Improving the measurement and detection of serious adverse drug reactions in databases of stored electronic health records

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Thesis submitted in accordance with the requirements for the degree of

Doctor of Philosophy of the University of London

July 2015

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Faculty of Epidemiology and Population Health

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

Funded by PROTECT (http://www.imi-protect.eu/)

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Declaration of authorship

I, Kevin Wing, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this had been indicated in the thesis.

Signed _____

Kevin Wing

Date _____

Use of published work

One paper has been published based on work undertaken for this thesis (see Published work appendix). This paper was a feature article that included proposals and discussion of some of the concepts and ideas included in this thesis. Work for this paper was carried out as part of the PhD and took place during the period of registration of the PhD. For this paper, Kevin Wing (KW) was the lead and corresponding author, and prepared all drafts of the paper. The co-authors' contributions to the manuscript were restricted to providing comments on the draft prepared by KW. Where appropriate, this paper is referenced from within the text of this thesis in the same way as any other referenced article.

Abstract

Background: Adverse drug reactions are responsible for a significant proportion of hospitalisations. This PhD aimed to develop and optimise methods for detecting serious adverse drug reactions in databases of electronic health records for use in pharmacoepidemiology and genetic epidemiology, with a focus on cholestatic liver injury.

Methods: A systematic review was performed before developing a multiple database source ("multisource") algorithm for identifying cholestatic liver injury. Multisource algorithm case status was used to guide the development of another algorithm using data from a standard UK Clinical Practice Research Datalink (CPRD) record only (the CPRD algorithm). Testing of the CPRD algorithm was performed within a cohort analysis of an established cause of the injury (flucloxacillin), before carrying out a case-control study investigating a number of putative associations (drug exposures carbamazepine, celecoxib, duloxetine, ramipril and risperidone).

Results: The majority of reviewed studies lacked a reproducible case definition, and case assignment generally required information external to database records. Secondary care (HES) data provided little additional information than that found in primary care (CPRD), meaning that the CPRD algorithm had a very good ability to discriminate between multisource algorithm cases statuses (ROC area under the curve 0.95). The flucloxacillin 45-day risk estimate obtained from the cohort study using the highest specificity CPRD algorithm (6.15 per 100 000 users, 95% CI 4.61 – 8.04) was very similar to previous studies. Celecoxib and risperidone were associated with cholestatic liver injury (celecoxib multivariablemultivariable RR recent vs. current users low specificity CPRD algorithm 1.89, 95% CI 1.11 – 3.22, risperidone multivariablemultivariable RR high specificity CPRD algorithm 2.59, 95% CI 1.41 – 4.75).

Conclusions: The CPRD algorithm detected similar flucloxacillin effects as (1) the multisource algorithm and (2) previous studies. Associations with risperidone and celecoxib were also detected. Algorithm characteristics that could facilitate (1) pharmacovigilance and (2) recruitment to genetic association studies include the ability to (a) detect cases without using information external to the EHR and (b) apply varying levels of specificity and sensitivity.

Acknowledgements

My supervisor and co-supervisor for this thesis were Ian Douglas and Krishnan Bhaskaran, and I would like to express how grateful I am to both of them for their patience, expertise, insight and guidance. I would also like to thank Tjeerd van Staa and Liam Smeeth for the support, advice and time that they provided on specific aspects of the project.

Two collaborators on the pharmacoepidemiological studies within this thesis that I would like to thank are Louise Pealing (who I discussed my results with) and Adrian Root (who was the clinician involved in review of potential cases). Olaf Klungel and Robert Reynolds were also helpful collaborators (providing input on the context of the work in relation to ongoing work by PROTECT http://www.imi-protect.eu/), as was Munir Pirmohamed (providing input related to previous work linking e-health to genetics).

Finally, I would like to thank my parents Alan and Joyce for their support, and Coby for her patience, love and understanding during the preparation of this thesis, particularly considering the imminent arrival of our first child.

Funding

Kevin Wing's PhD was funded by PROTECT. The PROTECT project has received support from the Innovative Medicine Initiative Joint Undertaking (www.imi.europa.eu) under Grant Agreement n° 115004, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. In the context of the IMI Joint Undertaking (IMI JU), the London School of Hygiene and Tropical Medicine received direct financial contributions from Pfizer.

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List of Abbreviations

ADR	adverse drug reaction
ALP	alkaline phosphatase
ALT	alanine-aminotransferase
BIFAP	(Spanish) Base de datos para la Investigación Farmacoepedemiológica en Atención Primaria
BMI	body mass index
BNF	British National Formulary
CIOMS	Council for International Organizations of Medical Sciences
CPRD	UK Clinical Practice Research Datalink
DAG	directed acyclic graph
DB	database
DILI	drug-induced liver injury
EHR	electronic health record
GP	general practitioner
GPRD	UK General Practice Research Database
HES	UK Hospital Episodes Statistics (database)
ICD	International Disease Classification
LFT	liver function test
LRT	likelihood ratio test
MHRA	UK Medicines and Healthcare products Regulatory Agency
ONS	UK Office of National Statistics
PPV	positive predictive value
PROTECT	Pharmacoepidemiological Research on Outcomes of Therapeutics by a European Consortium
R	(ALT/ULN)/(ALP/ULN)
ROC	receiver operating characteristics
SES	socioeconomic status
ULN	upper limit of normal
UTS	up to standard (data)
VAMP	Value Added Information Medical Products
WHO	World Health Organisation

1 Background

1.1 Introduction

In this chapter, the definition and epidemiology of adverse drug reactions are provided, along with an overview of drug safety and pharmacoepidemiological approaches adopted for their detection, analysis and prevention. Particular emphasis is provided on (1) the current use of databases of routinely collected electronic health records and (2) cholestatic liver injury caused by flucloxacillin and other drugs (two areas of focus of this Ph.D.). Finally, the aims and objectives of the thesis are described.

1.2 Adverse drug reactions

1.2.1 Definition, epidemiology and public health impact

The World Health Organisation defines adverse drug reactions (ADRs) as “Any response to a drug which is noxious and unintended and that occurs at doses used in man for prophylaxis, diagnosis or therapy” [1]. More common adverse reactions are classified as Type A reactions; serious (Type B or “idiosyncratic”) adverse reactions are less common, and may not be well predicted by pharmacology [2]. Type A reactions are normally due to the presence of drug levels within the body that are too high, which can be due to (1) the patient having been administered more of the drug than is advised or (2) the patient receiving a correct dose but then either metabolizing or excreting the drug slower than is normal, or being overly sensitive to the drug [3]. Reduction of dose can therefore normally be used as a treatment for Type A reactions. In contrast, Type B reactions usually require treatment to be stopped completely, and have a range of underlying causes which may be immunological (hypersensitivity reactions), inherited susceptibility, or unknown [3].

Examples of both types of reaction with an indication of the frequency at which they occur are provided by two of the known side-effects of the anti-epileptic carbamazepine. Greater than 1 in 10 users of carbamazepine experience drowsiness, which can often be alleviated by dose-reduction (a Type A reaction), while fewer than 1 in 10,000 users experience the life-threatening skin reaction Stevens-Johnson syndrome (a Type B reaction) [4] .

It has been estimated that adverse drug reactions are responsible for over 5% of hospital admissions in the EU and US [5, 6]. An additional important public health impact relates to the availability of medicines. If a marketed drug is discovered to cause serious adverse reactions, drug regulatory authorities can enforce changes to the labeling in order to restrict its use in certain populations, introduce specific risk

minimisation measures and in extreme cases they may revoke the marketing authorisation. Approximately 150 drugs have been withdrawn from the market since 1960 due to safety issues [7], in some cases many years after initial approval [8].

1.2.2 The genetics of adverse drug reactions

Evidence is increasing for a genetic predisposition to a number of adverse reactions, with genes related to enzymes involved in drug metabolism, drug receptor proteins, immune response and mitochondrial functions all having been investigated in recent years [9]. A number of genetic variants that effect susceptibility to various different drugs have been successfully identified (e.g. the HLA-B*5701 allele and Abacavir hypersensitivity, see section 1.3.4), and the magnitude of association between gene mutation and reaction phenotype has generally been substantially greater than those observed during investigations into the genetic basis of complex diseases. Genetic associations for adverse reactions also have a tendency to involve far fewer genes than those for complex diseases, suggesting the potential for relatively rapid developments in this area [9, 10].

1.3 Drug safety, pharmacoepidemiology and databases of stored electronic health records

1.3.1 Detection of adverse drug reactions during drug development

International regulations related to medicinal products require that the quality, effectiveness and safety of a newly developed medicine should be demonstrated before sale [11, 12]. Three phases of clinical testing in humans are required [3, 13]. Phase 1 studies are “first-in-human” and typically include 20 – 100 healthy volunteers, with an aim to determine a safe dosage range and exclude any common toxic reactions. In Phase 2 studies, the medicine’s efficacy is evaluated for the first time in approximately 100 – 200 patients with the target disease, to assess first information on efficacy and also to try and detect more common adverse reactions. Finally, Phase 3 studies generally include 1000 – 5000 patients exposed to the drug and obtain further detailed information related to the drug’s efficacy (through randomised controlled trials) and any less common adverse reactions.

There are a number of challenges associated with identifying and characterising adverse reactions during drug development (Figure 1.1). Firstly, the number of people exposed to the drug by the end of Phase 3 is likely to be no more than around 5000, which would mean only those reactions that occur at a frequency of approximately 1-2 per 1000 users would be detected. This would exclude many of the rarer, more serious (Type B) reactions. Secondly, the duration of use of the drug during randomised clinical

trials in Phase 3 may well be shorter than its post-approval use. Finally, the individuals exposed to the drug during the carefully controlled experimental settings of randomised controlled trials are likely to be a very restricted subset of the actual underlying population. In particular, patients in drug trials are unlikely to include the very old, very young or pregnant women, are unlikely to be taking medications other than the one under study, and are unlikely to be suffering from more than one disease [14].

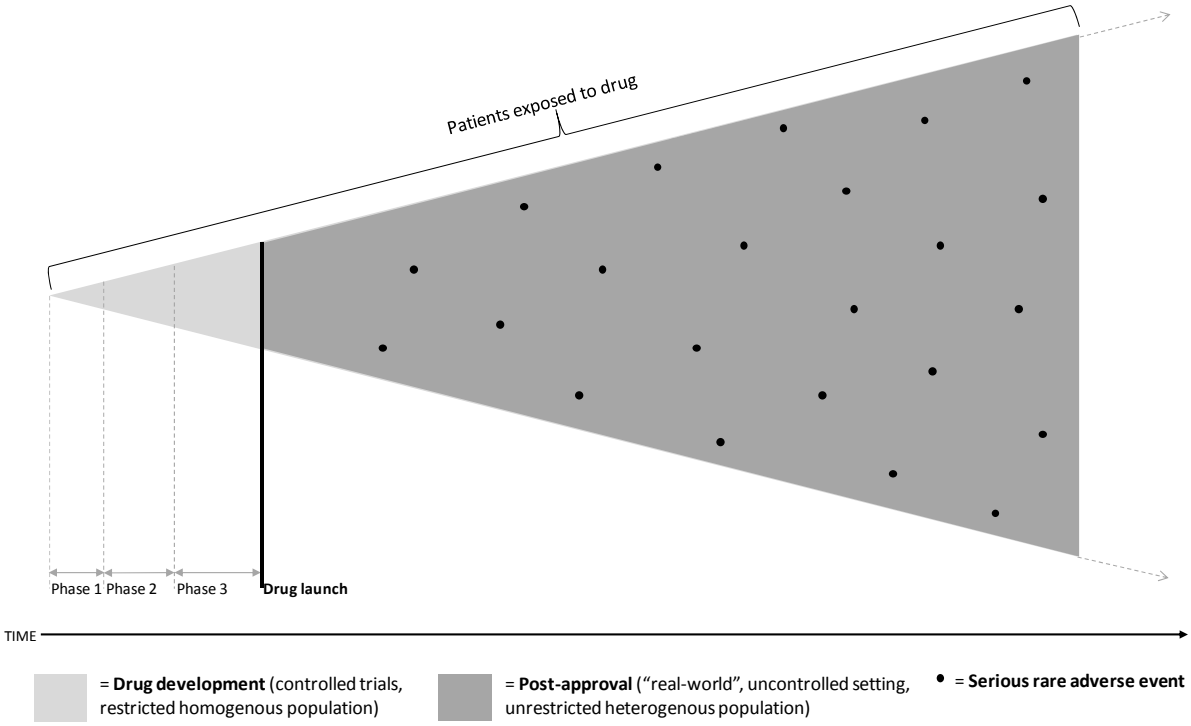


Figure 1.1: Drug development versus post-approval patient exposures – challenges for drug safety

Approx number of patients exposed to drug pre-approval: 3000 – 5000 (allowing detection of adverse events at frequencies of 1-2 per 1000 users). Post-approval exposure can involve millions of patients over the first few years of approval, meaning that adverse events occurring at frequencies less than 1 per 1000 occur and can have a substantial public health impact (in terms of direct patient impact but also due to the possibility of reduced availability of therapies due to restrictions on marketing authorisations)

1.3.2 Adverse event reporting and signal detection

An existing approach used for the detection of adverse drug reactions is the use of adverse event reporting and signal detection methods (also known as pharmacovigilance). Suspected adverse drug reactions are reported by clinicians or patients to national regulatory authorities, who enter the information into national and international databases of adverse drug reactions, such as the WHO VigiBase [15]. These databases can then be screened in order to allow potential drug adverse reaction associations that may require further analysis to be identified. The UK system for the reporting of suspected adverse drug reactions is known as the Yellow Card

Scheme, with information obtained via standardised forms entered into the UK Medicine and Healthcare products Regulatory Agency (MHRA) ADR database for signal detection [16].

Although spontaneous reporting systems such as this may enable early detection of previously unidentified serious adverse events, the information gathered is crude and subject to various types of error and bias. Outcome definition is non-standardised, information on the characteristics of the patient is often incomplete, an accurate measure of the frequency of the event cannot be obtained as the denominator is unknown and the numerator is unlikely to reflect the actual number outcomes, some outcome events are more likely to be reported than others (e.g. only more serious occurrences of a particular reaction) and there may be many external factors that influence whether the report is filed for a particular drug (e.g. reporting in the scientific literature or media).

1.3.3 Pharmacoepidemiological studies of adverse events and databases of stored electronic health records

In order to quantify and characterise the risk of an adverse reaction associated with a new (or currently marketed) medicine, large epidemiological studies of the adverse drug reaction are typically performed, also known as pharmacoepidemiological studies. The requirement for such a study may originate from a variety of sources: increased case reports in the scientific literature, detection of signals at regulatory authorities or by the WHO, clinicians noticing increased incidence or in some cases media reports. Studies may be performed by a variety of stakeholders, including pharmaceutical companies (at the request of regulatory agencies), universities working on drug epidemiology and public health, regulatory agencies themselves, not-for-profit organisations, clinical research organisations or charities.

