than research, but is being utilized to fulfill the objectives of this study.

4.1.1. General Practice Research Database (UK)

The UK General Practice Research Database (GPRD) is the largest on-going health care database available in the UK since 1987 (19). The database contains more than 5 million active patient data with data provided by primary care centres (more than 600 practices) based throughout the United Kingdom. Only those practices that meet quality standards are then used for research (about 10% of the practices that send data to GPRD do not meet the quality standards). Furthermore, validation

studies are conducted regularly by comparing GPRD data to written notes of general practitioners. The data covers 8.3 % of the population. Among recent additions to the database include external record linkage to other National Health Services (NHS) datasets, increased availability of free text information format via new automated system, the possibility of genetic linkage studies, prospective data collections such as questionnaires, copies of patient–based correspondence, the conduct of multi-country studies, and performing randomization studies within the database.

4.1.2. The Health Improvement Network (THIN)

The Health Improvement Network (THIN) is a collaboration between two companies, In Practice Systems Ltd. (INPS), developer of Vision software used by GPs in the UK, and EPIC, provider of access to data for use in medical research (21). THIN data are collected during routine practice and regularly delivered to THIN. THIN data collection started in 2003, currently contains the electronic medical records of almost 8 million patients (more than 3 million active patients) collected from over 386 general practices in the UK covering more than 5.7% of the population in the UK (21). Patient data are arranged in four standardised (Patient, Medical, Therapy and Additional Health Data and one linked (postcode variable indicators) files per practice. Further information is possible to obtain via the Additional Information Service (AIS) including: questionnaires completed anonymously by the patient or GP, copies of patient-based correspondence, a specified intervention (e.g. a laboratory test to confirm diagnosis) and death certificates.

4.1.3. BIFAP (Spain)

BIFAP (Base de datos Informatizada para estudios Farmacoepidemiologicos en Atencion Primaria – A computerised database of medical records of Primary Care) is a non-profit research project operated by the Spanish Medicines Agency (AEMPS), a public agency belonging to the Spanish Department of Health, with the collaboration of the Spanish Centre for Pharmacoepidemiological Research (CEIFE)(20). The project has started in 2003 having the goal to achieve a pool of collaborators in the range of 1000 general practitioners and paediatricians. Currently, 1190 physicians (995 GPs and 195 paediatricians) from 9 different autonomous communities in Spain collaborate with BIFAP and send their data to BIFAP every 6 months. BIFAP database includes clinical and prescription data from around 3.1 million patients covering around 6.8% of Spanish population. The AEMPS has renewed its funding to BIFAP for project consolidation, for validation of information included in the databases, in addition to performing epidemiological studies.

4.1.4. Bavarian Claims Database (Germany)

The Bavarian statutory health insurance physicians' association is based on accounting information of the Bavarian physicians. This German database includes a population-based data on diagnosis and medical services, covering 10.5 million people. It is a pharmacy (claims) database linked to outpatient treatment data through general practitioners and specialists. The database exists since 2001 and covers 84% of the Bavarian population excluding those with private insurance. A population-based study on asthma treatment resistance is done using this database(27).

4.1.5. Mondriaan (The Netherlands)

The Dutch Mondriaan project is a private-public collaboration funded by the Dutch TOP Institute Pharma. Under the umbrella of Mondriaan, the participating databases currently include: the Dutch

General Practitioner (LINH) database, The Almere Health Care (ZGA) database, The General Practitioners of Utrecht (HNU) database and The Leidsche Rijn Julius Health Centre (LRJG) database. The cumulative number of persons having data in Mondriaan reached around 1.4 million comprising mainly of general practitioner (GP) data complemented by pharmacy dispensing data and linkages to survey data. The four databases within Mondriaan have different starting dates and scope of data. LINH is the Netherlands Information Network of General Practice and it holds a longitudinal data on morbidity, prescription, and referrals. The GPs record data on all patient contacts, including diagnoses, referrals and prescriptions. The ZGA is a GP and pharmacy database. The HNU is a GP database set up in 1995 and includes data dating till the end of 2005. The LRJG is a GP database with a linkage to additional survey records. Survey information is periodically up-dated through follow-up, including information on a wide range of health and lifestyle related variables.

4.1.6. National Databases (Denmark)

The Danish registries include computerized medical records of general practitioners and all hospital contacts, medication use, and causes of death for the entire population (5.5 million inhabitants). The National Bureau of Statistics keeps computerized records of income, degree of education, working status, and civil status. The Ministry of Interior keeps records of all inhabitants and their migrations and date of birth and death. The information on outcomes will come from the National Hospital Discharge Register. The National Hospital Discharge Register was founded in 1977. It covers all inpatient contacts from 1977 to 1994 and from 1995 also all outpatient visits to hospitals, outpatient clinics, and emergency rooms. Upon discharge, the physician codes the reason for the contact using the ICD system. The code used is at the discretion of the individual physician. The register has a nationwide coverage and an almost 100% capture of contacts. In general, the validity of registrations is high. The National Health Service keeps a register of all contacts to general practitioners for reimbursement purposes. The register does not contain ICD codes for the contacts but codes for the nature of the contact (regular check-up visit, routine vaccination in children).

The Danish Medicines Agency keeps a nationwide register of all drugs sold at pharmacies throughout the country from 1996 onward (National Pharmacological Database run by the Danish Medicines Agency). Any drug bought is registered with ATC code, dosage sold, and date of sale for the period January 1, 1996, to December 31, 2009. As all sales are registered to the individual who redeemed the prescription, the capture and validity are high.

All registers can be linked through the use of a person specific code (the civil person number) given to all inhabitants, and used for all of the registrations mentioned before. The validity of fracture reports in general is high (around 97%, although differences may exist between different fracture types).

4.2. Period of valid data collection

Each data source (GPRD, THIN, BIFAP) has a period of valid data collection, from the left censoring date, up to the right censoring date. This is defined as follows:

The left censoring date is the latest of the following: the date that a practice became up to research standard, the date that a patient enrolled into a practice or the date that a practice was enrolled into the database, whichever came last. The right censoring date is the earliest of the following: the date a patient died, the date a patient was transferred out of the practice, the end of the database's data collection, or the date that the practice left the database. Death may not be always well recorded in

the Spanish data (BIFAP); alternatively we may consider to censor a patient to the right in these databases on the patients' latest recorded event date or the date that a practice left the database, whichever came first.

4.3. Study period

The study period will start in January 2004 and end in December 2009. Information on the use of antibiotics and occurrence of acute liver injury will be obtained from individual databases comprising of medical records of GPs and/or claims data where prescription and diagnosis data are recorded.

4.4. Source population

The primary study population will be 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. Patients must have attained one year of enrolment with the GP and one year of computerized prescription history

4.5. Study population

From the aforementioned source population, two study cohorts will be selected (see figure 1).
The first cohort will include 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) defines the start of follow-up (start date), for the exposed cohort.
The second cohort will be 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 their entry date (date when the patient meet all the eligibility criteria and enter in the study contribution). For these patients we will assign a random date during the study period. For this cohort of non-users the random date generated after meeting the eligibility criteria will be used as the start of follow-up (start date).
All subjects from the study population with one of the codes listed in table 1a,b, c (outcome definition) or one of the diagnoses included in table 2 (exclusion criteria: cancer, alcoholism, alcohol related problems, gallbladder disease, pancreatic disease, and other chronic liver diseases not included in outcome definition) prior to start date will be excluded.

4.6. Outcome ascertainment

All patients from the study population will be followed from the start date (date of first antibiotic prescription or random date in the comparison cohort until the first occurrence of one of the following endpoints: a code from tables 1a, b, c (outcome), death or end of the study period. Patients will be censored when a code for one of the exclusion criteria is recorded during the follow up (cancer, alcoholism, alcohol related problems, gallbladder disease, pancreatic disease, and other chronic liver diseases with clear aetiology such as viral, alcoholic, autoimmune)
We will ascertain patients with the first recorded occurrence of idiopathic acute liver injury (outcome) and the date of diagnosis will be their index date (outcome date). For these patients the computerized patient profiles and free text comments (available only in BIFAP) will be reviewed individually. Personal identifiers will be suppressed and information on drug exposure will be removed to allow for a blinded review by the investigators. All subjects identified as potential cases

through this initial computer search who have a code of pregnancy within two months of the index date will not be included.

4.7. Outcome definition

The outcome for this study is the first recorded occurrence of idiopathic acute liver injury. To initially identify cases, we used a list of codes (tables 1a,1b,1c) some of them are specific of liver disease or sumptoms (e.g. Hepatitis , Acute Hepatic Failure, Ictericia) and others are not specific (e.g: Liver Function Tests Abnormal, Increased Transaminasas). Patients will be 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 and the patient presents with 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 tables 1a, 1b, 1c) 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 tables 1a, 1b, 1c.

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 definite case are missing, applying different algorithms as follows:

Probable case 1: Patients identified with a specific READ or ICPC code of liver disease 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 READ or ICPC code of liver disease with complete laboratory criteria for acute liver injury B or C, and with a related visit to a specialist or hospitalization.

We will manually review available information of subgroups of probable cases, in BIBAP database in order to confirm their final status. In GPRD only a sample of these probable cases will be reviewed.

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 will be considered non-cases. We will not consider 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).

In BIFAP database a review of the free text comments will be conducted for all identified definite and probable cases, as well as for the group of non-cases with incidental laboratory findings. In GPRD this

review will be only done for a random sample of definite and probable cases. Information from free-text comments in BIFAP will help to better define the cases according to our operational definition, and for confirmation as part of the case ascertainment process. In GPRD, free text review will be used to evaluate the impact of this issue on the case status assignment, as this extra information will only be available for a random sample of patients. In GPRD and where available, a review of Hospital Episode Statistics (HES), using ICD-10 codes listed in table 1b, will be performed to ascertain definite case. We will compute the analysis comparing outcomes in different databases, using only similar cases based on the algorithms defined above.

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

hepatocellular; when there is 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 is 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 will be 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.

An assessment of Hy's Law (22,23) will also be conducted on the probable mixed cases, in order to facilitate comparison with the public-private US initiative Observational Medical Outcomes Partnership (OMOP).

5. Study Designs

The proposed designs described in this section will be conducted in different data sources by different partners.

DATA SOURCE	STUDY DESIGN	PRIORITY	PARTNER
BIFAP	Descriptive	High	BIFAP-CEIFE
BIFAP	Retrospective Cohort	High	BIFAP-CEIFE
BIFAP	Nested case-control	High	BIFAP-CEIFE
GPRD	Descriptive	High	Amgen
GPRD	Retrospective Cohort	High	Amgen
GPRD	Nested case-control	High	Amgen
GPRD	Population base case-control	Low	Amgen
GPRD	Case-Crossover	Low	Amgen
GPRD	Self-controlled case series	High	Amgen
THIN	Descriptive#	High	EMA
Bavaria	Descriptive#	High	LU_MUNCHEN
Mondrian	Descriptive#	High	UU
DKMA	Descriptive#	High	DKMA

Descriptive analysis of antibiotic use, but not outcome

5.1. Descriptive studies

A descriptive study will be done in the primary study population that will include patients of all ages with an active or died registration status during the study period of January 1st 2004 to December

31st 2009, with at least one year of enrolment with the GP and one year of computerized prescription. In BIFAP, THIN and GPRD databases, the primary study population will be used for descriptive studies. For Bavarian, Mondrian and DKMA the decision is still pending on final confirmation.

The following assessments will be done for the entire study period 2004-2009:

Prevalence of antibiotic drug use stratified by age (using ten-year categories, i.e. 0-9, 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89, 90+ years) and sex. This shall include a point prevalence (i.e. assessment on 01 Mar, 01 Jun, 01 Sep, 01 Dec 2004, 2005, … 2009) to take seasonal variations into account as well as a one year period prevalence (e.g. 01 Jan – 31 Dec 2004, 01 Jan – 31 Dec 2005, …, 2009) assessment. The denominator (preferred) will be the number of people that are present in the database at mid-year. As a second option we will use as denominator, the number of person-years in the study period, overall and in each age and sex specific categories.

Point and one year period prevalence of antibiotic drug use stratified by indication. Indications will be classified into the following three main groups: a) Respiratory/ORL infections; Genitourinary tract infections; c) Others. In BIFAP, GPRD, THIN there is no specific link between indication and prescriptions, and indication could only be assessed by searching for specific computer codes or free text within 2 weeks prior to the first prescription in the year of interest (period prevalence): this will produce an important misclassification. A more appropiate assesment would be done by manually reviewing the information available in a small sample of users of antibiotics (this will only be done if deadlines and time permits it). The denominator (preferred) will be the number of people that are present in the database at mid-year. As a second option we will use as denominator, the number of person-years in the study period, overall and in each age and sex specific categories.

One year period prevalence of ever antibiotic drug use in the year of interest (e.g. 01 Jan – 31 Dec 2004, 01 Jan – 31 Dec 2005, …, 2009) stratified by number of prescriptions (0, 1, 2-4, 5-11, ≥ 12 Rx) and stratified by major antibiotic groups . The denominator (preferred) will be the number of people that are present in the database at mid-year. As a second option we will use as denominator, the number of person-years in the study period, overall and in each age and sex specific categories

'Lifetime' prevalence of unspecifiified liver injury /liver disease (based on recorded codes related to liver disease, as shown in tables 1a,1b and 1c, without further manual review) in 2004 stratified by age (in 10 year categories; see above) and sex. The 'lifetime prevalence' assessment is based on all available follow-back information for an individual in the database prior to 01 Jan 2004. The denominator should be the number of people that are present in the database at mid-year 2004.

Cumulative yearly incidence of first-time acute liver failure by age (in 10 year categories; see above) and sex per calendar year (2004, 2005, …, 2009). The follow-back period to determine whether the recorded episode is a 'first-time' event includes all available database information prior to 01 Jan of the year of interest. Incidence rate will be estimate using as numerator the cases of Acute Liver Injury (based on the definition proposed in the protocol, after manual review of the initially computer-identified potential cases). The denominator corresponds to the number of people that are present in the database at start of the calendar year of interest (e.g. 01 Jan 2004) and who do not have a recorded history of acute liver failure prior to Jan 1 of that year. If – for instance – a patient has a recorded acute liver failure event in 2005 and another in 2008, these occurrences account only for the first-time incidence in 2005. In 2008 this person is excluded from the denominator as he is not "a person at risk" for getting his first event. As a second option we will use as denominator, the number of person-years in each, overall and in each age and sex specific categories.

5.2. Population based Retrospective Cohort study

5.2.1 Exposure definition for the cohort analysis

For the antibiotic cohort, the total follow up time will be divided into periods of non, current and past use of antibiotics with patients moving between these periods according to their use. The expected duration of each prescription/dispensing will be estimated using the prescribed quantity and the prescribed daily dose. When the estimated duration of use is missing for a patient, we will impute the median duration of all non-missing antibiotic prescriptions. A new period of current use starts, when a new antibiotic is prescribed. When a current prescription is not renewed within 14 days after the estimated end of the supply, a patient will automatically become a past user from the estimated end date of a current use prescription. A window of 30 days instead of 14 days will be used as a secondary definition of current use. A new period of current use starts, when a new antibiotic is prescribed.

5.2.2 Analyses

Hazard ratios will be produced by comparing the hazard rates of acute liver injury during current and past exposed periods with the hazard rates of acute liver injury during unexposed periods. We will estimate the risk associated with exposure to different antibiotic drug classes and the risk associated with individual antibiotic agents. Whenever possible exposure to antibiotics will be categorized based on major ATC/BNF pharmacologic groups and grouped in the following categories for the analysis:

1. tetracycline
2. beta-lactam antibacterias, penicillins
3. other beta-lactams:cephalosporins
4. macrolides, lincosamides and streptogramins
5. aminoglycosides
6. quinolones
7. Other antibiotics (anphenicols, suphonamides & others combinations)

Exposure to individual drugs or combinations with special interest (e.g. such amoxicillin & clavulanic) will be identified and analysed independently.