Randomised controlled trials designed to study the adverse effect of drugs are often not feasible; the low frequency of events for any particular drug would likely mean a very large trial at very high cost and possibly for a long duration. There may also be ethical issues in assigning patients to treatment groups for the purpose of measuring harmful effects. Therefore, pharmacoepidemiological studies tend to be very large observational studies (such as cohort, case control or self-controlled case series) performed using routinely collected health records. There are a number of characteristics of (particularly Type B) adverse reactions that present challenges for epidemiology, including: their relatively low incidence, their clinical presentation, their time of onset, the characteristics of the patients in which they may occur, and the possibility of similarity of symptoms to the indication being treated. These factors mean

that study sizes often have to be very large, classification of outcome is often difficult, and confounding by concomitantly administrated medicines or indication can be problematic (see Table 1-1).

Table 1-1: Characteristics of (Type B) adverse drug reactions and associated challenges for epidemiological studies

#	Adverse drug reaction characteristic	Challenge for pharmacoepidemiological studies
1.	Low incidence for any specific drug	Very large sample sizes required
2.	Symptoms similar to other diseases	Risk of measurement error (outcome misclassification)
3.	Delayed symptoms	Risk of measurement error (outcome misclassification)
4.	Patients likely to be prescribed multiple drugs	Confounding by other medications likely
5.	Indication for treatment may be related to likelihood of experiencing future health outcomes	Confounding by indication likely

1.3.3.1 Databases of routinely collected electronic health records

Over the past 25 years, routinely collected health records stored in very large databases from primary and secondary care have become the predominant setting for pharmacoepidemiological studies. These electronic records are typically longitudinal records of health care for patients, capturing information routinely, and not for the purpose of a predesigned research study or survey [17]. A typical set of information in an electronic health record will include (anonymised) demographic information, drug prescription and diagnostic information and information on subsequent referrals. Databases are increasingly being linked, providing the potential for detailed patient profiles to be created.

Major advantages of electronic health record databases include the ability to perform very large studies at relatively low cost, a relatively complete record of drug prescriptions and prior or subsequent clinical diagnoses, the presence of lifestyle information to allow assessment of the role of confounders, minimisation of observer or participant bias, and the ability to assess the effect of drugs in the settings, populations and for the duration that they are actually being used (the “real-world”). Conversely, one major challenge is being able to accurately classify and measure exposures and outcomes in relation to any particular study question. Due to the routine nature of the data, substantial data management and development of complex algorithms using multiple variables is often required in order to allow assignment of exposure and outcome status, in addition to careful review of electronic records by medically qualified

professionals [18-20]. Furthermore, studies performed within these databases have been particularly susceptible to time-related biases [21, 22].

Three important databases for pharmacoepidemiology include the US Kaiser Permanente database, the Spanish BIFAP database and the UK Clinical Practice Research Datalink (CPRD).

Kaiser Permanente medical care programs is a U.S. private health insurance scheme covering approximately 8 million members [23]. Multiple databases covering drug prescriptions, hospital discharge information, outpatient diagnoses and laboratory test results are linked by a unique id for each patient [24]. Recent pharmacoepidemiological studies using the data have included the effectiveness and safety of Spironolactone for systolic heart failure and [25] and the cardiovascular risk associated with different rheumatoid arthritis therapies [26].

The Spanish BIFAP (Base de datos para la Investigación Farmacoepidemiológica en Atención Primaria) is a major project to evaluate the feasibility of a computerised database aggregating information provided by general practitioners and paediatricians in the Spanish National Health Service, with a particular focus on data provision for pharmacoepidemiological studies, which currently has aggregated information from around 2.2 million patients [27]. Inspiration for the project came from the UK Clinical Practice Research Datalink (CPRD, formally The General Practice Research Database), which contains anonymised data on patients from over 625 primary care practices across the UK (approximately 12 million total patients). The electronic record of every patient in the database includes a unique CPRD patient id, the date the patient first contributed data up to research-level standard (start UTS), the date the patient transferred out of the database (if applicable) and patient demographics (such as gender and date of birth). Information for each patient is then added to the electronic record by general practitioners as part of routine clinical care and includes all consultations, diagnoses, prescribed drugs, out-patient referrals and some lifestyle information [28]. Links to other databases (such as hospital records) are possible, and examples of recent pharmacoepidemiological studies on drug safety include the use of metformin and the risk of lung cancer [29] and the risk of fracture associated with bisphosphonates [30].

1.3.4 Predictive genetic testing for drug safety

The discovery of a genetic basis for many adverse drug reactions (see section 1.2.2) has enabled the possibility of predictive genetic tests to be developed for drug safety. Such tests can be performed on people who have been identified as candidates for receipt of a particular drug, in order to assess the likelihood of them having a reaction

and directing subsequent treatment accordingly. This provides the potential for the risk-benefit profile of drugs that cause serious adverse events to be improved.

One high-profile success in this area is Abacavir, an antiretroviral launched in 1998 that was found to be associated with a severe hypersensitivity reaction in approximately 8% of patients [31]. Genetic association studies were able to demonstrate the critical role of the HLA-B*5701 allele in susceptibility to the reaction [32, 33], and following development of a genetic test, randomised controlled trials were able to demonstrate the test efficacy [32]. Abacavir remains available and testing is now routinely performed.

Despite this example, the development and use of predictive genetic tests for drug safety in clinical practice is limited. The FDA currently only mandates genetic testing for four drugs (the oncology drugs cetuximab, trastuzumab and dasatinib and the antiretroviral maraviroc [34]), and these are tests for the identification of responders, rather than for drug safety. Due to that fact that drugs that cause serious adverse reactions for which there is no predictive genetic test may be removed from the market (or have their development program stopped), the public health impact of not developing predictive genetic tests is potentially very large. The unfortunate cessation of the development program of the anti-malarial Lapdap due to safety issues associated with G6PD deficiency (a testable genetic trait) is one such example [35].

Possible reasons for the lack of development and use of predictive genetic tests include the difficulty/expense associated with accurately identifying true cases for inclusion in genetic association studies, the inadequate size of these studies, the prohibitively high cost of setting up randomised controlled trials of predictive genetic test effectiveness, and the inadequate assessment of co-existing clinical and environmental determinants of the reaction [36], [37].

1.3.5 Adaptive licensing

The current drug approval process is effectively a “binary” decision system: (0) pre-approval the drug is not considered safe and effective and is therefore not available for use to the general public (1) post-approval there is a presumption of effectiveness and safety that allows use by the general public. Pharmacoepidemiological studies of drug safety are likely to be performed some time after approval of the drug, or may not be performed at all. Algorithms as described in section 1.3.4 have typically been applied to database study populations at a single time-point following drug registration, in order to retrospectively identify sufficient cases for inclusion in well-powered epidemiological studies [36].

Adaptive drug licensing is a proposal to move away from such binary regulatory categorisation to a model involving iterative data-gathering and regulatory assessment, with corresponding phased approvals (or restrictions) [38]. Observational studies of drug-safety would be likely to fit more formally into regulatory requirements associated with an adaptive licensing framework. Furthermore, approaches that could allow early identification of people who have suffered a serious reaction associated with the drug of interest from within electronic health record databases could mean that updated safety information is available much earlier than is typical within the current non-adaptive models.

1.4 Serious drug-induced liver injury

Serious (idiosyncratic, Type B) drug-induced liver injury (DILI) is the leading cause of withdrawal of drugs from the market [39, 40], and has been estimated in one study to cause hospitalisations at a frequency of 13.9 per 100,000 inhabitants [41]. Over 50 drugs are known to have some potential for causing DILI, and these include analgesics, anti-hypertensives, antipsychotics and many antimicrobials [39, 42]. Two recent important examples include troglitazone for Type 2 diabetes and the antibiotic trovafloxacin. Troglitazone was approved by the FDA for the treatment of Type 2 diabetes in 1997 and withdrawn in 2000 due to the occurrence of liver failure at a frequency of around 1 per 10,000 users [43], while trovafloxacin was approved in 1998 and its use severely restricted after two years due to the occurrence of serious hepatic events (including liver transplantation and deaths) at a frequency of around 6 per 100,000 users [44].

A number of articles have been published for assisting with the identification and classification of drug-induced liver injury, including a widely-cited paper published under the auspices of the Council for International Organizations of Medical Sciences (CIOMS) [45]. A subsequent update and consolidation article was prepared by the DILI Expert Working Group of the Phenotype Standardization Project [46], a project aiming to improve and standardize phenotype definitions to facilitate the development of predictive genetic tests for drug safety [47]. Both articles classify liver injury into three types based on the underlying cellular mechanism: cholestatic, hepatocellular or mixed. Classification relies on the results of biochemical analysis of liver enzymes (liver tests), as shown in Table 1-2. Liver biopsy or ultrasound investigations can help in determining the type of injury, but are secondary evidence to liver test results [46].

Table 1-2: Classification of DILI using liver test results

#	Type of liver injury	Liver test result
1.	Any DILI	ALT ≥ 5 x ULN or ALP ≥ 2 x ULN or ALT ≥ 3 x ULN and Bil > 2 x ULN
2.	Hepatocellular type of DILI	R* ≥ 5
3.	Mixed type of DILI (=cholestatic hepatitis)	R > 2 and < 5
4.	Cholestatic type of DILI	R ≤ 2

**R=(ALT/ULN)/(ALP/ULN), where ALT=alanine aminotransferase, ALP=alkaline phosphatase, Bil=bilirubin and ULN=upper limit of normal*

1.4.1 Drug-induced cholestatic liver injury

Drug-induced cholestatic liver injury can be defined as liver injury caused by drug therapy in which all or some of the pathology is due to impairment of the flow of bile, known as cholestasis [48]. Injury can be purely cholestatic in nature, or include a hepatocellular component and be classified as mixed (also known as cholestatic hepatitis, see Table 1-2). Common non-drug causes of cholestasis include pregnancy, alcoholism, and some cancers (with bile duct cancer commonly mistaken for drug-induced cholestasis [48]). Up to half of all hepatic drug toxicity may be accounted for by a cholestatic type of adverse reaction, although prevalence estimates for population subgroups that are likely to be more susceptible (such as the elderly) are lacking [49].

Classes of drugs that have been linked to cholestatic liver injury include non-steroidal anti-inflammatory drugs, antihypertensives, antidiabetics, anticonvulsants, lipid-lowering agents and psychotropic drugs [49]. Cholestatic reactions have a tendency to be prolonged after discontinuation of treatment, and clinical symptoms vary by whether the reaction is pure or mixed, and by the specific drug [48, 49]. Pure cholestasis is typical of synthetic steroids, and patients are likely to present with debilitating pruritus, with severe cases exhibiting jaundice. Symptoms appear 2 – 3 months after starting therapy, but may be delayed for up to 9 months. Cholestatic hepatitis is typical of numerous drugs, including: chlorpromazine, tricyclic ant-depressants, erythromycins, oxy-penicillins, amoxicillin-clavulanate, flouroquinolones and some neuroleptic agents [48]. Clinical symptoms usually occur 1 – 6 weeks after starting treatment, and can include jaundice, pruritus, fever, anorexia, upper abdominal pain, nausea, pale stools and dark urine. In most patients, symptoms normally resolve within 1 month of cessation of therapy, although complications include bile duct injury and a “vanishing bile duct syndrome” that can persist and may be fatal [48].

1.4.1.1 Flucloxacillin

Flucloxacillin is an antibiotic of the penicillin class, that has a broad range of uses in the treatment of gram-positive bacterial infections, including the treatment of skin and soft tissue infections, respiratory tract infections, urinary-tract infections and meningitis in addition to prophylaxis during some major surgical procedures [50]. First available in 1960, case reports appeared in the 1980's of a serious adverse drug reaction in which the patient developed cholestatic hepatitis, which in some cases could be fatal [51]. The vast majority of reports of the reaction were from Australia, a type of "mini-epidemic" caused by a rapid increase in use and increase in publicity of the adverse reaction leading to a large increase in spontaneous report [48, 52]. Intense promotion of alternative therapies has decreased the use of flucloxacillin in Australia, while in the UK it remains heavily prescribed [53]. In the U.S., and some European countries, flucloxacillin is not marketed, but alternative therapies that are perceived to have a better safety profile are used (such as dicloxacillin).

Studies have shown that flucloxacillin causes cholestatic hepatitis at a frequency of around eight per 100,000 users, and is characterised by cholestatic liver test results, pruritus and jaundice, which may occur up to 45 days from initiation of treatment (and in some cases after discontinuation of therapy) [52-54]. Age, duration of use and gender may be related to susceptibility [53, 55, 56]. A genetic association study demonstrated a very strong association between the flucloxacillin-induced adverse reaction phenotype and the HLA-B*5701 allele, suggesting the reaction has an immune-related mechanism [57, 58]. Despite the strong association, the numbers needed to screen to prevent one case (upwards of 13,000) mean that a predictive genetic test is not yet feasible, and further work on the characterisation of environmental and genetic susceptibility is required [59].

1.5 Thesis aim, rationale and objectives

1.5.1 Aim

The aim of this thesis is to develop new methods for the measurement and detection of serious adverse drug reactions in databases of stored electronic health records, with a focus on cholestatic liver injury caused by (1) flucloxacillin (2) other drugs.

1.5.2 Rationale

Methods developed during the work on this thesis should facilitate pharmacoepidemiological studies in databases of routinely collected electronic health records, the development of predictive genetic tests and adaptive licensing.

1.5.3 Objectives

(1) To perform a systematic literature review on the identification of individuals with cholestatic liver injury in epidemiological studies utilising databases of routinely collected electronic health records
(2) To develop a “multi-source” algorithm using the UK clinical practice research datalink (CPRD) and other sources to identify cases that are potentially drug-induced cholestatic liver injury
(3) To develop a CPRD algorithm that uses data only within the CPRD database for the identification of cases of potential drug-induced cholestatic liver injury, and to validate this against the “multi-source” algorithm from (2)
(4) To test the CPRD algorithm within an epidemiological study on a known drug cause of cholestatic liver injury (flucloxacillin) and improve the understanding of the epidemiology of this reaction
(5) To use the CPRD algorithm to study drugs that are putatively associated with drug-induced cholestatic liver injury

1.5.4 Organisation of the thesis

Chapter 2 describes a systematic review performed to understand (i) how studies on cholestatic liver injury have been performed in databases of electronic health records. Chapter 3 describes the data sources used and overall methods for research. Chapter 4 provides results related to development of the new algorithm for identifying cholestatic liver injury within the CPRD. Chapters 5 and 6 provide detailed results for the epidemiological studies performed using the new methods. Chapter 7 summarizes and discusses the overall findings in relation to the background, rationale and context of the work.