The incidence rate of acute idiopathic liver injury will be first estimated among the whole study population, meaning both cohorts (antibiotic and comparison non-exposed) as selected from the source population. The rate will be calculated by using the total number of identified acute liver injury cases as the numerator and the total number of person-years in the study population as the denominator. Secondly, incidence rate of acute liver injury will be estimated among non-exposed individuals by using the number of identified cases with liver injury during the follow-up time of the non-exposed cohort as the numerator and the total number of person-years in the non-exposed study population as the denominator. Lastly, in BIFAP and GPRD, incidence rates of acute liver injury associated with use of antibiotics (or particular class of antibiotics) will be computed using the number of cases among current users of antibiotics (or antibiotic class) as the numerator and the number of patients exposed, prescriptions or person time corresponding to current use as the denominator. Incidence among current users will be compared with past and non-users. Incidence rate ratios will be calculated by comparing the incidence rate of acute liver injury in the exposed cohort (current and past) with the incidence rates of acute liver injury in the unexposed cohort during unexposed person-time (in BIFAP/GPRD respectively).

All incidence estimates will be calculated by age, sex, and calendar year categories. Separate estimates will be calculated using only definite cases (narrow-specific definition) in a first step and definite and probable cases (broad, non-specific definition covering other scenarios) as a second step in order to compare cases detected in different databases.
We will compute incidence rate ratios and 95% confidence intervals of acute liver injury associated with current use of antibiotics (as a group and different classes and individual drugs when possible) as compared to non-use with Poisson regression. Time-to-event will be analysed using a Cox proportional hazards regression model for multivariate adjustment of potential confounders (mainly age, sex, and concomitant medications). In BIFAP and GPRD covariates will be measured at baseline and in GPRD time dependent variables could be used, (this analysis will have low priority). The effect of covariates will be examined by constructing a series of bivariate models. Likelihood Ratio tests will be used to compare models. We will fit a model only including general confounders (age, sex, body mass index, smoking, alcohol use and health care use) and also fit a full model, including all potential confounders: general confounders and more specific ones such as prescribed drugs used and comorbidity.

5.3. Nested case-control study

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 UTS period from January 2004 until December 2009 will be identified. The date of diagnosis (acute liver injury) will be considered the index date of the case. Separate analyses will be done using only definite cases as a first step and definite and probable cases as a second step.
Controls will be sampled from the patients at risk at the time of occurrence of the case (incidence density sampling). Thus, for a given case, potential controls are all non-cases at the time of the occurrence of the event, including future cases. Controls can therefore be cases later on. Controls will be matched to cases by age (within on year), sex calendar date (month & year) and practice (only in GPRD). We will select up to five controls per case.

5.3.1 Exposure definition for the case-control analysis

We will define 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 in cases and random date in controls). We will use a window of 30 days as a secondary definition of current use. We will define 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 will be estimated by comparing the odds of past and current users with the odds of non-users. Non-use of antibiotics will be used as reference. We will study the effect of dose and duration of treatment among current users. Duration of use will be defined by the treatment period covering consecutive prescriptions. Prescriptions will be considered consecutive when less than 14 days elapse between them.

We will estimate the risk associated with different antibiotics drug class categorized in seven groups based on major ATC/BNF categories: tetracycline, penicillins & betalactamic, cephalosporin, macrolides, aminoglycosides, quinolones and other antibiotics and combinations.

5.3.2. Statistical Analyses

We will compute 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 will be examined. Separate analyses will be done using only definite cases in a first step and definite and probable cases as a second step. Several strategies to select confounders will be 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 will be examined by constructing a series of bivariate models. Likelihood Ratio tests will be used to compare models. We will also fit a full model, including all of the potential confounders.
We will construct different regression models, first including general confounders (age, sex, BMI, alcohol, smoking, and visits to GP). And in a second step we will also fit a full model including all other potential confounders (comorbidities and drugs).

5.4. Case-crossover analysis

Data for this study will be obtained from GPRD (low priority), BIFAP and THIN (low priority) databases.
Case-crossover design is similar to case control design but only among cases with control moments from the same patient (24). Cases and index dates are the same as in the nested case-control design. The study population will comprise all antibiotic users with an acute liver injury episode during follow-up.
The index date will be the day of the acute liver injury. Exposure to antibiotics will be specified using 4 periods preceding the index date. Assuming a lag of 14 days between end of antibiotic intake and onset of first clinical symptoms, the at risk period for each patient in the study population will be defined as the 30 days immediately before the index date. Four successive 14 days control periods starting immediately prior to the at-risk period will be selected. Sensitivity analysis will be performed using other windows (i.e. 30 days).
It is analogous to a matched case-control study design, where one compares a ‘case’ person-moment with a series of ‘control’ person-moments from different subjects. In the case-crossover design, the ‘control’ person-moments will come from the same subject. Each of these person-moments covers a duration that is the length of a time window. (25)
This case-crossover design will be analysed using conditional logistic regression, as it accounts for the matched nature of the data. The risks will be calculated in terms of odds ratios (OR) with corresponding 95% confidence interval (CI). Other risk factors that will change over time such as changes in co-medication will be added as covariates in these models. Analyses of acute liver injury that occurred during antibiotic use will be stratified according to indication.

5.5. Self-controlled case series analysis

In the self-controlled case series method, patients in the exposed cohort, who received an antibiotic agent during the up to standard (UTS) follow-up, and experienced acute liver injury during the study period, will be included and will act as their own control. Because comparisons are made within individuals, fixed confounding factors, such as gender, are controlled for (26).

Person-time will be divided into several time-periods depending on the start of treatment with antibiotic agents and the remaining treatment period. Each individual's observation time will be divided into risk windows as follows: 1) from 0 to 7 days after the start of the treatment, 2) from 8 to 14 days after the start of the treatment and 3) from 15 to 30 days after the start of treatment and 4) the remaining exposed time, followed by 5) a wash-out period of 30 days. The remaining person-time will be used as a baseline comparison period. The thirty-day washout period will be divided into three 10-day periods after treatment.

We will include periods after treatment as we cannot be sure when treatment is stopped, and these periods will represent a gradual shift from full exposure, to a washout period, and finally to an entirely unexposed state. Relative incidence ratios can then be calculated by comparing the rate of acute liver injury experienced during risk periods with the rate of events during baseline time.

To determine whether the reported association between antibiotic agents and acute liver injury could partly be explained by the limited ability of a study design to control bias, we will compare the results of a self-controlled case series study with a case-control and retrospective cohort study.

We will also assess the potential impact of including both exposed and unexposed cases of liver injury to further confirm that adding unexposed cases can possibly increase the power of the self-controlled case series, but will not change the main results

Statistical Analyses

Data analyses will be performed using STATA software. Conditional Poisson regression will be used for the self-controlled case series analysis, in accordance with standard practice for the self-controlled case series method (24). Follow up for each patient will be divided into strata determined by antibiotic exposure status and current age. The incidence rate of idiopathic acute liver injury will be calculated during periods of exposure to antibiotic agents and compared with the incidence in the absence of exposure, adjusted for age. The analysis will be repeated considering all patients (both the antibiotic exposed and unexposed patients).

The case crossover and self controlled case series analyses both provide some advantages over the more traditional designs. Because comparisons are made within persons rather than between persons, with individuals acting as their own controls, case-only designs can provide powerful estimates of the effect of exposure on an outcome. Fixed confounding variables do not affect the results as these are implicitly controlled for. On the other hand, these study designs are better placed to assess the effect of short /acute treatments than chronic treatment, and also several assumptions need to be met when using case-only designs. An important assumption of all case-only designs is that the exposure of interest needs to be transient in nature. The 'antibiotics and acute liver injury' study is therefore the ideal drug-event pair to perform a case crossover and a self-controlled case series design. Exchangeable exposure time is an assumption of the case crossover design that needs to be met. The self-controlled case series method would provide some advantages over the case-crossover design when the exposure distribution in successive time periods is not exchangeable. With respect to the use of antibiotic agents it is likely that this assumption will be met as long as seasonality does not affect exposure too much. More important is perhaps the assumption of the self-controlled case series method that an event should not affect exposure. There is the possibility that liver injury may affect the likelihood of later exposure to antibiotic agents. If cases are less likely to receive antibiotic agents after acute liver injury, then the time included after the liver injury will be heavily skewed towards non-exposed time thereby introducing bias (increased RR). The case crossover study will clearly not be affected by bias after the event as follow-up time is censored at

the time of the event, but if prodromal symptoms anteceding the index date of case onset, this could determinate the use of antibiotics and might introduce bias that should be taken into account in the estimates of case-only studies, sensitivity analyses with different time windows will help to control for this.

In short, the case-crossover and self controlled case series design both provide both some advantages and disadvantages over more traditional designs. Both designs have assumptions that need to be met before they are used. As some of the assumptions differ per case-only design, it is of interest within this particular drug-event pair to investigate which design is more suitable to use. From this experience, recommendations for future case-only studies can be made

6. Blinding of Results

During the statistical analysis and reporting phase, database analysts will be blinded to the interim results from different study databases on the same drug-AE pair. The results of the association studies will be communicated directly to Utrecht University, WP2 Project Manager. When all results have been collected, they will be forwarded to the drug-disease teams to evaluate and compare the results between the databases.

7. Covariates and potential confounders

Potential confounders will be measured at baseline (start of study period) for the cohort analysis, and at index date (date of acute liver injury in cases and random date in controls) in the case control analysis.

The crude estimates of risk will be adjusted for age and sex in a first step. A further adjusted analysis will be 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 will also be considered as potential confounders.

Specific possible confounders include 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 will be identified by the recording of a Read/ICD code in the patient's clinical or referral files, as will data on treatment with any concomitant medication. A list of confounders is shown in table 4.

8. Possible limitations

We must consider that we are only able to capture individuals who consulted their GP regarding their symptoms and received a diagnosis of liver disorder. These 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. To further assess the sensitivity and specificity of our classification, patient profiles and free text comments of all definite and probable cases identified in BIFAP and a random sample of cases in GPRD will be extracted and manually reviewed by expert clinicians. Their classification of these cases into definite and probable cases will be compared to our classification using our defined rules. Sensitivity, specificity and positive predictive values will be produced. It should be acknowledged that none of the database would be able to assess with 100% sensitivity/specificity the diagnosis of liver injury. The nature of the database also means that we will not be able to detect patients diagnosed outside primary care (i.e. by a specialist, in hospital or by private doctors), unless referral letters and free text information is available. This may cause a slight underestimation of the prevalence of the outcome compared with that in the general population, but it is applicable to and representative of the primary care setting. Information on hospitalization and/or referrals is not available in all databases, and may need to be performed by linkage to hospital data or by review of all free text comment entered by the physicians and only available in some databases.

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. Free text review of some of the cases 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 in Europe for antibiotics. But we must consider the lack of systematic recording of concomitant use of over-the-counter medicine, such as paracetamol, 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. However it should be noted that elderly individuals are not charged for the receipt of medicine from the age of 60 onwards in UK and 65 + in Spain, which indicates a high likelihood to collect the prescription. Therefore, in GPRD, THIN, BIFAP database we expect that we can better capture concomitant drugs use in the older age groups.

We must also considered that prescription made outside the general practice setting or by private doctors 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 databases is that some information may be incomplete or not available. There could be incomplete recording in data on life style factors (weight, height, smoking or alcohol consumption) as well as data on socioeconomic level.

A key assumption of the case series method is that the time that passes after the observation stops, should be missing completely at random and should not be dependent on the occurrence of an event. If a patient experiences an idiopathic acute liver injury and dies, the assumption that the actual observation period for each individual is independent of event times is violated(19). The case-

series method has recently been extended to deal with censoring of follow-up time due to short-term mortality following the event. The extension accounts for the censoring of observation time post-event and removes the need to assume the observation period is independent of event time. The new extension to the case series method will be further explored in this study.

9. Instrumental variable analysis

A method that potentially controls for both observed and unobserved confounding is instrumental variable (IV) analysis [Martens 2006, Hernan 2006]. An IV is a variable that is strongly related to exposure, and only related to the outcome through exposure. Hence, an IV should neither directly nor indirectly through (unobserved) confounders be associated with the outcome. Importantly, if the IV is independent of observed confounders, it is assumed to be independent of unobserved confounders. This is in analogy with the comparability of observed and unobserved prognostic variables between the intervention and control group achieved by randomization in a trial.

A key example of instrumental variable approach in pharmacoepidemiology for the assessment of gastrointestinal complications in relation to COX-2 inhibitors compared to non-selective non-steroidal anti-inflammatory drugs (NSAIDs) has illustrated this approach [Brookhart 2006]. It may however not be possible to identify valid IVs for every pharmacoepidemiologic research question [Groenwold 2010].

It is proposed to use IV analysis to assess the unconfounded association between prescriptions for antibiotics and acute liver injury. Several potential IVs will be evaluated, including physician preference (e.g. as indicated by the prescription to the previous patient with a prescription for the same indication), regional variation (e.g. different regions or countries, possibly with different prescribing guidelines), and calendar time (e.g., periods prior to and after establishment of new guidelines) [Brookhart 2007, Chen 2010]. These variables may be related to prescriptions for antibiotics, yet are unlikely to be directly related to acute liver injury, nor indirectly through the potential confounder(s) listed in the paragraph “potential confounders”. Estimation will be conducted via a two-stage instrumental variable model [Rassen 2009]. This analysis will be a separate from the main analyses described in this proposal and focuses on the (methodological) application of IV analysis in pharmacoepidemiology.

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11. Tables and figures

Table 1a: READ codes for idiopathic acute Liver injury/disease (GPRD/ THIN)

READ code	Description
44d2.00	Liver Function Tests Abnormal
44E2.00	Serum Bilirubin Raised
44g2.00	Liver Enzymes Abnormal
J60..00	Acute And Subacute Liver Necrosis
J600.00	Acute Necrosis Of Liver
J600000	Acute Hepatic Failure
J600011	Acute Liver Failure
J600100	Acute Hepatitis - Noninfective
J600200	Acute Yellow Atrophy
J600z00	Acute Necrosis Of Liver Nos
J601.00	Subacute Necrosis Of Liver
J601000	Subacute Hepatic Failure
J601100	Subacute Hepatitis - Noninfective
J601200	Subacute Yellow Atrophy
J601z00	Subacute Necrosis Of Liver Nos
J60z.00	Acute And Subacute Liver Necrosis Nos
J622.00	Hepatic Coma
J622.11	Encephalopathy - Hepatic
J625.00	[X] Hepatic Failure
J625.11	[X] Liver Failure
J62y.11	Hepatic Failure Nos
J62y.12	Liver Failure Nos
J62y.13	Hepatic Failure
J633.00	Hepatitis Unspecified
J633000	Toxic Hepatitis
J633z00	Hepatitis Unspecified Nos
J635.00	Toxic Liver Disease
J635000	Toxic Liver Disease With Cholestasis
J635100	Toxic Liver Disease With Hepatic Necrosis
J635200	Toxic Liver Disease With Acute Hepatitis
J635X00	Toxic Liver Disease, Unspecified
J636.00	Central Haemorrhagic Necrosis Of Liver
J63y.00	Other Specified Liver Disorder
J63y100	Nonspecific Reactive Hepatitis
J63yz00	Other Specified Liver Disorder Nos
J66y600	Obstructive Jaundice Nos
R024.00	[D]Jaundice (Not Of Newborn)