1.6 Chapter 1 Summary

- This chapter provided the background to the project, including discussion of: adverse drug reactions, drug safety and pharmacoepidemiology, databases of stored electronic health records and cholestatic liver injury

- Adverse drug reactions are responsible for a significant proportion of all hospitalisations and can impact the availability of medicines

- Databases of stored electronic health records are used to study the epidemiology of adverse drug reactions and predictive genetic tests for drug safety have been developed for a limited number of drugs

- Serious drug-induced liver injury is the most common type of serious adverse drug reaction, with cholestatic liver injury caused by the antibiotic flucloxacillin an important example

- This PhD will develop new methods for detecting serious adverse drug reactions in the UK clinical practice research database that can improve pharmacoepidemiological studies, facilitate predictive genetic test development and be used in an adaptive drug licensing model, with a focus on cholestatic liver injury caused by flucloxacillin and other drugs

2 Systematic review on the identification of individuals with cholestatic liver injury for epidemiological studies utilising databases of stored electronic patient health records

2.1 Introduction and Aims

The primary objective of this review was to provide an overview and understanding of how cholestatic liver injury has been defined as an outcome and how individuals have been assigned as having cholestatic liver injury in epidemiological studies that utilise pre-existing population-based databases of patient health records. The specific questions of interest were:

1. What are the diagnostic codes, laboratory test cut-offs and clinical descriptions that have been used to define cholestatic liver injury in the study?
2. Which data sources were used to obtain information related to the diagnosis of cholestatic liver injury?
3. How were the diagnostic criteria in (1) applied to the data sources in (2), and how reproducible was the case-definition?

2.2 Methods

2.2.1 Search strategy

2.2.1.1 Databases and sources

Two very large medical journal databases, MEDLINE (1946 to present) and EMBASE (1947 to present) were searched electronically on the 30th June 2014 for studies that were performed within databases of stored (routinely collected) electronic health records and had cholestatic liver injury (or a synonym) mentioned as the main or one of a number of outcomes. In order to obtain any potential "grey literature" the websites of the electronic health record databases or electronic health record database research groups were manually searched for publications: The General Practice Research Database, The Health Improvement Network Database, The Hospital Episode Statistics database, The Base de datos para la Investigación Farmacoepidemiológica en Atención Primaria (BIFAP), The PHARMO institute for Drug Outcomes Research and The Boston Collaborative Drug Surveillance Program. The reference list of selected publications found using the above methods were also searched for relevant articles, and any found were used to amend the search terms as necessary and update the search. Reference lists of any review articles obtained were also searched.

2.2.1.2 Search keywords and terms

In designing the search strategy, the research question was divided into two main categories of terms: (1) terms related to cholestatic liver injury and (2) terms related to databases of stored electronic health records. Terms within each category were combined by "or" statements while the two categories were combined by an "and" statement.

Within Medline (indexed according to the MeSH terms), the following MeSH keywords were identified and used:

CHOLESTASIS or DRUG-INDUCED LIVER INJURY or CHOLESTASIS, INTRAHEPATIC **and** DATABASE MANAGEMENT SYSTEMS or DATA COLLECTION or INFORMATION STORAGE AND RETRIEVAL

Within EMBASE (indexed according to its own hierarchy of terms), the following EMBASE terms were identified and used:

CHOLESTATIC LIVER INJURY **and** DATA BASE

A number of terms were then identified as being relevant to the search, but not available for selection as headings in either of the databases' indexes. These were therefore included as search terms of article title, abstract or keywords in both of the databases, and were as follows (with "*" indicating that any subsequent ending is acceptable, "adj" specifying the number of permissible separating words before or after the terms and "#" meaning any letter is permissible at this position):

CHOLESTAT* adj1 HEPAT* or CHOLANGIOL#TIC adj1 HEPAT* or CHOLESTAT* LIVER adj1(FAIL* or DISEASE* or DAMAGE*, INJUR*) or CHOLANGIOL#TIC* LIVER ADJ1(FAIL* or DISEASE* or DAMAGE*, INJUR*) **and** ADMINISTRATIVE or DATAB* or PRACTICE MANAG* adj1(SYSTEM or SOFTWARE) or AUTOMAT* adj1(RECORD*) or COMPUTER* adj7(GENERAL PRACT*) or RECORD* adj5(COMPUTER*) or ARIANNA or BIFAP or BASE DE DATOS PARA LA INVESTIGACION FARMACOEPEDEMIOLOGICA ATENCION PRIMARIA or BOSTON COLLABORATIVE DRUG SURVEILLANCE PROGRAM or BRIDGE DATABASE OF DATABASES or CALLIOPE or CCEP or CPRD or DAPI or DANISH MEDICAL REGISTRIES or EFEMERIS or ELECTRONIC MEDICAL RECORDS DATA or GECEM INVENTORY or GEPARD or GPRD or GROUP HEALTH COOPERATIVE or HOSPITAL CDM-US or HOSPITAL EPISODE STATISTICS or HES or HARVARD PILGRIM HEALTH CARE or HEALTHCORE or HENRY FORD HEALTH SYSTEMS or HEIS or HCUP or HMORN or IMS or INDIANA HEALTH INFORMATION EXCHANGE or IMPCI or INTERACTION DATABASE or IADB or INTEGRATED PRIMARY CARE INFORMATION DATABASE or KAISER PERMENENTE NORTHWEST or KAISER

PERMANENTE MEDICAL CARE PROGRAM or LIFELINK or MANITOBA HEALTH RESEARCH or MEDICAID or MEDICARE or MEDICINES MONITORING UNIT or MIS or ODENSE UNIVERSITY PHARMACOEPIDEMIOLOGICAL DATABASE or OPED or PCCIU-R or PEDIANET or PEM or PHARM or PHARMO RECORD LINKAGE SYSTEM or PHARMACOEPIDEMIOLOGICAL PRESCRIPTION DATABASES OF NORTH JUTLAND or PDNJ or POPULATION DATA BC or POPULATION HEALTH RESEARCH UNIT or PRESCRIPTION CLAIMS DATA or PRESCRIPTION EVENT MONITORING DATABASE or QRESEARCH or SASKATCHEWAN HEALTH SERVICES or SEER-MEDICARE LINKED DATABASE or SIDIAP or SWEDISH CENTRE FOR EPIDEMIOLOGY or UNITED HEALTH GROUP or TAYSIDE MEDICINES MONITORING UNIT or THE HEALTH IMPROVEMENT NETWORK or THIN adj7(DATA*) or THIN adj7(COMPUTER*) or VALUE ADDED MEDICAL PRODUCTS or VACCINE SAFETY DATALINK or VETERANS ADMINISTRATIVE DATABASES.

Note that the terms “COMPUTER* adj7 GENERAL PRACT*” and “RECORD* adj5 COMPUTER*” were added to the search terms after a number of documents had been found through searching database websites/reference lists of the retrieved articles that had not been retrieved by the database searches. Updating the search terms accordingly for each database allowed these documents (and additional ones using similar terms) to be retrieved.

2.2.1.3 Inclusion and exclusion criteria

Inclusion criteria

The following inclusion criteria were applied:

Any type of study design.
Outcome (primary or otherwise) of the study is cholestatic liver injury (or a synonym).
Study population and outcome/exposure information have been obtained from a database of stored electronic health records that were collected prior to the design of the study.

Exclusion criteria

The electronic search strategy was designed with a high sensitivity but low specificity, in order to ensure that all relevant articles would be retrieved. The following exclusion criteria were applied by manually reviewing the title, abstract (and full document where necessary) of retrieved articles in order to increase the specificity of the search method.

Studies involving: (1) pharmacovigilance (signal-detection or spontaneous reporting) databases or (2) databases that were not population-based, for example databases:

- containing records related to a specific disease (or diseases) only
- holding records on a single or selected hospital departments only (such as a hepatology clinic)
- relating to a specific procedure (for example, liver transplants)
- holding information only on medical inpatients
- created specifically for a clinical trial.

2.2.1.4 Procedure

Titles and abstracts of all retrieved articles were screened for an initial assessment of eligibility for inclusion. For the articles that fulfilled the inclusion criteria defined in section 2.2.1.3 (or articles for which eligibility was still unclear), the full text was obtained for review. Eligibility decisions were finalised based on the full-text review as necessary. The list of information extracted from each included article is provided in Table 2-1, along with a rationale for why the information was considered important. Reference was made to existing standards for the reporting of observational studies [60] when deciding what information to extract, with a particular focus on case-identification methods and their reproducibility [61, 62].

Table 2-1: Information extracted from reviewed studies, with rationale

Description of information extracted	Rationale
1. First author and year of publication	Descriptive information about the study
2. Database(s)	Descriptive information about the study
3. Study design	Descriptive information about the study
4. Outcome(s)	Descriptive information about the study
5. Exposure(s)	Descriptive information about the study
6. Main study results (for cholestatic injury type) including number in exposure groups, frequency of cholestatic liver injury and risk, rate or odds ratio (where possible)	Allows the impact of different case identification approaches on the study results to be assessed.
7. Age range criteria	Descriptive information about the study and allows understanding of how comparable results are across studies
8. Database diagnostic terms/codes used	Use of different diagnostic codes across studies for outcome definition is likely to influence the number of cases identified. Providing lists of terms allows comparisons to be made across studies and facilitates reuse of an electronic health record case

Description of information extracted	Rationale
	identification method.
9. Any liver test criteria used	Liver test results that may be recorded in databases of electronic health records provide a way of assessing the presence of liver injury (including cholestatic injury). Publication of the biochemical criteria used to define liver injury facilitates the comparison of results obtained across studies and the reuse of the case identification method by other research groups.
10. Whether external standards were referenced	Use of external standards for case identification can reduce measurement error and increase reproducibility of methods by other research groups. For example, external standards for defining type of liver injury.
11. Time interval between diagnostic code and liver test	Due to the routine nature of the data used, the time interval that defines that a diagnostic code and a subsequent (or preceding) liver test are part of the same clinical episode is applied at the discretion of the researchers. Varying time intervals could influence whether somebody is assigned as a case or not by one case identification method compared to another.
12. How the data were used (e.g. diagnostic codes, full computer record, external sources)	Sources of data used in studies of electronic health records might include diagnostic codes/liver test results, other information in the electronic record and information requested directly from clinicians (e.g. paper records external to the database). Case identification methods within a study may rely on specific combinations of these sources, and the data quality and availability associated with each source is likely to lead to different results across studies.
13. Whether blinding to exposure status was performed	A lack of blinding to exposure status can lead to observer bias, where case status assignment may become subjective rather than objective. This could undermine the case identification process.
14. Response rate for external note requests and comment on impact of low response rate	If a case-identification process relies heavily on external (paper) notes requested directly from clinicians and there is a very low response rate, this could be a factor in the number of cases that were identified in the study.
15. Whether appropriate reviewers were clearly identified (e.g. medical qualifications, speciality)	Provides an indication as to whether appropriate steps of case identification involved people with expertise in the disease area being studied (i.e. liver-related conditions)
16. Whether exclusions were applied appropriately	Applying exclusions inappropriately can lead to bias in cohort studies (if follow-up time is not correctly assigned) and in case-control studies (if different criteria are applied to the potential case and control populations). Clear descriptions of how exclusions were handled facilitates comparison of results obtained across studies that may have used similar case-identification methods.
17. Whether there was an attempt to measure method validity	A comparison of the case identification method used with another standard can help demonstrate how

Description of information extracted	Rationale
	well a case-identification method has performed.
18. Whether more than one case level was defined	Where there is clinical uncertainty around case identification (often due to a lack of information in routinely collected data for all patients), using multiple defined case levels can assist in interpretation of results obtained and facilitate comparisons across different studies.

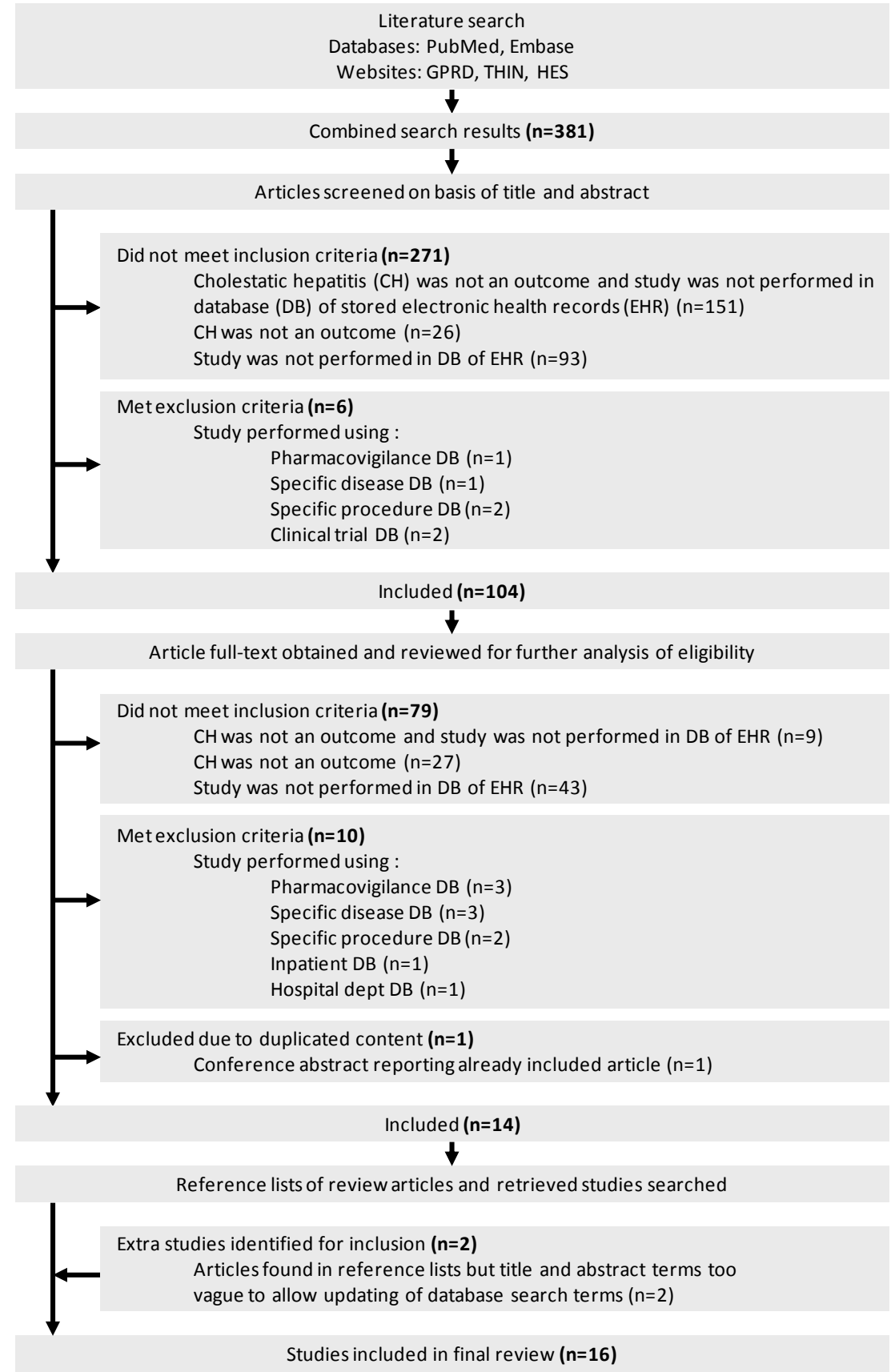
The search was initially conducted in October 2011. An auto-update was setup so that the search then ran automatically (monthly) during the preparation of the other sections of this project. The final update/run of the search was performed in July 2014.