R024000	[D]Cholaemia Nos
R024100	[D]Icterus Nos
R024111	[D]Jaundice
R024z00	[D]Jaundice (Not Of Newborn) Nos
R148.00	[D]Abnormal Liver Function Test
R148000	[D]Abnormal Liver Scan
R148.11	[D]Lft's Abnormal
R148z00	[D]Abnormal Liver Function Test Nos
7804200	Open Wedge Biopsy Of Lesion Of Liver
7807000	Diagnostic Laparoscopic Examination And Biopsy Liver LESION
780A000	Percutaneous Transvascular Biopsy Of Lesion Of Liver
780A100	Percutaneous Biopsy Of Lesion Of Liver Nec
780A111	Menghini Needle Biopsy Of Liver
780A112	Needle Biopsy Of Liver Nec
780A113	Sheeba Needle Biopsy Of Liver
780B000	Biopsy Of Liver Nec
780B011	Biopsy Of Lesion Of Liver Nec
44G3100	Alt/Sgpt Level Abnormal
44H5100	Ast/Sgot Level Abnormal
44H5200	Ast/Sgot Level Raised
14C5.00	H/O: Liver Disease
14C6.00	H/O: Jaundice
25G3.00	O/E -Liver Moderately Enlarged
25G4.00	O/E - Liver Grossly Enlarged
44G2.00	Liver Enzymes Abnormal
D307000	Deficiency Of Coagulation Factor Due To Liver Disease
J624.00	Hepatorenal Syndrome
Jyu7000	[X]Toxic Liver Disease With Other Disorders Of Liver
Jyu7600	[X]Toxic Liver Disease
SP14200	Hepatic Failure As A Complication Of Care
SP14300	Hepatorenal Syndrome As A Complication Of Care
ZC2CH11	Dietary Advice For Liver Disease
J63z.00	Liver disease NOS
J635700	Acute hepatic failure due to drugs

Table 1b: ICD-10 codes for Acute Liver injury

READ code	Description
K71	Toxic liver disease Includes: drug-induced: · idiosyncratic (unpredictable) liver disease · toxic (predictable) liver disease Excludes: alcoholic liver disease (K70.-)
K71.0	Toxic liver disease with cholestasis

	Cholestasis with hepatocyte injury"Pure" cholestasis
K71.1	Toxic liver disease with hepatic necrosis
	Hepatic failure (acute)(chronic) due to drugs
K71.2	Toxic liver disease with acute hepatitis
K71.9	Toxic liver disease, unspecified
K72	Hepatic failure, not elsewhere classified Includes: hepatic:· coma NOS · encephalopathy NOS . hepatitis:· acute · fulminant · malignant NEC, with hepatic failure liver (cell) necrosis with hepatic failure yellow liver atrophy or dystrophy Excludes: alcoholic hepatic failure (K70.4) hepatic failure complicating: · abortion or ectopic or molar pregnancy (O00-O07 , O08.8) · pregnancy, childbirth and the puerperium (O26.6)icterus of fetus and newborn (P55-P59)viral hepatitis (B15-B19)with toxic liver disease (K71.1)
K72.0	Acute and subacute hepatic failure
K72.9	Hepatic failure, unspecified

Table 1c: CIAP(ICPC) codes for Idiopathic Acute Liver injury (BIFAP)*

CIAP*(ICPC) code	Description
D72.19	Hepatitis
D97.3	Coma hepatico nc
D97.4	Enfermedad hepatica nc
D97.5	Hepatitis aguda
D97.7	Hepatitis nc (no viral)
D97.12	Hepatopatia nc
B85.6	Anormal prueba sanguinea transaminasas nc
B85.9	Anormalidad prueba sanguinea (inexplicable) transaminasas
B85.35	Hipertransaminassemia nc
B85.37	Transaminitis
B85.39	Augment, augmentat transaminases nc
B85.42	Transaminasas elevadas
B85.44	Transaminasas elevadas
B85.45	Elevacion no especifica de transaminasas/ldh
B85.49	Hipertransaminasemia
Signos/síntomas	
D13.0	Ictericia
D13.1	Cambio (en) (de) piel, nc color icterico

D13.2	Ictericia nc
D13.3	Subictericia
D13.4	Ictericia
F29.4	Ictericia conjuntival nc
B85.46	Hiperbilirrubinemia
D96.1	Aumento, aumentado. Higado
D96.2	Hepatomegalia
D06.31	Hepatalgia
D01.28	Colico hepático

* CIAP Clasificación Internacional de la Atención Primaria (ICPC-International Classification of Primary Care)

Table 2: Exclusion criteria for outcome definition

(See list of codes READ/ICPC, in data specification document)

2.1 Cancer
2.2 Alcoholism / alcohol abuse / alcohol related disease
2.3 Gallbladder diseases/ Cholelithiasis/ cholecystitis/ Cholangitis
2.4 Diseases of pancreas/ Pancreatitis/ Other diseases of pancreas
2.5 Other chronic liver disease (including autoinmune hepatitis, see data specification document)

Table 3a: List of antibiotics /BNF classification

BNF	BNF description	Protocol Group
05.01.01.01	Benzylpenicillin & phenoxymethylpenicillin	2
05.01.01.02	Penicillinase-resistant penicillins	2
05.01.01.03	Broad-spectrum penicillins	2
05.01.01.04	Antipseudomonal penicillins	2
05.01.01.05	Mecillinams	2
05.01.02.01	Cephalosporins	3
05.01.02.02	Carbapenems	3
05.01.02.03	Other beta-lactam antibiotics	3
05.01.03.00	Tetracyclines	1
05.01.04.00	Aminoglycosides	5
05.01.05.00	Macrolides	4
05.01.06.00	Clindamycin	4
05.01.07.00	Some other antibacterials	7
05.01.07.01	Chloramphenicol	7
05.01.07.02	Fusidic acid	7
05.01.07.03	Vancomycin and teicoplanin	7
05.01.07.04	Daptomycin	7
05.01.07.05	Linezolid	7
05.01.07.06	Quinupristin and dalfopristin	7

05.01.07.07	Polymyxins	7
05.01.08.00	Sulphonamides & trimethoprim	7
05.01.09.00	Antituberculosis drugs	7
05.01.10.00	Antileprotic drugs	7
05.01.11.00	Metronidazole and tinidazole	7
05.01.12.00	Quinolones	6
05.01.13.00	Urinary-tract infections	7

*Plus any combinations with the antibiotic agents listed above.

Table 3b: List of antibiotics by groups /ATC classification

PROTOCOL GROUP 1: tetracycline

ATC codes	ATC Description
J01A	TETRACYCLINES
J01AA	Tetracyclines
J01AA01	demeclocycline
J01AA02	doxycycline
J01AA03	chlortetracycline
J01AA04	lymecycline
J01AA05	metacycline
J01AA06	oxytetracycline
J01AA07	tetracycline
J01AA08	minocycline
J01AA09	rolitetracycline
J01AA10	penimepicycline
J01AA11	clomocycline
J01AA12	tigecycline
J01AA20	combinations of tetracyclines
J01AA56	oxytetracycline, combinations

PROTOCOL GROUP 2: beta-lactam antibacterias, penicillins

ATC code	Name
J01C	BETA-LACTAM ANTIBACTERIALS, PENICILLINS
J01CA	Penicillins with extended spectrum
J01CA01	ampicillin
J01CA02	pivampicillin
J01CA03	carbenicillin
J01CA04	amoxicillin
J01CA05	carindacillin
J01CA06	bacampicillin
J01CA07	epicillin
J01CA08	pivmecillinam
J01CA09	azlocillin

J01CA10	mezlocillin
J01CA11	mecillinam
J01CA12	piperacillin
J01CA13	ticarcillin
J01CA14	metampicillin
J01CA15	talampicillin
J01CA16	sulbenicillin
J01CA17	temocillin
J01CA18	hetacillin
J01CA19	aspoxicillin
J01CA20	combinations
J01CA51	ampicillin, combinations
J01CE	Beta-lactamase sensitive penicillins
J01CE01	benzylpenicillin
J01CE02	phenoxymethylpenicillin
J01CE03	propicillin
J01CE04	azidocillin
J01CE05	pheneticillin
J01CE06	penamecillin
J01CE07	clometocillin
J01CE08	benzathine benzylpenicillin
J01CE09	procaine benzylpenicillin
J01CE10	benzathine phenoxymethylpenicillin
J01CE30	combinations
J01CF	Beta-lactamase resistant penicillins
J01CF01	dicloxacillin
J01CF02	cloxacillin
J01CF03	meticillin
J01CF04	oxacillin
J01CF05	flucloxacillin
J01CG	Beta-lactamase inhibitors
J01CG01	sulbactam
J01CG02	tazobactam
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors
J01CR01	ampicillin and enzyme inhibitor
J01CR02	amoxicillin and enzyme inhibitor
J01CR03	ticarcillin and enzyme inhibitor
J01CR04	sultamicillin
J01CR05	piperacillin and enzyme inhibitor
J01CR50	combinations of penicillins

PROTOCOL GROUP 3: other beta-lactams: cephalosporins

ATC code	Name

J01D	OTHER BETA-LACTAM ANTIBACTERIALS
J01DB	First-generation cephalosporins
J01DB01	cefalexin
J01DB02	cefaloridine
J01DB03	cefalotin
J01DB04	cefazolin
J01DB05	cefadroxil
J01DB06	cefazedone
J01DB07	cefatrizine
J01DB08	cefapirin
J01DB09	cefradine
J01DB10	cefacetrile
J01DB11	cefroxadine
J01DB12	ceftezole
J01DC	Second-generation cephalosporins
J01DC01	cefoxitin
J01DC02	cefuroxime
J01DC03	cefamandole
J01DC04	cefaclor
J01DC05	cefotetan
J01DC06	cefonicid
J01DC07	cefotiam
J01DC08	loracarbef
J01DC09	cefmetazole
J01DC10	cefprozil
J01DC11	ceforanide
J01DC12	cefminox
J01DC13	cefbuperazone
J01DC14	flomoxef
J01DD	Third-generation cephalosporins
J01DD01	cefotaxime
J01DD02	ceftazidime
J01DD03	cefsulodin
J01DD04	ceftriaxone
J01DD05	cefmenoxime
J01DD06	latamoxef
J01DD07	ceftizoxime
J01DD08	cefixime
J01DD09	cefodizime
J01DD10	cefetamet
J01DD11	cefpiramide
J01DD12	cefoperazone
J01DD13	cefpodoxime

J01DD14	ceftibuten
J01DD15	cefdinir
J01DD16	cefditoren
J01DD17	cefcapene
J01DD54	ceftriaxone, combinations
J01DD62	cefoperazone, combinations
J01D	OTHER BETA-LACTAM ANTIBACTERIALS
J01DE	Fourth-generation cephalosporins
J01DE01	cefepime
J01DE02	cefpirome
J01DE03	cefozopran
J01DF	Monobactams
J01DF01	aztreonam
J01DF02	carumonam
J01DH	Carbapenems
J01DH02	meropenem
J01DH03	ertapenem
J01DH04	doripenem
J01DH05	biapenem
J01DH51	imipenem and enzyme inhibitor
J01DH55	panipenem and betamipron
J01DI	Other cephalosporins
J01DI01	ceftobiprole medocaril
J01DI02	ceftaroline fosamil

PROTOCOL GROUP 4: macrolides, lincosamides and streptogramins

ATC code	Name
J01F	MACROLIDES, LINCOSAMIDES AND STREPTOGRAMINS
J01FA	Macrolides
J01FA01	erythromycin
J01FA02	spiramycin
J01FA03	midecamycin
J01FA05	oleandomycin
J01FA06	roxithromycin
J01FA07	josamycin
J01FA08	troleandomycin
J01FA09	clarithromycin
J01FA10	azithromycin
J01FA11	miocamycin
J01FA12	rokitamycin
J01FA13	dirithromycin
J01FA14	flurithromycin
J01FA15	telithromycin

J01FF	Lincosamides
J01FF01	clindamycin
J01FF02	lincomycin
J01FG	Streptogramins
J01FG01	pristinamycin
J01FG02	quinupristin/dalfopristin

PROTOCOL GROUP 5: aminoglycosides

ATC code	Name
J01G	AMINOGLYCOSIDE ANTIBACTERIALS
J01GA	Streptomycins
J01GA01	streptomycin
J01GA02	streptoduocin
J01GB	Other aminoglycosides
J01GB01	tobramycin
J01GB03	gentamicin
J01GB04	kanamycin
J01GB05	neomycin
J01GB06	amikacin
J01GB07	netilmicin
J01GB08	sisomicin
J01GB09	dibekacin
J01GB10	ribostamycin
J01GB11	isepamicin
J01GB12	arbekacin
J01GB13	bekanamycin

PROTOCOL GROUP 6: quinolones

ATC code	Name
J01M	QUINOLONE ANTIBACTERIALS
J01MA	Fluoroquinolones
J01MA01	ofloxacin
J01MA02	ciprofloxacin
J01MA03	pefloxacin
J01MA04	enoxacin
J01MA05	temafloxacin
J01MA06	norfloxacin
J01MA07	lomefloxacin
J01MA08	fleroxacin
J01MA09	sparfloxacin
J01MA10	rufloxacin
J01MA11	grepafloxacin
J01MA12	levofloxacin

J01MA13	trovafloxacin
J01MA14	moxifloxacin
J01MA15	gemifloxacin
J01MA16	gatifloxacin
J01MA17	prulifloxacin
J01MA18	pazufloxacin
J01MA19	garenoxacin
J01MA21	sitafloxacin
J01MB	Other quinolones
J01MB01	rosoxacin
J01MB02	nalidixic acid
J01MB03	piromidic acid
J01MB04	pipemidic acid
J01MB05	oxolinic acid
J01MB06	cinoxacin
J01MB07	flumequine

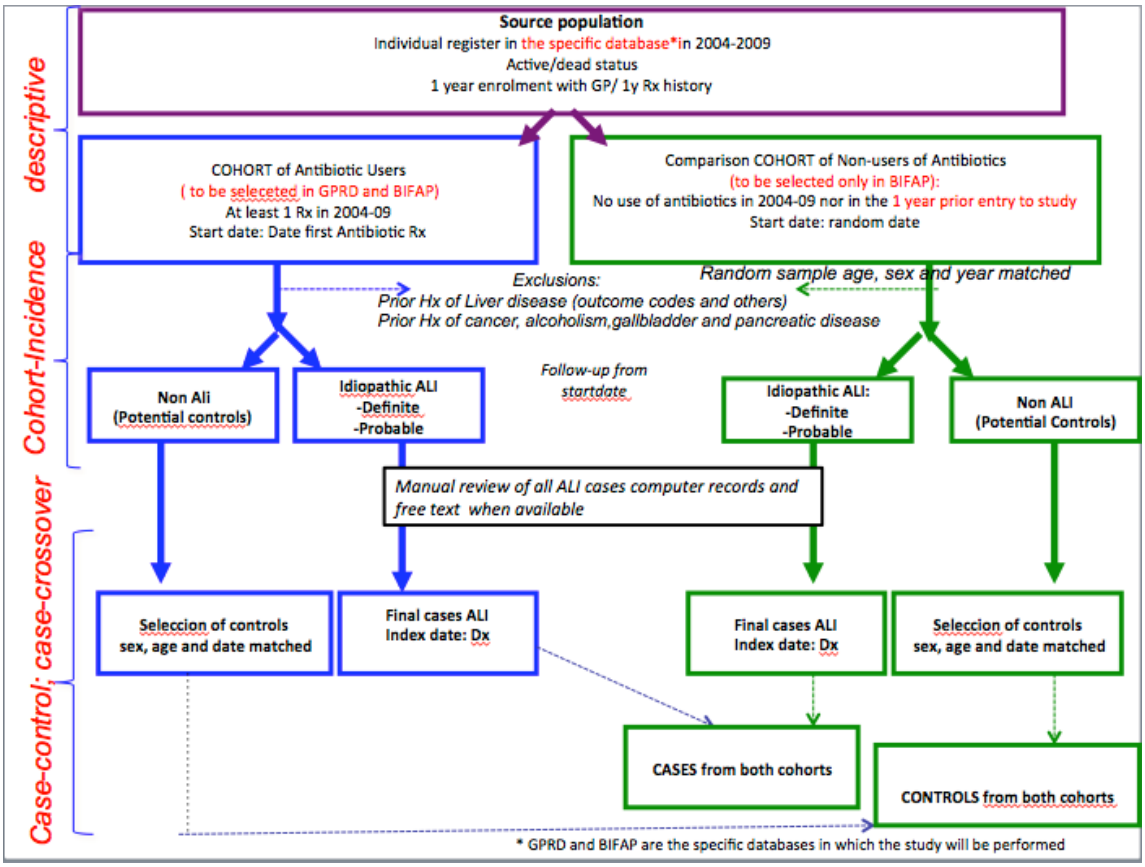
PROTOCOL GROUP 7: Other antibiotics (anphenicols, suphonamides & others combinations)