2.3 Results

The automated searches of the Medline and EMBASE databases retrieved 412 articles, 36 of which were duplicated between databases, leaving 376 unique articles. Manual searching of the websites specified in section 2.2.1.1 retrieved a further 5 articles, giving a combined search result of 381 articles. Based upon title and abstract review, 277 articles were excluded because they either did not meet one or both of the inclusion criteria (271) or met the exclusion criteria (6). The full text of the remaining 104 documents was then obtained for further review. Review of full text led to the exclusion of a further 90 articles, 79 that did not meet either or both of the inclusion criteria, 10 that met the exclusion criteria and 1 that was a duplicated report (conference proceedings) of the results of another included article.

During the search, 2 eligible studies were obtained from a review of the reference lists and included in the final number of articles for review (updating of the search terms based on these two studies was not possible due to the vague terms used for electronic health record databases in the abstract and title of these articles). An overview of these results is provided in Figure 2.1.

Figure 2.1: Flow diagram of search strategy used



Flow diagram format adapted from PRISMA guidelines for systematic review and meta-analyses [63]

2.3.1 Description of studies included in the final review

A total of sixteen studies performed between 1992 and 2014 were included in the final review [53, 55, 64-77]. Table 2-2 contains a description of the studies. The earliest was performed in 1992 [64] and the most recent in 2014 [77]. Of these sixteen studies, one was performed using records from the Saskatchewan Health Plan Database, Canada [64], two in the Kaiser Permanente Southern California (KPSC) electronic medical record system, USA [76, 77] and the remaining thirteen were performed using either the General Practice Research Database (GPRD) in the UK [53, 69-75] or its predecessor (the Value Added Information Medical Products Ltd, VAMP) [55, 65-68]. Twelve of the sixteen studies utilised a cohort design only. Of the four that used other designs, three cohort studies that included a secondary nested case control analysis [66, 68, 71], while the other was a case-control study only [74].

Five studies had cholestatic liver injury as the main outcome [53, 55, 65, 67, 75] while in the remaining studies, cholestatic liver injury was one of a number of outcomes under a broader aim of the whole study (looking at acute liver injury, for example). The drug exposures under study included non-steroidal anti-inflammatory drugs, flucloxacillin, oxytetracycline, erythromycin, chlorpromazine, isoniazid, trimethoprim-sulfamethoxazole, amoxicillin, cimetidine, ketoconazole, macrolides, tetracyclines, metoclopramide, betahistine, sulphasalizine, azathioprine, diclofenac and antiepileptics. Three specific drug-exposures were studied multiple times: the antibiotics flucloxacillin and amoxicillin & clavulanic acid, and the antipsychotic chlorpromazine.

Table 2-2: Description of studies performed in databases of electronic health records that have cholestatic liver injury as an outcome

First author and year of publication	Electronic health record database	Study design	Outcome(s)	Exposure(s)	Number of people in exposure group (number of cases of cholestatic liver injury)[1]	Frequency of cholestatic liver injury (by exposure)[2]	Risk, rate or odds ratio[3]
García Rodríguez 1992 [64]	Saskatchewan Health Plan Database, Canada	Cohort	Acute liver injury (including cholestatic injury)	NSAIDs	5 different NSAIDs 177 550 (11) No NSAIDs 467 906 (9)	5 different NSAIDs 6.2 per 100 000 (95% CI 3.1 – 11.1) No NSAIDs 1.9 per 100 000 (0.9 – 3.7)	3.2 (1.3 - 7.8)
Derby 1993a [55]	VAMP, UK	Cohort	Cholestatic hepatitis (2 case definitions: "***characteristic"*** and ***"consistent"***)	Flucloxacillin compared with oxytetracycline	*"characteristic"* Flucloxacillin 132 087 (6) Oxytetracycline 145 844 (3) *"characteristic"* + *"consistent"* Flucloxacillin 132 087 (10) Oxytetracycline 145 844 (3)	*"characteristic"* Flucloxacillin 4.5 per 100 000 (1.7 – 9.9) Oxytetracycline 2.1 per 100 000 (0.4 – 6.0) *"characteristic"* + *"consistent"* Flucloxacillin 7.6 per 100 000 (3.6 – 13.9) Oxytetracycline 2.1 per 100 000 (0.4 – 6.0)	*"characteristic"* 2.2 (0.6 – 8.8) *"characteristic"* + *"consistent"* 3.3 (0.9 – 12.2)
Derby 1993b [65]	VAMP, UK	Cohort	Cholestatic hepatitis (2 case definitions: "***characteristic"***	Erythromycin	*"characteristic"* Erythromycin 366 064 (10) *"characteristic"* + *"consistent"* Erythromycin	*"characteristic"* Erythromycin 2.7 per 100 000 (1.3 – 5.0) *"characteristic"* + *"consistent"* Erythromycin	*No comparator group*

First author and year of publication	Electronic health record database	Study design	Outcome(s)	Exposure(s)	Number of people in exposure group (number of cases of cholestatic liver injury)[1]	Frequency of cholestatic liver injury (by exposure)[2]	Risk, rate or odds ratio[3]
			and ***"consistent"***)		366 064 (13)	3.6 per 100 000 (1.9 – 6.1)	
Derby 1993c [66]	VAMP, UK	Cohort & nested case control	Liver toxicity (including cholestatic jaundice) (2 case definitions: "***probable"*** and ***"possible"***)	Chlorpromazine, isoniazid	*"probable"* Chlorpromazine 10 502 (3) Isoniazid 921 (3) *"probable"* + *"possible"* Chlorpromazine 10 502 (3) Isoniazid 921 (4)	*"probable"* Chlorpromazine 28.6 per 100 000 (5.9 – 83.5) Isoniazid 325.7 per 100 000 (67.2 – 951.9) *"probable"* + *"possible"* Chlorpromazine 28.6 per 100 000 (5.9 – 83.5) Isoniazid 434.3 per 100 000 (118.3 – 1112.0)	*No comparator group*
Jick 1994 [67]	VAMP, UK	Cohort	Cholestatic hepatitis	Flucloxacillin compared with oxytetracycline	Flucloxacillin 77 552 (5)	Flucloxacillin 6.4 per 100 000 (2.1 – 15.0)	*No comparator group*
García Rodríguez 1994 [68]	VAMP, UK	Cohort & nested case control	Acute liver injury (including cholestatic injury)	NSAIDs	12 different NSAIDs 625 307 (17)	12 different NSAIDs 2.7 per 100 000 (1.6 - 4.4)*	*No comparator group*

First author and year of publication	Electronic health record database	Study design	Outcome(s)	Exposure(s)	Number of people in exposure group (number of cases of cholestatic liver injury)[1]	Frequency of cholestatic liver injury (by exposure)[2]	Risk, rate or odds ratio[3]
Jick 1995 [69]	GPRD, UK	Cohort	Serious drug toxicity (including cholestatic hepatitis)	Trimethoprim-sulfamethoxazole, trimethoprim, cephalexin	TMP-SMZ 232 390 (5) Trimethoprim 266 951 (4) Cephalexin 196 397 (3)	TMP-SMZ 2.2 per 100 000 (0.7 - 5.0) Trimethoprim 1.5 per 100 000 (0.4 - 3.8) Cephalexin 1.5 per 100 000 (0.3 - 4.5)	*No comparator group*
García Rodríguez 1996 [70]	GPRD, UK	Cohort	Acute liver injury (including cholestatic liver injury)	Amoxicillin and clavulanic acid (combination) compared with amoxicillin	Amoxicillin & clavulanic acid 93 433 (19) Amoxicillin 329 213 (7)	Amoxicillin & clavulanic acid 20.3 per 100 000 (12.2 – 31.8) Amoxicillin 2.1 per 100 000 (0.9 – 4.4)	9.6 (4.0 – 22.7)
García Rodríguez 1997 [71]	GPRD, UK	Cohort & nested case control	Acute liver injury (including cholestatic liver injury)	Cimetidine and other acid-suppressing anti-ulcer	Anti-ulcer drugs 108 981 (18)	Anti-ulcer drugs 16.5 per 100 000 (9.8 – 26.1)	*No comparator group*[5]
García Rodríguez 1999 [72]	GPRD, UK	Cohort	Acute liver injury (including cholestatic liver injury)	Ketoconazole and other antifungal drugs	Fluconazole 35 833 (0) Griseofulvin 6 731 (0) Itraconazole 19 488 (2)	Fluconazole 0.0 per 100 000 (0.0 – 10.3) Griseofulvin 0.0 per 100 000 (0.0 – 54.8) Itraconazole 10.3 per 100 000 (1.2 – 37.1)	*No comparator group*[5]

First author and year of publication	Electronic health record database	Study design	Outcome(s)	Exposure(s)	Number of people in exposure group (number of cases of cholestatic liver injury)[1]	Frequency of cholestatic liver injury (by exposure)[2]	Risk, rate or odds ratio[3]
					Ketoconazole 1 052 (2) Terbinafine 13 430 (1)	Ketoconazole 190.1 per 100 000 (23.0 – 686.8) Terbinafine 7.4 per 100 000 (0.2 – 41.5)	
Huerta 2002 [73]	GPRD, UK	Cohort	Acute liver injury (including cholestatic injury)	Diabetes and antidiabetic [70, 74, 77]drugs	Diabetes 98 726 (8) No diabetes 148 028 (5) Sulfonylureas 17 151 (5) Metformin 10 471 (3) Arcabose 1 550 (1) Insulin 8 880 (2)	Diabetes 8.1 per 100 000 (3.5 - 16.0) No diabetes 3.4 per 100 000 (1.1 - 7.9) Sulfonylureas 29.1 per 100 000 (9.5 – 68.0) Metformin 28.7 per 100 000 (5.9 – 83.7) Arcabose 64.5 per 100 000 (1.6 – 359.5) Insulin 22.5 per 100 000 (2.7 – 81.4)	Diabetes vs. no diabetes: 1.6 (0.8 – 3.4) *Adjusted for age, sex, calendar year and antidiabetic drug use.*
De Abajo 2004 [74]	GPRD, UK	Case-control	Drug-induced liver injury (including cholestatic injury)	Wide range of drugs	Amoxicillin & clavulanic acid 151 942 (8) Flucloxacillin 155 185 (3) Tetracyclines	Amoxicillin & clavulanic acid 5.3 per 100 000 (2.3 – 10.4) Flucloxacillin 1.9 per 100 000 (0.4 – 5.6) Tetracyclines	*No comparator group*

First author and year of publication	Electronic health record database	Study design	Outcome(s)	Exposure(s)	Number of people in exposure group (number of cases of cholestatic liver injury)[1]	Frequency of cholestatic liver injury (by exposure)[2]	Risk, rate or odds ratio[3]
					162 417 (4) Macrolides 243 832 (3) Sulpiride 1 241 (2) Chlorpromazine 4 432 (6) Sulfasalazine 5 335 (3) Azathioprine 2 204 (2) Metoclopramide 41 689 (3) Chlorpheniramine 43 137 (2) Betahistine 15 780 (2) Diclofenac 157 721 (7)	2.5 per 100 000 (0.7 – 6.3) Macrolides 1.2 per 100 000 (0.3 – 3.6) Sulpiride 161.2 per 100 000 (19.5 – 582.2) Chlorpromazine 135.4 per 100 000 (49.7 – 294.7) Sulfasalazine 56.2 per 100 000 (11.6 – 164.3) Azathioprine 90.7 per 100 000 (10.9 – 327.8) Metoclopramide 7.2 per 100 000 (1.5 – 21.0) Chlorpheniramine 4.6 per 100 000 (0.6 – 16.7) Betahistine 12.7 per 100 000 (1.5 – 45.8) Diclofenac 4.4 per 100 000 (1.8 – 9.1)	
Russman 2005	GPRD, UK	Cohort	Cholestatic liver	Flucloxacillin compared	Flucloxacillin	Flucloxacillin	11.1 (1.5 – 82.2)

First author and year of publication	Electronic health record database	Study design	Outcome(s)	Exposure(s)	Number of people in exposure group (number of cases of cholestatic liver injury)[1]	Frequency of cholestatic liver injury (by exposure)[2]	Risk, rate or odds ratio[3]
[53]			disease	with oxytetracycline	283 097 (24) Oxytetracycline 131 189 (1)	8.5 per 100 000 (5.4 – 12.6) Oxytetracycline 0.8 per 100 000 (0.02 – 4.3)	
Li 2009 [75]	GPRD, UK	*Same as Derby 1993a*	*Same as Derby 1993a*	*Same as Derby 1993a*	Flucloxacillin 346 072 (21) Penicillin 1 179 360 (4) Co-fluampicil 159 215 (6)	Flucloxacillin 6.1 per 100 000 (3.8 – 9.3) Penicillin 0.3 per 100 000 (0.1 – 0.9) Co-fluampicil 3.8 per 100 000 (1.4 – 8.2)	*Flucloxacillin compared with penicillin* 17.9 (6.1 – 52.1)
Shin 2013 [76]	KPSC, USA	Cohort	Drug-induced liver injury (including cholestatic injury)	14 drugs frequently associated with hepatotoxicity	14 different drug exposures[6] 601 125 (68)	14 different drug exposures 11.3 per 100 000 (8.9 – 14.3)	*No comparator group*
Cheetham 2014 [77]	KPSC, USA	Cohort	Drug-induced liver injury (including cholestatic injury) (2 case definitions:	15 drugs frequently associated with hepatotoxicity	*"probable or highly probable"* 15 different drug exposures[6] 1 239 071 (1 222) *"possible" + "probable or highly probable"* 15 different drug exposures[6] 1 239 071 (5 923)	*"probable or highly probable"* 15 different drug exposures 98.6 per 100 000 (93.2 – 104.3) *"possible" + "probable or highly probable"* 15 different drug exposures 478.0 per 100 000 (465.9 – 490.4)	*No comparator group*

First author and year of publication	Electronic health record database	Study design	Outcome(s)	Exposure(s)	Number of people in exposure group (number of cases of cholestatic liver injury)[1]	Frequency of cholestatic liver injury (by exposure)[2]	Risk, rate or odds ratio[3]
			"***possible"*** and ***"probable or highly probable"***)				

[1]**Cholestatic liver injury:** Cholestatic liver injury includes any liver injury with a cholestatic component i.e. pure cholestatic and/or cholestatic hepatitis (mixed).
[2]**Frequency of cholestatic liver injury** All bracketed figures are 95% Confidence Intervals. Frequency estimates extracted from those in each article or if not present for cholestatic liver injury, calculated from the number of cases of cholestatic liver injury reported in each exposure group. 95% confidence intervals were calculated assuming a Poisson distribution. All denominators are number of people exposed, apart from García Rodríguez 1992/1999 and Huerta 2002 which are person-years (choice of denominator used in this table dependent on the information provided in the article).
[3]**Risk, rate or odds ratio:** Unadjusted unless specified otherwise. Where these figures were not included in the article, they were calculated from the published or calculated frequencies in each exposure group.
[4]**Jick 1994:** This study was an extension of the Derby 1993b study
[5]**No comparator group (García Rodríguez 1997/1999, De Abajo 2004):** Comparator groups of non-drug-use are discussed in García Rodríguez 1997/1999 but cases within this exposure group are not broken down by liver injury type. Furthermore, in the García Rodríguez 1997 and the De Abajo 2004 articles, odds ratios for separate drugs estimated by case control analysis are presented, but the case-control analysis includes any liver injury type (not just cholestatic).
[6]**Multiple different drug exposures (Shin 2013, Cheetham 2014):** Incidence of cholestatic injury only presented for the entire cohort in the article, not broken down by drug

2.3.2 Overall approach to identification of individuals with cholestatic liver injury

The general methods used for defining and obtaining cases of cholestatic liver injury were similar across the majority of studies. An overview is provided in Figure 2.2, and may be described as follows:

Step 1: Definition of diagnostic criteria: authors identified (1) a broad list of relatively non-specific database diagnostic codes and (2) more specific laboratory test result parameters and/or descriptive clinical information (other than database codes) to use as criteria for selecting individuals with cholestatic liver injury.