ATC code	Name
J01E	SULFONAMIDES AND TRIMETHOPRIM
J01EA	Trimethoprim and derivatives
J01EA01	trimethoprim
J01EA02	brodimoprim
J01EA03	iclaprim
J01EB	Short-acting sulfonamides
J01EB01	sulfaisodimidine
J01EB02	sulfamethizole
J01EB03	sulfadimidine
J01EB04	sulfapyridine
J01EB05	sulfafurazole
J01EB06	sulfanilamide
J01EB07	sulfathiazole
J01EB08	sulfathiourea
J01EB20	combinations
J01EC	Intermediate-acting sulfonamides
J01EC01	sulfamethoxazole
J01EC02	sulfadiazine
J01EC03	sulfamoxole
J01EC20	combinations
J01ED	Long-acting sulfonamides
J01ED01	sulfadimethoxine

J01ED02	sulfalene
J01ED03	sulfametomidine
J01ED04	sulfametoxydiazine
J01ED05	sulfamethoxypyridazine
J01ED06	sulfaperin
J01ED07	sulfamerazine
J01ED08	sulfaphenazole
J01ED09	sulfamazone
J01ED20	combinations
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives
J01EE01	sulfamethoxazole and trimethoprim
J01EE02	sulfadiazine and trimethoprim
J01EE03	sulfametrole and trimethoprim
J01EE04	sulfamoxole and trimethoprim
J01EE05	sulfadimidine and trimethoprim
J01EE06	sulfadiazine and tetroxoprim
J01EE07	sulfamerazine and trimethoprim
J01B	AMPHENICOLS
J01BA	Amphenicols
J01BA01	chloramphenicol
J01BA02	thiamphenicol
J01BA52	thiamphenicol, combinations
J01R	COMBINATIONS OF ANTIBACTERIALS
J01RA	Combinations of antibacterials
J01RA01	penicillins, combinations with other antibacterials
J01RA02	sulfonamides, combinations with other antibacterials (excl. trimethoprim)
J01RA03	cefuroxime, combinations with other antibacterials
J01RA04	spiramycin, combinations with other antibacterials
J01X	OTHER ANTIBACTERIALS
J01XA	Glycopeptide antibacterials
J01XA01	vancomycin
J01XA02	teicoplanin
J01XA03	telavancin
J01XA04	dalbavancin
J01XA05	oritavancin
J01XB	Polymyxins
J01XB01	colistin
J01XB02	polymyxin B
J01XC	Steroid antibacterials
J01XC01	fusidic acid
J01XD	Imidazole derivatives
J01XD01	metronidazole
J01XD02	tinidazole

J01XD03	ornidazole
J01XE	Nitrofuran derivatives
J01XE01	nitrofurantoin
J01XE02	nifurtoinol
J01XX	Other antibacterials
J01XX01	fosfomycin
J01XX02	xibornol
J01XX03	clofoctol
J01XX04	spectinomycin
J01XX05	methenamine
J01XX06	mandelic acid
J01XX07	nitroxoline
J01XX08	linezolid
J01XX09	daptomycin
J01XX10	bacitracin

FIGURE 1: Flow- chart of study design and analyses



12. Appendix 1 (Amendments)

Amendment 1

Protocol: PROTECT_WP2 Final Protocol Antibiotics-LiverInjury_29Nov2011.doc
Amendment number: *Nº 1*
Amendment date: 10 May 2012
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Reason(s) for Amendment:
This protocol amendment serves to the following purposes:
Correcting an error in cohort ascertainment (Study population)
Clarifications of the outcome definition, adapted to the data availability
Preferred exposed window for cohort, case control and case-crossover analyses (based on data obtained from preliminary descriptive results)

Protocol Section(s) Amended (highlighted)

Correcting an error in cohort ascertainment (Study population)

Change from:

Study population
From the aforementioned source population, two study cohorts will be selected.
The first cohort will include 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) defines the start of follow-up (start date), for the exposed cohort.
The second cohort will be selected from the same source population among patients who have not received an antibiotic prescription during the study period and in the year before the entry date (date when the patient meet the eligibility criteria and enter in the study). For these patients we will assign a random date during the study period, and we will select a random sample of these patients not exposed to antibiotics ("non users") frequency-matched by age, sex and calendar date (month and year, if possible) to the cohort of antibiotic users. For this cohort of non-users the random date generated after meeting the eligibility criteria will be used as the start of follow-up (start date).
All subjects from the study population with one of the codes listed in table 1a,b, c (outcome definition) or one of the diagnoses included in table 2 (exclusion criteria: cancer, alcoholism, alcohol related problems, gallbladder disease, pancreatic disease, and other chronic liver diseases not included in outcome definition) prior to start date will be excluded.

Change to:

Study population
From the aforementioned source population, two study cohorts will be selected (see figure 1).
The first cohort will include 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) defines the start of follow-up (start date), for the exposed cohort.
The second cohort will be composed of all members belonging to ~~the selected from~~ 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 their entry date (date when the patient meet all the eligibility criteria and enter in the study contribution). For these patients we will assign a random date during the study period~~,. and we will select a random sample of these patients not exposed to antibiotics ("non users") frequency-matched by age, sex and calendar date (month and year, if possible) to the cohort of antibiotic users.~~ For this cohort of non-users the random date generated after meeting the eligibility criteria will be used as the start of follow-up (start date).

b) Clarifications of the outcome definition, adapted to the data availability

Change from:

Outcome definition

The outcome for this study is the first recorded occurrence of idiopathic acute liver injury. Patients will be 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 and the patient presents with at least with one of the following conditions (A+B or A+C):

A - A diagnosis of liver injury (codes listed in tables 1a, 1b, 1c) with a referral to a specialist or hospital.

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 tables 1a, 1b, 1c.

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 definite case are missing, applying different algorithms as follows:

2.2. - Probable case referred to specialist/or hospitalised: Patients identified with a READ or ICPC codes listed in table 1a 1b, 1c, with a hospitalization or visit to a specialist but without complete laboratory criteria. We will further classify these patients depending on the information on laboratory test results (patients with at least one recorded abnormal liver test but not meeting the criteria B or C; patients with normal test results and those with no laboratory test results)

2.3. - Probable case without a referral to specialist/or hospital: Patients identified with a READ or ICPC codes listed in table 1a 1b, 1c, without a referral to a hospital/specialist, and with or without complete laboratory data. We will further classify these patients depending on the information on laboratory test available (patients with at least one recorded abnormal liver test, but not meeting the criteria B or C; patients with normal test results or no laboratory test results, and those with complete laboratory criteria for acute liver injury B or C).

We will 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 will be considered non-cases.