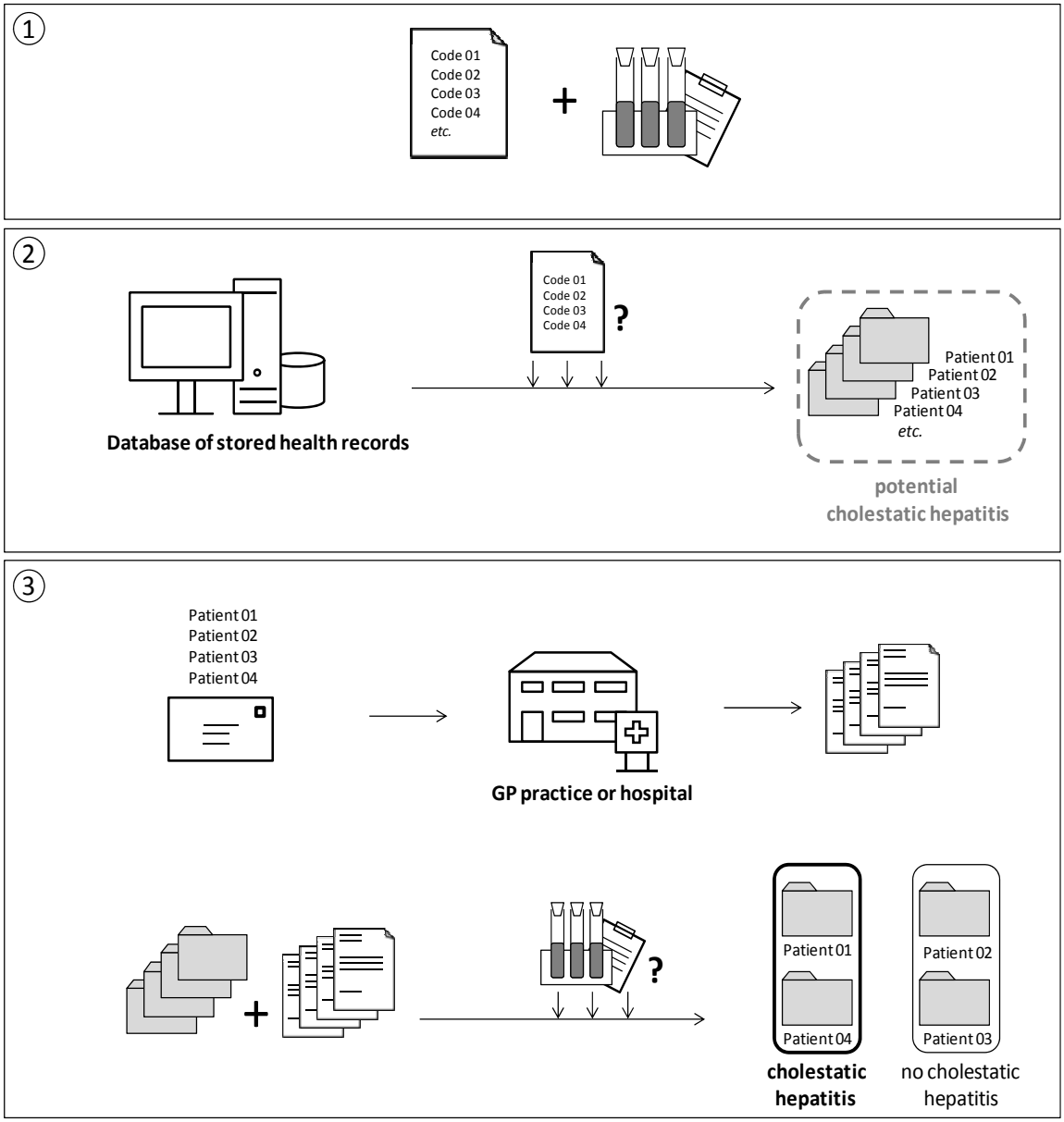
Step 2: Identification of database records of potential cases: the patient id and full (anonymised) electronic records of patients with any of the computer database codes in the codelist were obtained. Additional processing of the computer records of potential cases may have been performed to exclude patients with certain conditions, and some exclusions may also have been applied to the entire study population, based on the presence of specific codes prior to start of follow-up.

Step 3: Finalisation of cases status based on information external to the database: general practices (or hospitals) were contacted by post (or in person) to request additional information for the patients with the patient ids identified by the broad computer diagnostic code search. The information provided by GPs was reviewed, and this information considered with the full database record in order to assign individuals identified as possible cases in the computer search as (definite) cases or non-cases. Analysis could then be performed using the designated case status and information on (e.g. drug) exposures contained in the full database record.

For the two studies that applied a slightly different method, cases were identified solely on the basis of liver test results (without the use of diagnostic codes) [77, 78].

All of the steps applied across all studies will be considered in greater detail in the following sections.

Figure 2.2: Overview of methodology employed for identifying individuals with cholestatic liver injury in 14 of the 16 included studies



Step 1: Definition of diagnostic criteria
A broad list of database disease codes and more specific laboratory/clinical criteria are identified as diagnostic criteria for cholestatic liver injury.

Step 2: Identification of database (DB) records of potential cases
The list of database disease codes is used to identify indiviudals with records in the database who potentially have the outcome and their full electronic record is retrieved. Exclusion criteria may also be applied to potential cases at this stage (e.g. by excluding those with specific codes).

Step 3: Finalisation of case status based on information external to the DB
Additional information (e.g. medical notes) for the patients with patient ids identified in step (2) are requested and obtained from GP practices or hospitals, and the the laboratory/clinical diagnostic criteria are applied during a review of medical notes and computer record in order to provide a definite classification of cholestatic liver injury. Analysis using e.g. drug exposure information held within the electronic record is then performed to obtain frequency and effect measures in relation to cholestatic liver injury.

2.3.3 Step 1: Definition of diagnostic criteria

2.3.3.1 Database codelists

Fourteen of the sixteen studies initially used a list of database disease codes to select patients that could potentially have acute liver injury. Providing the actual list of codes used in the published studies provides benefit by (1) allowing readers to assess how accurate/biased the studies might be and (2) allowing the studies to be reproduced in order to support development of an evidence base. Within the fourteen studies, the specific type of code used depended upon the design of the database in which the study was performed. The study performed by García Rodríguez et al in the Saskatchewan Health Plan database [64] therefore used International Disease Classification (ICD) codes (version 9), while all GPRD/VAMP based studies relied on the OXMIS coding system [53, 55, 65-75], with one GPRD study additionally including READ codes [53]. Only three of the fourteen studies did not either (1) include a list of all codes used or (2) clearly reference another study which contained the codes used. The codelists for these studies were therefore obtained from the authors by contacting them personally [53, 70, 74]. A complete list of all codes and their frequency of use (across all studies and across those analysing only cholestatic liver injury) is provided in Table 2-3, while the list of database codes used by each of the studies can be found in Table 2-5 (Step 1 column). Chapter 2 Appendix (Table A1) contains all the diagnostic codes used listed alphabetically.

Table 2-3: Frequency of database codes used by all (14) studies that applied a code search and by the (5) studies that analysed only cholestatic liver injury

Database code *(OXMIS unless specified)*	**Diagnosis** (OXMIS terms)	**Frequency across all studies** (OXMIS, other)	**Frequency across cholestatic liver injury studies**
570XX*, J633.00 (READ), 573.3 (ICD-9)	Hepatitis/liver necrosis[a]	14 (12, 1, 1)	5 (4,1)
7852XX, R024111 (READ), 782.4 (ICD-9)	Jaundice[b]	14 (12, 1, 1)	5 (4,1)
576A, J66y600 (READ)	Obstructive jaundice[c]	12 (11, 1)	5 (4, 1)
L3264AB	Abnormal hepatic function	12	4
L3263AB	Abnormal liver enzymes	12	4
L3260AB	Abnormal liver function test	12	4
L3262AB	Biochemical liver dysfunction	12	4
070F	Fulminant hepatitis	12	4
L3263H	Liver enzymes raised	12	4
573XX	Other liver disorders	12	4
785CP	Pale stools	11	4
5730D	Hepatocellular damage	9	4
5719CH	Chronic hepatitis	7	4
070	Infectious hepatitis	7	4
K501	Liver biopsy	7	4
L3260	Liver function test	7	4
575XX	Other gallbladder disorders (cholangitis/cholecystisis)	7	4
5710HA	Alcoholic hepatitis	6	4
7516JA	Familial intrahepatic cholestasis	6	4
574XX	Gallbladder disorders	6	4
K5091	Hepatostomy	6	4
070N	Non-A non-B hepatitis	6	4
L109H	ALT raised	5	0
L1002CR	Aspartate aminotransferase level raised	5	0
L110H	AST raised	5	0
L1151NA	Bilirubin serum level abnormal	5	0
9779PN	Drug-induced jaundice	5	0
7851XX	Enlarged liver	5	0
K501XX	Liver biopsy	5	0
9669XX	Abnormal drug reactions/effects	1	0
L4720N	Alkaline phosphatase level	1	0
L1151	Bilirubin serum level normal	1	0
574AL	Cholelithiasis	1	0
57300	Hepatocellular damage	1	0
All		*249*	*91*

*XX = category including a range of diagnoses

[a]**Hepatitis/liver necrosis:** García Rodríguez et al 1992 [64]specified ICD-9 codes (570: Necrosis of the liver and 573.3: Unspecified hepatitis) which are counted as a single code search for Hepatitis/liver necrosis in this table. The READ code of J633.00 specified by Russman et al [53] has been considered as appropriate for the OXMIS heading of Hepatitis/liver necrosis in this table

[b]**Jaundice:** Russman et al [53] specified five diagnostic codes for jaundice. These are counted as a single code search for Jaundice in this table and the READ code for Jaundice (R024111) is included.

[c]**Obstructive jaundice:** Russman et al [53] specified codes for obstructive jaundice(/obstructive jaundice nos) using both OXMIS and READ codes, this is counted as a single code search for obstructive jaundice in this table and the READ code is included.

The most common codes selected by all studies were those for hepatitis/liver necrosis and for jaundice (Table 2-3), which were included in every study. These two codes were also used most frequently among those studies that only included cholestatic liver injury as an outcome, in addition to the code for obstructive jaundice. The next most frequent group of codes across all studies included those indicating results for laboratory tests for liver functions, the code for fulminant hepatitis and the code for other liver disorders (used by 12/14 studies). Codes of note that were used by studies that were looking at acute liver injury but not by those assessing only cholestatic liver injury included drug-induced jaundice and liver biopsy.

As four of the five articles in which cholestatic liver injury was studied as the only outcome used the same codelist, the difference in frequency of codes across these studies was attributable only to the study that utilised a different codelist (Russman et al, 2009 [53]). This article included a much more restricted list than the other four cholestatic liver injury studies (Table 2-5), and the list provided was described as "the codelists of **included** cases"[53]. In a personal communication with the author, he stated that he thought this was also the list used to search for cases although could not be "100% sure" if additional codes weren't used for searching.

2.3.3.2 Liver test results

For disease outcomes that can be measured by laboratory analysis of (e.g.) blood samples, the use and reporting of such standards can help minimise bias caused by measurement error. Use of the same standards across studies may also allow meta-analyses to obtain reliable pooled point estimates of effect across homogenous studies (facilitating evidence-based medicine). Standard criteria were published for defining cholestatic liver injury in 1990, in a paper reporting on an international consensus meeting on the criteria of drug-induced liver disorders published under the auspices of CIOMS [45], and again in 2011 (by an international DILI working group [46]). Table 2-5 lists the laboratory standards used for each study (step 1 column) and Table 2-6 displays which of the studies referenced diagnostic standards. Of the sixteen included studies, nine referenced the CIOMS paper [64, 68, 70-74] or the working group paper [76, 77] . Of those that didn't reference these articles, six described (or referenced another study describing) laboratory test parameters as "predominant elevation of bilurubin and alkaline phosphatase" [53, 55, 65, 67, 69, 75], while the remaining study described using laboratory test results but did not include details of the parameters used [66].

2.3.3.3 Other test results, clinical descriptions and exclusions

Biopsy and ultrasound examination are two other tests that may be used to strengthen the diagnosis of cholestatic liver injury [46]. Only two of the fourteen studies that used information other than just liver test results did not specify anywhere that biopsy results had been considered [53, 64]. In the case of Russman et al [53], it is possible it was included as an initial code search (see comment from author in section 2.3.3.1). Six of the fourteen studies described consideration of ultrasound results somewhere during review of computer records or complete medical notes [55, 65, 67, 69, 74, 75].

Six of the studies provided (or referenced a study who had provided) some additional descriptive information that was used to define a case in their study (in addition to the diagnostic codelists and laboratory test results), specifying that an individual was considered to be suffering drug-induced cholestatic liver injury if they had “painless jaundice” with positive lab test results (and no history of other causes of cholestasis) [53, 55, 65, 67, 69, 75].

Details of the exclusions applied were provided by all the studies, although of note was that none of the studies included a list of database diagnostic codes that were considered exclusions but instead included more general descriptions of conditions/diseases without identifying any specific database codes. A complete list of the exclusion criteria used across all studies is provided in Table 2-4, which also shows frequency of use, and an alphabetical list is provided in Chapter 2 Appendix (Table A2). Table 2-5 illustrates which exclusion criteria were applied at the database records (step 2 column of the table) or during the review of notes external to the database (step 3 column of the table) for each of the studies. For the five studies in which cholestatic liver injury was the only outcome, there were only two exclusions described: normal liver function test results and other liver disorders (Table 2-4) [53, 55, 65, 67, 75]. The other studies provided more extensive lists of exclusions, with alcoholism, congestive heart failure, and other well defined pathology or disease the three exclusions cited most frequently (Table 2-4). Four of the studies stated that patients who were not referred to a specialist or admitted to hospital would be excluded [64, 71-73].