Change to:

Outcome definition

The outcome for this study is the first recorded occurrence of idiopathic acute liver injury. To initially identify cases, we used a list of codes (tables 1a,1b,1c) some of them are specific of liver disease or symptoms (e.g. Hepatitis , Acute Hepatic Failure, Ictericia) and others are not specific (e.g: Liver Function Tests Abnormal, Increased Transaminasas). Patients will be 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 and the patient presents with 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 tables 1a, 1b, 1c) 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 tables 1a, 1b, 1c.

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 definite case are missing, applying different algorithms as follows:

Probable case 1: Patients identified with a specific READ or ICPC code of liver disease 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 READ or ICPC code of liver disease with complete laboratory criteria for acute liver injury B or C, and a related visit to a specialist or hospitalization.

We will manually review available information of subgroups of probable cases, in BIBAP database in order to confirm their final status. In GPRD only a sample of these probable cases will be reviewed.

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 will be considered non-cases. We will not consider 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).

In BIFAP database a review of the free text comments will be conducted for all identified definite and probable cases, as well as for the group of non-cases with incidental laboratory findings. In GPRD this review will be only done for a random sample of definite and probable cases

c) Preferred exposed window for cohort, case control and case-crossover analyses

Change from:
Population based Retrospective Cohort study
Exposure definition for the cohort analysis
For the antibiotic cohort, the total follow up time will be divided into periods of non, current and past use of antibiotics with patients moving between these periods according to their use. The expected duration of each prescription/dispensing will be estimated using the prescribed quantity and the prescribed daily dose. When the estimated duration of use is missing for a patient, we will impute the median duration of all non-missing antibiotic prescriptions. A new period of current use starts, when a new antibiotic is prescribed. When a current prescription is not renewed within 30 days after the estimated end of the supply, a patient will automatically become a past user from the estimated end date of a current use prescription. A window of 14 days instead of 30 days will be used as a secondary definition of current use. A new period of current use starts, when a new antibiotic is prescribed.
Nested case-control study
Exposure definition for the case-control analysis
We will define patients as current users if a prescription for the drugs of interest lasts until the index date or ends within 30 days prior to the index date (i.e. date of onset of liver injury in cases and random date in controls). We will use a window of 14 and 30 days as a secondary definition of current use. We will define patients 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. The association between the use of antibiotics and the experience of acute liver injury will be estimated by comparing the odds of past and current users with the odds of non-users. Non-use of antibiotics will be used as baseline. We will study the effect of dose and duration of treatment among current users. Duration of use will be defined by the treatment period covering consecutive prescriptions. Prescriptions will be considered consecutive when less than 30 days elapse between them.
Case-crossover analysis
Data for this study will be obtained from GPRD (low priority), BIFAP and THIN (low priority) databases.
Case-crossover design is similar to case control design but only among cases with control moments from the same patient (24). Cases and index dates are the same as in the nested case-control design. The study population will comprise all antibiotic users with an acute liver injury episode during follow-up.
The index date will be the day of the acute liver injury. Exposure to antibiotics will be specified using 4 periods preceding the index date. Assuming a lag of 30 days between end of antibiotic intake and onset of first clinical symptoms, the at risk period for each patient in the study population will be defined as the 30 days immediately before the index date. Four successive 30-day control periods

starting immediately prior to the at-risk period will be selected. Sensitivity analysis will be performed using other windows (i.e. 14 days).

Change to:

Population based Retrospective Cohort study

Exposure definition for the cohort analysis

For the antibiotic cohort, the total follow up time will be divided into periods of non, current and past use of antibiotics with patients moving between these periods according to their use. The expected duration of each prescription/dispensing will be estimated using the prescribed quantity and the prescribed daily dose. When the estimated duration of use is missing for a patient, we will impute the median duration of all non-missing antibiotic prescriptions. A new period of current use starts, when a new antibiotic is prescribed. When a current prescription is not renewed within ~~30~~ 14 days after the estimated end of the supply, a patient will automatically become a past user from the estimated end date of a current use prescription. A window of ~~14~~ 30 days instead of ~~30~~ 14 days will be used as a secondary definition of current use. A new period of current use starts, when a new antibiotic is prescribed.

Nested Case-control study

Exposure definition for the case-control analysis

We will define patients as current users if a prescription for antibiotics lasts until the index date or ends within ~~30~~ 14 days prior to the index date (i.e. date of onset of liver injury in cases and random date in controls). We will use a window of 30 ~~14~~ days as a secondary definition of current use. We will define patients as past users if the prescription ends between ~~30~~ 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 will be estimated by comparing the odds of past and current users with the odds of non-users. Non-use of antibiotics will be used as reference ~~baseline~~. We will study the effect of dose and duration of treatment among current users. Duration of use will be defined by the treatment period covering consecutive prescriptions. Prescriptions will be considered consecutive when less than 14 ~~30~~ days elapse between them.

Case-crossover analysis

Data for this study will be obtained from GPRD (low priority), BIFAP and THIN (low priority) databases.

Case-crossover design is similar to case control design but only among cases with control moments from the same patient (24). Cases and index dates are the same as in the nested case-control design. The study population will comprise all antibiotic users with an acute liver injury episode during follow-up.

The index date will be the day of the acute liver injury. Exposure to antibiotics will be specified using 4 periods preceding the index date. Assuming a lag of 14 ~~30~~ days between end of antibiotic intake and onset of first clinical symptoms, the at risk period for each patient in the study population will be defined as the 30 days immediately before the index date. Four successive 14 ~~30~~ days control periods starting immediately prior to the at-risk period will be selected. Sensitivity analysis will be performed using other windows (i.e. ~~14~~ 30 days).

Amendment 2

Protocol: PROTECT_WP2 Final Protocol Antibiotics-LiverInjury_29Nov2011.doc
Amendment number: *Nº 2*
Amendment date: 20 July 2012
Protocol Owners: Ana Ruigomez[1] & Luis Alberto García Rodríguez[1]

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Reason(s) for Amendment :
This protocol amendment serves to the following purposes:
Describe in more detail the rationale and importance of the proposed comparison between the self-controlled case series and case crossover design

Protocol Section(s) Amended (highlighted)

The following new statement has been introduced at the end of Section 5 (Study Designs):

The case crossover and self controlled case series analyses both provide some advantages over the more traditional designs. Because comparisons are made within persons rather than between persons, with individuals acting as their own controls, case-only designs can provide powerful estimates of the effect of exposure on an outcome. Fixed confounding variables do not affect the results as these are implicitly controlled for. On the other hand, these study designs are better placed to assess the effect of short /acute treatments than chronic treatment, and also several assumptions need to be met when using case-only designs. An important assumption of all case-only designs is that the exposure of interest needs to be transient in nature. The 'antibiotics and acute liver injury' study is therefore the ideal drug-event pair to perform a case crossover and a self-controlled case series design. Exchangeable exposure time is an assumption of the case crossover design that needs to be met. The self-controlled case series method would provide some advantages over the case-crossover design when the exposure distribution in successive time periods is not exchangeable. With respect to the use of antibiotic agents it is likely that this assumption will be met as long as seasonality does not affect exposure too much. More important is perhaps the assumption of the self-controlled case series method that an event should not affect exposure. There is the possibility that liver injury may affect the likelihood of later exposure to antibiotic agents. If cases are less likely to receive antibiotic agents after acute liver injury, then the time included after the liver injury will be heavily skewed towards non-exposed time thereby introducing bias (increased RR). The case crossover study will clearly not be affected by bias after the event as follow-up time is censored at the time of the event, but if prodromal symptoms anteceding the index date of case onset, this could determinate the use of antibiotics and might introduce bias that should be taken into account in the estimates of case-only studies, sensitivity analyses with different time windows will help to control for this.
In short, the case-crossover and self controlled case series design both provide both some advantages and disadvantages over more traditional designs. Both designs have assumptions that need to be met before they are used. As some of the assumptions differ per case-only design, it is of interest within this particular drug-event pair to investigate which design is more suitable to use. From this experience, recommendations for future case-only studies can be made