Table 2-4: Frequency of exclusions used by all (16) studies and by the (5) studies that analysed only cholestatic liver injury

Exclusion description	Frequency across all studies	Frequency across cholestatic liver injury studies
Alcoholism	9	0
Congestive heart failure	9	0
Other well defined pathology or disease	9	0
Other liver disorders	8	5
Normal liver function test results	7	5
Malignant neoplasm	7	0
Cholelithiasis	7	0
Viral hepatitis (based on serology)	6	0
Gallbladder or pancreatic disease	5	0
Chronic liver disease	4	0
Rheumatoid arthritis	3	0
Not referred to a specialist or admitted to hospital	3	0
Cirrhosis	2	0
Hepatitis after blood transfusion	2	0
Sarcoidosis	2	0
Systemic lupus	2	0
Viral infection (serologically confirmed)	2	0
Cancer of the liver	2	0
Cancer of the gallbladder	1	0
Cancer of the pancreas	1	0
Cholecystitis	1	0
Crohn's disease	1	0
HIV infection	1	0
Inflammatory bowel disease	1	0
Pancreatic disease	1	0
Ulcerative colitis	1	0
Well-defined systemic condition affecting the liver	1	0
Liver disease	1	0
Other co-morbidity associated with liver chemistry elevations	1	0
ALT or AST elevations above normal levels during 1 year prior	1	0
Concomitant use of medications associated with hepatotoxicity	1	0
All	*102*	*8*

Table 2-5: Summary of overall methodology used by each study for the identification of individuals with cholestatic liver injury, including diagnostic criteria/exclusions applied and data sources used

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
García Rodríguez 1992 [64]	ICD-9 codes (3) 570: necrosis of the liver 573.3: unspecified hepatitis 782.4: jaundice	Increase of over twice the upper limit of the normal range in alkaline phosphatise (AP) alone or when the ratio (R) of serum activity of alanine aminotransferase to serum activity of alkaline phosphatase was ≤ 2	Outcome definition included admission to hospital. Unclear what aspects of computer record were used and what criteria were applied – only exclusions mentioned are those for step 3, results say that "patients were excluded based on computer and hospital records".	Excluded those with no liver disease or the following other causes for liver disease: alcoholism, malignant neoplasm, cholelithiasis, viral hepatitis based on serology, chronic liver disease, cirrhosis, congestive heart failure, hepatitis after blood transfusion, other well defined pathology and then used laboratory test results of those remaining to assign final cholestatic liver injury status.
Derby 1993a [55]	OXMIS codes (22) 070: Infectious hepatitis 070F: Fulminant hepatitis 070N: Non-A non-B hepatitis K501: Liver biopsy 570XX: Hepatitis/liver necrosis 573XX: Other liver disorders 574XX: Gallbladder disorders 575XX: Other gallbladder disorders 576A: Obstructive jaundice 785CP: Pale stools L3260: Liver function test L3260AB: Abnormal liver function test L3262AB: Biochemical liver	Elevation of bilirubin and alkaline phosphatase levels or biopsy showing cholestatic jaundice	Identified those "with a first-time diagnosis of a liver disorder that might represent intrahepatic cholestatic jaundice" and requested their GP records, but no specifics on exactly how identification was achieved.	Excluded individuals if other causes of liver injury were well defined, the history and lab findings were not suggestive of chol-hep or the disorder appeared before drug use and considered the remaining as cases if they presented with painless jaundice and positive lab results.

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
	dysfunction L3263AB: Abnormal liver enzymes L3263H: Raised liver enzymes L3264AB: Abnormal hepatic function K5091: Hepatostomy 5710HA: Alcoholic hepatitis 5730D: Hepatocellular damage 7516JA: Familial intrahepatic cholestasis 7852XX: Jaundice 5719CH: Drug-induced jaundice XX=category including a range of diagnoses			
Derby 1993b [65]	*Same as Derby 1993a (22)*	*Same as Derby 1993a*	*Same as Derby 1993a*	*Same as Derby 1993a*
Derby 1993c [66]	*Same as Derby 1993a (22)*	Laboratory results used but no laboratory parameters provided.	Based on "review of available computer data", excluded individuals who had an illness that was likely to account for the liver disorder ("conditions such as cancer of the pancreas, pre-existing liver disease") and requested GP records for those that remained.	Applied unspecified exclusions, although results state that those excluded based on notes review had: gallbladder disease, serology-confirmed hepatitis A, alcoholic liver disease or jaundice secondary to cardiac failure. Then categorised as having probable or possible drug-induced liver disease based on lab results, biopsy, timing and "extensiveness of documentation".
Jick 1994 [67]	*Same as Derby 1993a (22)*	*Same as Derby 1993a*	*Same as Derby 1993a*	*Same as Derby 1993a*

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
García Rodríguez 1994 [68]	OXMIS codes (15) L3263AB: Abnormal liver enzymes L3260AB: Abnormal liver function test L3264AB: Abnormal hepatic function 070F: Fulminant hepatitis 5719CH: Chronic hepatitis 070: Infectious hepatitis L3262AB: Biochemical liver dysfunction K501: Liver biopsy L3263H: Liver enzymes raised 570XX: Hepatitis/liver necrosis 574AL: Cholelithiasis 575XX: Cholangitis/cholecystisis 573XX: Other liver disorders 7852XX: Jaundice 9669XX: Abnormal drug reactions/effects	Increase of more than twice the upper limit of the normal range in AP alone or R≤2.	Used "computerized patient profile" (specifics unclear) to "eliminate" those with clear causes for their liver injury ("such as viral infection, cholelithiasis, cancer of the liver, and cancer of the gall bladder") and requested GP notes for the remaining individuals.	Excluded patients with cholelithiasis, malignant neoplasm, viral hepatitis (serologically confirmed), chronic liver disease, congestive heart failure, alcoholism, or other well defined disease and then used laboratory test results to define outcome status.
Jick 1995 [69]	*Same as Derby 1993a (22)*	"Strong consideration" applied to lab findings but no parameters provided.	Identified those with a "first-time diagnosis of one of the study outcomes for which no apparent cause was noted" (specifics of which data used not provided) and requested GP notes for these individuals.	Authors gave "strong consideration to the details of all clinical and laboratory findings as well as the clinical diagnoses made by the attending physicians" in order to assign final outcome status. Exclusions specified only in results but included: "no referral/hospitalisation and other clinical diagnosis present".

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
García Rodríguez 1996 [70]	OXMIS codes (19) (*obtained from authors*) 070F: Fulminant hepatitis 570XX: Hepatitis/liver necrosis 573XX: Other liver disorders 5730D: Hepatocellular damage 576A: Obstructive jaundice 785CP: Pale stools 7851XX: Enlarged liver 7852XX: Jaundice 9779PN: Drug-induced jaundice K501XX: Liver biopsy L1151NA: Bilirubin serum level abnormal L3260AB: Abnormal liver function test L3262AB: Biochemical liver dysfunction L3263AB: Abnormal liver enzymes L3263H: Liver enzymes raised L3264AB: Abnormal hepatic function L109H: ALT raised L110H: AST raised L1002CR: Aspartate aminotransferase level raised	Alkaline phosphatase over twice the limit of normal or R>2*. **R>2 likely to be a typo, as CIOMS paper is referenced which states R≤2.*	Excluded individuals from the total study population (prior to start of study follow-up) who had codes for neoplasm of the liver, diseases of the liver, jaundice, or RA and specified age range requirement of 10 - 79 years. Excluded potential cases with DB codes for neoplasm, cholecystitis, alcohol-related conditions or alcohol mentioned in the free-text. Then assigned individuals as non-cases based on presence of normal liver function test results (or minor elevations), viral hepatitis, cholelithiasis, congestive heart failure, or other well defined pathological findings found during "manual review of complete computerized patient profile". Notes for the remaining ("undetermined") individuals were requested from GPs.	Based on information within medical records, excluded patients with cholelithiasis, malignant neoplasm, viral hepatitis (serologically confirmed), chronic liver disease, congestive heart failure, alcoholism, or other well defined disease then used lab test results to identify cases from the remaining individuals.

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
García Rodríguez 1997 [71]	OXMIS codes (21) 070F: Fulminant hepatitis 570XX: Hepatitis/liver necrosis 573XX: Other liver disorders 576A: Obstructive jaundice 785CP: Pale stools 7851XX: Enlarged liver 7852XX: Jaundice 9779PN: Drug-induced jaundice K501XX: Liver biopsy L1151: Bilirubin serum level L1151NA: Bilirubin serum level abnormal L3260: Liver function test L3260AB: Abnormal liver function test L3262AB: Biochemical liver dysfunction L3263AB: Abnormal liver enzymes L3263H: Liver enzymes raised L3264AB: Abnormal hepatic function L4720N: Alkaline phosphatase level L109H: ALT raised L110H: AST raised L1002CR: Aspartate aminotransferase level raised	Increase of over twice the upper limit of the normal range of Alkaline Phosphatase alone or the ratio of serum activity of Alanine AminoTransferase (ALT) over AP (R) ≤2.	Excluded individuals from the total study population (prior to start of study follow-up) who had any of the codes listed in step 1 and additionally: cancer, other liver disease, jaundice, gallbladder or pancreatic disease, congestive heart failure, alcoholism, rheumatoid arthritis, sarcoidosis, systemic lupus, Crohn's disease or ulcerative colitis and specified age range requirement of 20 - 74 years Excluded potential cases not referred to a specialist or admitted to hospital. Then “manually reviewed the patients computerized profile” to select those for whom medical records should be requested from GPs, based on absence of alcoholism, malignant neoplasm, cholelithiasis, viral hepatitis based on serology, chronic liver disease, cirrhosis, congestive heart failure, hepatitis after blood transfusion or other well defined pathology.	Based on medical records, excluded patients with other well defined causes for liver injury (other liver disorders, cancer, cholelithiasis, serologically confirmed viral hepatitis, congestive heart failure, alcoholism or a well-defined systemic condition affecting the liver) and then used laboratory test results to define outcome status.

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
García Rodríguez 1999 [72]	OXMIS codes (19) 070F: Fulminant hepatitis 570XX: Hepatitis/liver necrosis 573XX: Other liver disorders 5730D: Hepatocellular damage 576A: Obstructive jaundice 785CP: Pale stools 7851XX: Enlarged liver 7852XX: Jaundice 9779PN: Drug-induced jaundice K501XX: Liver biopsy L1151NA: Bilirubin serum level abnormal L3260AB: Abnormal liver function test L3262AB: Biochemical liver dysfunction L3263AB: Abnormal liver enzymes L3263H: Liver enzymes raised L3264AB: Abnormal hepatic function L109H: ALT raised L110H: AST raised L1002CR: Aspartate aminotransferase level raised	Increase of more than twice the upper limit of the normal range in APh alone or R≤2.	Excluded individuals from the total study population (prior to the start of study follow-up) who had a history of liver injury in the preceding 5 years and any of the following: cancer, liver disease, gallbladder disease, pancreatic disease, heart failure, alcohol abuse, HIV infection, rheumatoid arthritis, sarcoidosis, systemic lupus or inflammatory bowel disease (likely to be DB codes but this is not specified) and had an age inclusion criteria of 20-79 years.	Assigned definite case status to those presenting with symptoms suggestive of liver disorder, referred to a specialist or admitted to hospital, free of exclusion criteria mentioned in step 2 and with lab test results showing cholestatic injury.
Huerta 2002 [73]	OXMIS codes (19) 070F: Fulminant hepatitis 570XX: Hepatitis/liver necrosis 573XX: Other liver disorders 57300: Hepatocellular damage	An increase of more than 2 times the upper limit of normal in alkaline phosphatase alone or the ratio of	Excluded individuals from the total study population (prior to start of study follow-up) who had any of the codes listed in step 1 and any of the following: other liver diseases,	Questionnaire used to “validate the diagnosis”, same exclusion criteria applied as in step 2, laboratory results then used to assign final case status.

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
	576A: Obstructive jaundice 785CP: Pale stools 7851XX: Enlarged liver 7852XX: Jaundice. excluding 7852PF 9779PN: Drug-induced jaundice K501XX: Liver biopsy L1151NA: Bilirubin serum level abnormal L3260AB: Abnormal liver function test L3262AB: Biochemical liver dysfunction L3263AB: Abnormal liver enzymes L3263H: Liver enzymes raised L3264AB: Abnormal hepatic function L109H: ALT raised L110H: AST raised L1002CR: Aspartate aminotransferase level raised	the serum activities of ALT was 2 or less.	gallbladder or pancreatic disease, alcoholism, or congestive heart failure (time window not-specified, also doesn't specify codes, although likely to be these). Included only those between 20 - 79 years. Reviewed "computerised patient profiles" manually to exclude all patients not referred to a specialist or hospital, patients with a recorded liver function test result of normal, or with minor elevations, patients with serologically confirmed viral infection, cancer, cholelithiasis, congestive heart failure, and/or alcoholism and requested notes on those remaining.	
De Abajo 2004 [74]	*Same as García Rodríguez 1996 (19)*	Increase of over twice the upper limit of the range in AP alone or R≤2.	Excluded individuals from the total study population (prior to start of study follow-up) who had any of: liver-related diagnosis, cancer, gallbladder or pancreatic disease, alcohol-related disorders (no time window provided). Included only those aged 5-75 years. Reclassified potential cases as noncases if they had minor	Excluded patients with no confirmed liver injury, antecedents of liver disease, or presented primary causes then used laboratory values to identify cases of cholestatic liver injury.

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
			elevations in serum enzymes, a primary cause was identified (chronic liver disease, malignant neoplasm, viral hepatitis, cholelithiasis, alcoholism, congestive heart failure or other well-defined pathology of the liver) or the patient was not referred to a consultant or hospital or had died. Medical notes from GPs were requested for the remaining potential cases.	
Russman 2005 [53]	OXMIS codes (3) 7852: Jaundice 576A: Obstructive jaundice 7852JC: Cholestatic jaundice READ codes (5) J66y600: Obstructive jaundice nos 1675.11: Jaundice – symptom J633.00: Hepatitis unspecified R024.00: Jaundice (not of newborn) R024111: Jaundice	Predominant elevation of alkaline phosphatase and bilurubin concentrations.	Requested GP notes for those who had “computer-recorded data consistent with idiopathic cholestatic liver disease” (unclear specifically what this was based upon). If notes unavailable, used computer record to assign final case status.	Excluded all subjects where a causal relationship was unlikely and classified remaining individuals as cases of drug-induced cholestatic liver disease when they showed painless jaundice with predominant elevations of alkaline phosphatase and bilirubin concentrations.
Li 2009 [75]	*Same as Derby 1993a (22)*	*Same as Derby 1993a*	*Same as Derby 1993a*	*Same as Derby 1993a*
Shin 2013 [76]	*None (used laboratory test criteria*	AP ≥2 x ULN and	Identified cases as people over the	*Step not performed (case status*

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
	only)	R≤2[76]	age of 18 without underlying non-drug causes of liver injury who had clinically significant elevations in liver chemistry tests (ALT, AST, and alkaline phosphatase (AP)) that were temporally associated with drug use. Liver chemistry elevations were required to meet laboratory threshold criteria within clearly defined (drug-specific) high-risk period s. Exclusions were people with (i) an ICD-9 diagnosis code for liver disease or other co-morbidity associated with liver chemistry elevations starting from the year prior to the index date until the liver injury date (or until the end of the drug exposure if liver injury was not identified); (ii) ALT or aspartate aminotransferase (AST) elevations above normal levels anytime during the year prior to the index date; or (iii) concomitant use of medications, besides the 14 selected drugs, that were associated with hepatotoxicity.	*finalised in step 2 using database records only)*
Cheetham 2014 [77]	*None (used laboratory test criteria only)*	AP ≥2 x ULN and R≤2	Identified cases (probable or possible) based solely on screening for elevated liver enzyme levels temporally associated with drug	*Step not performed (case status finalised in step 2 using database records only)*

First author and year of publication	Step 1: Definition of diagnostic criteria		Step 2: Identification of database records of potential cases (see note[1])	Step 3: Finalisation of case status based on information external to the database
	Database diagnostic codes	Laboratory test criteria		
			initiation. Then applied 7 criterion based on the RUCAM method [45] to determine case status. Excluded patients with a diagnosis of liver disease or co-morbidity that could cause liver enzyme elevations in the 12 months prior to the index date or patients with concomitant medications [other than the 14 study drugs] potentially associated with liver injury.	

[1]**Note for Step 2:** A common step for all studies was to use the list of DB diagnostic codes from step 1 to retrieve computer records of potential cases (i.e. individuals for further analysis in case identification process). The Step 2 column contains methods that were applied **in addition** to this common method.

2.3.4 Step 2: Identifying database records of potential cases

For each of the studies, a single database of stored electronic health records was used for the retrieval of records, application of exclusions and initial review of disease information in order to identify potential cases. Two of these studies then finalised case status using information within the database only [76, 77]. An overview of the differing approaches adopted in (1) the use of diagnostic codes prior to start of study follow-up and (2) the use of diagnostic codes/full database electronic health record from the beginning of study follow-up can be found in Table 2-5 (Step 2 column), and is discussed in the following section.

2.3.4.1 Exclusions of electronic records prior to start of follow-up

The use of diagnostic codes within electronic health record databases allows individuals with a history of specific diseases to be excluded from a study population prior to the start of follow-up using quick automated searches. Application of exclusions prior to start of follow-up allows individuals who may have conditions other than the one under study to be removed, therefore reducing misclassification of outcomes. In studies looking at (for example) drug-induced cholestatic liver injury, this could therefore include other conditions that are likely to cause or be mistaken for cholestatic liver injury (such as alcoholism or some cancers).

Only seven of the sixteen studies describe using the database diagnostic codes for the application of exclusions from the total study population prior to start of follow-up [70-74, 76, 77] (see Table 2-5 and Table 2-6). Although those that do not apply such exclusions do subsequently apply exclusions based upon information in the full computer record and/or medical notes obtained from the general practitioner, this means that exclusions are applied only to a subset of the study population (those that have been selected as possibly having the outcome based upon diagnostic codes, as described in section 2.3.4.2). This represents a particular problem for the two case control studies where this occurs [66, 68], because it could have introduced a selection bias, where the controls are being selected from a group that is not representative of the population from which the cases were selected, as the same exclusions have not been applied.

An additional point of note that varied between studies was whether an age-range criteria was applied when selecting patients for inclusion in the study. Only seven of the sixteen specified an age-range inclusion criteria (Table 2-6), with age ranges

of 10 – 79 [70], 20 – 74 [71], 20 – 79 [72, 73], 5 – 75 [74] and over 18 [76, 77] (Table 2-5, Step 2 column).

2.3.4.2 Use of computer records from start of follow-up

For the two studies that relied only on liver test results to define case status [76, 77], only information within the database was used to assign case status (the liver test result values). One of the methods in these studies then applies detailed steps based on the RUCAM methodology [45] focusing on assigning causality to a specific drug cause.

For all of the remaining studies, the single source of stored electronic health records was queried using the broad disease codelist described in section 2.3.3.1 to obtain the full anonymised electronic health record of each patient (identifiable by a unique patient identifier). The study performed by García Rodríguez et al using the Saskatchewan Health Plan database [64] utilised electronic records created at hospitals (from the hospital plan services database component of this database), while all the other (GPRD based) studies utilised electronic health records routinely entered by personnel at general practices in the UK. The studies describe various different ways of utilising the electronic records in assisting with case definition (Table 2-5, Step 2 column).

In the studies performed by García Rodríguez et al in 1992 [64] and 1999 [72] the authors used the broad list of diagnostic codes only to obtain full computer records for individuals that represented possible cases of acute liver injury. Limited information is provided on whether information in the computer record was used to remove those unlikely to be cases (either of cholestatic or of any liver injury) prior to obtaining notes from hospital/general practice.

Studies performed by Derby et al [55, 65, 66], Jick et al [67], Jick and Derby [69], García Rodríguez et al in 1994 [68], Russman et al [53] and Li et al [75] describe some further use of the computer record. In the first two Derby et al studies in 1993 [55, 65] and the study by Li et al [75], for example, the authors reviewed the electronic health record medical information to "identify those with a first-time diagnosis of a liver disorder that might represent intrahepatic cholestatic jaundice", although further information on what criteria were applied in doing this are not provided. Russman et al describe a similar approach [53]. When performing a cohort study using stored electronic health records in which groups with (e.g.) differing drug exposures are being compared, ensuring that reviewers are blinded to exposure status during record review can minimise observer bias (where a reviewer may be influenced by knowledge of exposure to assign somebody to a

particular outcome). This is particularly the case if the information being reviewed is of a subjective nature. In the first study performed by Derby et al [55] and the study performed by Russman et al [53] two drug exposures were compared (flucloxacillin, suspected of causing cholestatic liver injury and oxytetracycline, a comparator drug not known to cause cholestatic liver injury) and blinding of reviewers to exposure status during the review of the electronic records was not specified (Table 2-6).

The most recent of the studies performed by Derby et al [66] describes using "the available computer data to exclude subjects who had an illness that was likely to account for the liver disorder (e.g. cancer of the pancreas, pre-existing chronic liver disease)"; the studies performed by García Rodríguez et al in 1994 [68] and Jick and Derby [69] describe similar approaches. Of these three studies, only García Rodríguez et al 1994) [68] specify that reviewers were blinded to medication exposure status during review of electronic health records. The study by Jick and Derby [69] was one of the four studies who excluded patients not referred to a specialist or hospitalized (see section 2.3.3.3), and this criteria was applied based upon review of the electronic computer record.

A final group of studies [70, 71, 73, 74] include the most detail on how the electronic health records were used to assist with outcome identification. In these studies, there is clear definition that exclusion criteria were applied based on information in the database prior to study start date and during the study period, and detail is provided on how computer records allowed identification of potential cases, for which additional information was required from other sources. García Rodríguez et al (1996) [70] firstly used the computer search to identify all study members who had a code of liver disorder recorded for the first time within 45 days after a study drug prescription was written, they then applied exclusions, which included use of free-text comments (for identifying alcoholics). The complete computerized patient profile without information on drug use was then manually reviewed to categorize the individual into either a non-case or an undetermined case (for which further information from GPs was needed to assign case status). Liver function test (LFT) results were used at this stage (normal LFT result = non-case). García Rodríguez et al (1997) [71], Huerta et al [73] and de Abajo et al [74] described very similar approaches to this, although without the use of GPRD freetext. Blinding of drug exposure status in the computer record was emphasised in each of these studies (Table 2-6).

2.3.5 Step 3: Finalisation of case status based on information external to the database

2.3.5.1 Request for additional information from general practices

The percentage of notes received that provide adequate information to allow a final outcome status to be assigned is useful information in a case identification approach which relies heavily upon feedback or additional information from GPs (or other health centres) to assign case status. If notes or additional information sufficient to designate case status were received for a low percentage of the total number of individuals for which notes were requested, this would result in a large proportion of individuals for whom the data for classifying as a case is either not available or is being obtained from a single source (the database alone), rather than from two sources (the database and the external source). This could increase the likelihood that the individual's outcome status is being incorrectly assigned and introduce bias in the subsequent epidemiological study. The direction and magnitude of the bias could be small and more likely towards the null if it occurs completely independent of exposure status or, alternatively, larger and in either direction if it does not. This is likely to be dependent upon the drug exposure being studied - if the drug caused or was indicated for a condition with high mortality and extended morbidity, for example, the chances of receiving the notes could be reduced due to death of the patient or the requirement for photocopying a large set of notes at the GP surgery [79]. If exposure was related to the likelihood of notes being received in this way, for case control studies a selection bias could be introduced (where unexposed individuals could be more likely to be included in the study as cases due to availability of notes) and in cohort studies a differential information bias could occur, where those exposed to the drug of interest would be more likely to have their disease status wrongly assigned, leading to under-ascertainment of exposed cases.

Of the fourteen studies that used data external to the database, all of the studies except the Canadian study performed in the Saskatchewan Health Plan database requested and received information by post from GP surgeries in the UK. In the Canadian study (performed by García Rodríguez et al [64]), no response rate is included, but the method of collection of notes involved nurses travelling to the hospital themselves, which may have meant that the response rate was effectively 100%. For the other studies, two (both updates to a previous study sent as letters to the editor of a journal) do not publish a response rate [67, 75]. Of the remaining eleven, six studies obtained response rates over 90% [55, 65, 68, 69, 71-73], three others obtained response rates over 80% [66, 69, 70] while there were two with

response rates lower than 80% [53, 74]. The study by Russman et al [53] obtained the lowest response rate (64%). Of those writing to GPs, all requested existing notes apart from Huerta et al [73], who sent out a questionnaire for completion.

Only four of the fourteen studies that used external data included some discussion, comment or analysis related to the % of missing or/inadequate notes (Table 2-6). Derby et al 1993c [66] and Russman et al [53] both stated that for those individuals for whom notes were not received, they used information available in the computer record to assign a status (with Derby et al providing crude risk figures both with and without the inclusion of these individuals). García Rodríguez et al (1994 and 1996) assumed that those not-received would be likely to have the same risk of outcome as those received, but do not provide any further discussion as to whether the non-receipt could be related to exposure or outcome, as discussed above.

2.3.5.2 Review of medical notes to assign final outcome status

All studies that used data external to the database effectively relied on review of the notes to confirm the status of potential cases identified from information in the computer record. In contrast to database diagnostic codes, the information in medical notes is not categorical, meaning that clear identification of which aspects of the notes were used to facilitate decision-making can be helpful, as can a clear definition of who performed the review and of any exclusion criteria that were applied. Blinding of the reviewers to the exposure status of the individuals can decrease the likelihood of bias, as described previously (section 2.3.4.2). Including a check of the validity of the method used can provide confidence in the method, such as having somebody perform a new review of a set of notes that include a selection of those designated as cases and non-cases by the method used in the study, and comparing the results. Finally, the use of multiple case status levels such as (e.g.) possible or probable, can increase the transparency and interpretability of the results obtained in the study (and the impact on risks or rates of including/excluding possible cases can be assessed). Table 2-5 contains a description of how the medical notes were utilised by each study (step 3 column), while Table 2-6 describes whether reviewers and blinding were clearly described, whether validation of individuals not designated as potential cases was performed and whether multiple case-status levels were applied.

Three of the studies provided minimal information on which of the authors reviewed the notes, whether blinding to exposure status was performed during the review and on any attempt to test validity [64, 66, 70]. García Rodríguez et al

(1992 [64]) reviewed the hospital notes together with the computer record, and applied exclusions considering both sets of records. The same author adopted a different approach in his study performed in 1996 [70], with the computer records having already been used to remove potential cases before review of the notes took place. Despite not identifying reviewers clearly or specifying blinding, both studies provide clear descriptions of how the notes were used to define cases, stating that after application of exclusions, laboratory results were used to define outcome status (and the CIOMS international standard was clearly referenced). Although the 1993 study by Derby et al [66] lacked information on blinding, who performed review and validation of non-cases, the authors did include a probable/possible notation to indicate the availability/extensiveness of supporting documentation for assigning case status to an individual.

A further eight studies provided additional detail on the notes review methodology applied [53, 55, 65, 67, 69, 72-75]. Derby et al (1993a [55]), Jick et al [67] and Li et al [75] specified that at least three of the authors reviewed the notes and one was blinded. They also described how disagreements between blinded and unblinded reviewers were resolved and provided a clear definition of what they were looking for ("painless jaundice with predominant elevations of bilirubin and alkaline phosphatase levels with no history of alcoholism or other cause of cholestasis identified clinically"). A check of the validity of the method as described above was not performed, however. Jick and Derby [69] also described blinded review of the notes, and highlighted that clinical, laboratory and the diagnosis made by the attending physician were all used in identifying cases. García Rodríguez et al 1999 [72], Huerta et al [73], De Abajo et al [74] and Russman et al [53] all provided very clear definitions of what would be considered a case based on review of notes/questionnaires (for example García Rodríguez et al 1999 [72] described a case of acute liver injury as "a person presenting with symptoms suggestive of liver disorder (nausea, vomiting, abdominal pain and/or jaundice) referred to a specialist or admitted to hospital, and who was free of exclusion criteria" and then further assigned cholestatic status based upon laboratory test results). All four studies clearly identified who reviewed the notes, although only García Rodríguez et al 1999 [72] described blinding to drug exposure status at this stage. None of the four studies attempted to validate the method.

The two studies with the greatest level of detail on how the medical notes were used for case definition were two of the four case-control studies [68, 71]. García Rodríguez et al 1994 [68] applied additional exclusions (having applied exclusions already at the computer record stage) and gave a detailed description of what was

being considered a case and how the laboratory tests were used to help with the case definition at this stage. Blinding to drug exposure status was performed and the reviewers were clearly identified, and a random sample of non-cases was reviewed separately by a third party in an attempt to provide some indication of validity of the method. García Rodríguez et al 1997 [71] were also very clear with the criteria being used on notes review to decide how a case is identified, and on reviewers/blinding, although did not describe any attempt at measuring validity.

2.3.5.3 Note on appropriate application of exclusions according to study design

For the four case-control studies included in the review, a subsequent step following identification of cases (those with the outcome) was to select controls. As discussed previously (section 2.3.4.1), selection bias can be minimised by applying the same exclusion criteria to controls as has been applied to cases. Of the four studies including a case control component [66, 68, 71, 74], only De Abajo et al [74] specify that they applied the same exclusion criteria to the controls as had been applied to the cases (Table 2-6), meaning the results obtained for the case control analyses of the other studies are potentially biased. In addition, of the four studies, only De Abajo et al [74] specify that potential cases designated as not having the outcome during the case identification process were “classified as non-cases” (as opposed to excluded completely from the analysis). A final aspect of the case control studies that is of particular importance when considering database studies is that all of them describe detailed review of potential case records in order to identify final cases (and remove those considered non-cases due to other underlying conditions), but do not describe applying a similar review to the (potential) control population. This therefore creates a risk of bias due to the exposure distribution among controls being different to that in the source case population [80].

For the cohort studies, where potential cases are discounted as cases during the review of their computer records/notes due to the presence of an exclusion criteria (such as a code for congestive heart failure) that has occurred after the start of study follow-up, the person time from the start of follow-up until the occurrence of the exclusion event should be included in the analysis [78], as this represents time when these people should be considered at risk of the event. Only four of the thirteen studies that included a cohort analysis described clearly that this person-time was included in the analysis [70-73], with the remaining eleven either specifying only that these individuals were excluded, or not providing sufficient detail to understand if the pre-exclusion event person time had been included [53, 55, 64-69, 75].

Table 2-6: Summary of methodological characteristics for case identification and ratio of *potential cases:final cases* selected for each study

First author and year of publication	García Rodríguez 1992 [64]	Derby 1993a [55]	Derby 1993b [65]	Derby 1993c [66]	Jick 1994 [67]	García Rodríguez 1994 [68]	Jick 1995 [69]	García Rodríguez 1996 [70]	García Rodríguez 1997 [71]	García Rodríguez 1999 [72]	Huerta 2002 [73]	De Abajo 2004 [74]	Russman 2005 [53]	Li 2009 [75]	Shin 2013 [76]	Cheetham 2014 [77]
1. Specified an age range inclusion criteria?								✓	✓	✓	✓	✓			✓	✓
2. Provided list of diagnostic codes used to identify cases included in study?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	NA	NA
3. Liver test parameters described?	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4. External diagnostic standards for liver tests referenced?	✓					✓		✓	✓	✓	✓	✓			✓	✓
5. Time interval between diagnostic code and liver test described?															✓	
6. Excluded individuals based on DB codes prior to start of study?								✓	✓	✓	✓	✓			✓	✓
47. Blinding to exposure status at electronic record review?	✓		NA[1]			✓		✓	✓		✓	✓			[2]	[2]
8. GP/hospital notes response rate (%)	NP[3]	98	97	86	NP[3]	95	86	83	94	90	95	76	64	NP[3]	NA[1]	NA[1]

First author and year of publication	García Rodríguez 1992 [64]	Derby 1993a [55]	Derby 1993b [65]	Derby 1993c [66]	Jick 1994 [67]	García Rodríguez 1994 [68]	Jick 1995 [69]	García Rodríguez 1996 [70]	García Rodríguez 1997 [71]	García Rodríguez 1999 [72]	Huerta 2002 [73]	De Abajo 2004 [74]	Russman 2005 [53]	Li 2009 [75]	Shin 2013 [76]	Cheetham 2014 [77]
9. Analysis or comment on % of notes received?				✓		✓	✓	✓					✓		NA[1]	NA[1]
10. Reviewers of notes clearly defined?		✓	✓		✓	✓	✓		✓	✓	✓	✓	✓	✓	NA[1]	✓
11. Blinding to exposure status at notes review?		✓	NA[1]		✓	✓	✓		✓	✓				✓	NA[1]	NA[1]
12. Exclusions appropriate for study design?[4]		✓	✓		✓		✓	✓		✓	✓	✓	✓	✓		
13. Attempt to measure validity?						✓										✓
14. Use of >1 case status level?		✓	✓	✓												✓
Cases identified (potential cases:final cases, (%))[5]	127:20 (16%)	143:13 (10%)	28:3 (11%)	199:14 (7%)	166:17 (10%)	[6]	330:12 (4%)	177:26 (15%)	185:18 (10%)	73:9 (12%)	165:13 (8%)	1022:77 (8%)	36:30 (83%)	[6]	NA[1]	NA[1]

[1]**NA:** Garcia Rodriguez 1992 , Derby 1993b - not applicable as entire study population was exposed (there was no baseline exposure studied); Shin 2013, Cheetham 2014 – neither study relied on notes obtained from sources external to the database so no response rate or analysis. Shin 2013 did not perform any notes review, while Cheetham 2014 did review charts as part of validation (and reviewer was clearly identified). Blinding to exposure status not applicable to Cheetham 2014 because knowledge of the prescribed drug was part of the algorithm being assessed. Finally, as case identification did not involve identifying potential cases (but only final cases using liver test results), there was no potential case:final case ratio for either study.

[2]**:** Blinding not specified, but may not have been necessary as case identification procedure may have been completely automated (i.e. no manual record review)

[3]**NP:** No information provided on % of notes received or could not otherwise be calculated from results

[4]**Exclusion criteria appropriately for study design:** Cohort study – where a time-to-event analysis was being applied, for individuals with one of the exclusion criteria, the person-time contributed prior to the exclusion criteria event for that individual was included in the analysis; Case control study – any exclusion criteria applied to individuals during the case identification process was also applied to the controls (including whether the same detailed review of records was applied to the potential control set if it was also applied to the potential case set).

[5]**Cases identified:** Cases are the numbers of individuals identified at each stage in the format *number found by code search*:*number confirmed by full computer/notes review* (also shown as a %).

[6]**Jick 1994, Li 2009 cases:** Provided no information on numbers of cases identified at each stage.

2.4 Discussion

This systematic review has identified sixteen studies that were performed in databases of stored electronic health records in which cholestatic liver injury (or a synonym) was one of the outcomes of the study, and considered the, diagnostic criteria, data sources and methodology used in order to identify individuals with cholestatic liver injury.

The overall method was similar in fourteen of the sixteen studies, involving identification of potential cases with (generally) a broad search using a set of database diagnostic codes, retrieval and review of the database computer record of these potential cases in order to narrow the search, and then retrieval and review of the actual medical notes (or completed questionnaires) from GP/hospitals for remaining individuals in order to allow a final decision on case status to be made. The two remaining studies (performed most recently) used only data within the database with a particular focus on liver test results.

Two recent reviews of studies that have performed validation of General Practice Research Database diagnostic codes [81, 82] include some overlap of the considerations applied in this review (and retrieved four of the same studies [68, 72-74]). However, in contrast to those reviews, this one focused specifically on cholestatic liver injury as an outcome, and was not restricted to the GPRD. A second important difference was that the GPRD validation reviews were investigating the range of methods used to validate the coded diagnoses in the database record against other data sources identified as the “gold standard” (such as GP notes). In contrast, this review has focused on how any of a number of data sources (e.g. diagnostic codes, computer record, GP notes) have been utilised in order to identify cases, and was not considering whether a particular source might be a “gold standard” that was validating any of the other sources.

It is of note that one of the other reviews [82] compared the positive predictive value (PPV) for studies of a wide range of diseases/conditions, and the three with the lowest PPVs were those related to acute liver injury, also included in this review [72-74]. The relatively low PPVs obtained are very likely a reflection of (1) the fact that the authors of these three studies have deliberately used broad diagnostic codes as detection of cholestatic liver injury (and acute liver injury) based on diagnostic code alone is very difficult (requiring additional verification, such as LFT test results and application of a number of exclusions, as discussed previously in this review) and (2) the fact that PPV varies with prevalence, each disease will have a different prevalence, and acute liver injury is typified by a low prevalence compared to other conditions such as, e.g.

diabetes (which has the highest PPV in the review). In fact, although these three studies use codelists with a very low PPV, this is not the most suitable measure for the quality of the overall methodology for identifying cases of cholestatic liver injury when using multiple data sources, because a powerful method would likely have a highly sensitive codelist search (and codelist PPV is of less importance).

There were a number of strengths in the methodologies used for identifying cases of cholestatic liver injury that were consistently demonstrated across the studies in this review.

In general, studies used a codelist that would be able to identify all cases of cholestatic liver injury (or other acute liver injury outcomes) but would also return individuals who were unlikely to have cholestatic liver injury (i.e. a sensitive non- specific search). At the early stage of case identification, this approach may be sensible for a condition such as cholestatic liver injury, where LFTs and consideration of other conditions causing symptoms similar to cholestatic liver injury are likely to be needed to designate a final case status [46]. There was uncertainty on the exact codes used for only one of the studies [53]. All the remaining studies provided codelists, which increases method transparency and reproducibility, and may be in contrast to the majority of studies that use diagnostic codes to identify cases [81]. In the study using the most restricted list of codes (Russman et al [53]), 83% of the potential cases were eventually classified as final cases based on notes review (over six times more than the study with the next highest % of potential cases classified as final cases) (Table 2-6). Using a more restricted set of codes would reduce the resource required to perform the study due to lower total potential cases retrieved, but could increase the risk of true cases being missed.

The majority of studies describe review of/application of exclusions to both the full GPRD computer record and the information obtained from hospitals/GPs. This means that a total of at least three different sources were used to assist with case definition (database codes, full database computer record and medical notes or questionnaire) in these studies. Today, linkages between databases of electronic health records provide the potential for information contained in other databases to be used for assisting in case identification. Of those studies that did not just rely on liver test results, the most recent was performed in 2009 (and the earliest in 1992). The authors of these studies can be seen to have made good use of multiple available data sources in order to minimise misclassification.

Other positive characteristics of the methods utilised across all the studies included the citation of external standards for laboratory tests indicating cholestatic liver injury. The majority of the studies either referenced the CIOMS standard [45] or provided a clear

description of the laboratory criteria considered, minimising the likelihood of measurement error and maximising reproducibility. In general, the studies clearly identified reviewers of the data, allowing the reader to assess whether the review process was carried out by a sufficient number of appropriately qualified people in order to minimise measurement error. Clear descriptions of the exclusion criteria that were being applied at each stage of the review (for example computer record versus medical notes) were also present for the majority of studies. The majority of studies provided response rates for medical notes/questionnaires from GPs, providing an initial indication of how biased the results could be due to non-receipt of notes.

In a number of other areas, this review found that there were less consistent approaches applied across the studies, and some ambiguities/weaknesses could be observed.

Although the studies used multiple data sources (diagnostic codes, database computer records, information from GPs or hospital records), in each case there was only one database of electronic health records that was used. Thirteen of the sixteen studies were performed using primary care electronic health records stored in the UK GPRD (or its predecessor, VAMP), which contains a patient's medical history (prescriptions and diagnoses) as recorded at the General Practice that the person has attended. Other databases of electronic health records exist in the UK, and the information in these databases could be used to assist with case identification. The Hospital Episode Statistics (HES) database, for example, contains information on every NHS hospital admission in England, with metadata on patient demographics, hospital administrative information and clinical information related to diagnoses and operations or procedures performed. There is likely to be additional useful information in the HES record that could assist with identification of cholestatic liver injury when performing a study within the GPRD. During the time-period that the studies performed within the UK GPRD that were included in this review were performed, linkage of the information held within GPRD to HES data was not possible, which explains why this additional source of data was not utilised. From 2011 linkages between the GPRD database and the HES database have been possible (linking via the unique patient id), and could be utilised in future studies requiring the identification of cases of cholestatic liver injury.

There was likely to have been additional information that could have been used within the computer records for the studies reviewed. For example, the GPRD record contains a freetext component that is available for analysis, and only García Rodríguez et al (1996) [70] specify searching the freetext of the computer record during the process of identification of those with cholestatic liver injury (specifically, for identifying alcoholics who should be excluded). Obtaining freetext is expensive, which may have prohibited

use of this as a source of data within the computer record. In addition, none of the studies described assigning a hierarchy within the codelists (i.e. designating some codes as representing a stronger weight of evidence for the individual to be an actual case). Reviewers may well have done this implicitly (for example, considering somebody with a diagnosis of obstructive jaundice to be more likely to be a case than somebody with abnormal hepatic function). Including this level of detail could improve the reproducibility of the study.

Although the majority of the studies specified criteria for laboratory test results for confirmation of cholestatic liver injury and exclusions were clearly defined, a clear case-definition for cholestatic liver injury that was used to select cases at the final stage of reviewing all the information gathered was lacking in many of the studies. If this omission is due to the lack of a clear protocol, there is a risk of information bias having caused spurious results (incorrect measurement of outcome due to lack of clear definitions in a protocol). The reproducibility of the study (or the ability to assess heterogeneity in reviews of the work) is also limited.

There were a number of issues related to how exclusions were applied. The majority of the studies did not apply exclusions based on historical diagnostic codes, but applied them only to groups of individuals who had already been designated as potential cases due to the presence of specific diagnostic codes. As discussed, this approach is problematic for the case-control studies where this occurred, because if the same exclusion criteria are not applied to controls after selection, then the controls are being selected from a population that is systematically different from the population from which the cases were selected. For the cohort studies, omitting this step is unlikely to have biased the results, providing that when the exclusions were applied to the "potential case" subset, reviewers were blinded to exposure status. Blinding of reviewers to exposure status was not described in half the studies, however. This would create a risk of misclassification of outcome based on reviewer's knowledge of exposure for all these studies, and the two studies particularly susceptible to bias were those in which there was a lack of both reviewer blinding and no application of exclusion criteria to the whole study population based on historical codes [64] [53].

Exclusion criteria were inconsistently defined across studies, with no studies choosing to present a list of database diagnostic exclusion codes, as the majority did with their database diagnostic inclusion codes. A standardised approach with respect to provision of both inclusion and exclusion codes across studies utilising databases of stored electronic health records would be very helpful.

A number of studies excluded individuals that were not referred to a specialist or a hospital. Although it is unlikely that patients with cholestatic liver injury would not be

referred to a specialist or to hospital, using this criteria in an “all or nothing” fashion does risk omission of some cases of cholestatic liver injury who may actually be true cases (possibly meaning only severe cases would be included in the study). A preferable option might be to include this criteria as one of a number that would contribute to the weight of evidence for assigning the individual as one of a number of different case statuses. It should be noted, however, that a recent expert working group on drug-induced liver injury advised that including hospitalisation as a criteria for applying judgements of the severity of liver injury was not advisable, as whether or not people are hospitalised for a particular indication varies worldwide [46].

Finally, only a minority of studies described applying exclusions appropriately to fit the study design (section 2.3.5.3 and Table 2-6). One key point to emphasise that could be a particular issue for case-control studies performed in large databases of stored electronic health data is that where a case-identification/verification process requires detailed case review, it may not be possible to apply the same review steps to the potential control pool. This is due to (1) comparable information not being available for review (e.g. free text related to underlying issues with the liver may be more likely to be populated for potential cases) and (2) the large number of controls in a case control study. This further increases the risk that the controls will not be representative of the source case population, introducing a bias that is dependent upon case-status [80]. The potential for bias is difficult to predict as it will be related to the prevalence of exclusion events/conditions in the control population, and the degree to which they are associated with the medication being studied. Therefore, when estimating relative frequency in a case control study within an EHR database, a case definition based on a set of well-populated data elements may be advisable. If the aim of an analysis is to estimate an absolute rather than a relative effect (i.e. risk or rate rather than a ratio for liver injury), then detailed review of any and all additional case information can be performed to verify case status, rule out other causes of liver injury and allow accurate risk estimates.

The majority of studies described a sequential approach to definition of cases, where a group of individuals are selected based on diagnostic codes, computer records for this group are then reviewed in order to exclude those with/without certain criteria and reduce the size of the group before finally medical notes are reviewed in order to apply further exclusions and identify those who are considered to be true cases of cholestatic liver injury. There may be a number of potential issue with this approach.

Firstly, it may be removing possible cases from the study inappropriately. For example, if a patient is identified as not having cholestatic liver injury based upon criteria applied by the reviewers following review of the full computer record, additional information

would not be requested from the (e.g.) general practice. If the general practice notes contain additional information that was not present in the full database record that in fact provides strong evidence for cholestatic liver injury (such as a biopsy result), then this individual would have been classified as not having cholestatic liver injury because insufficient data was reviewed to classify him or her as such, and not because the true situation was that he or she did not have cholestatic liver injury. For an approach that relies on external information from GPs/hospitals, a less risky method could have been to request GP/hospital records for all of those obtained from the initial code search, and then consider the full computer record and the notes received from the GP concomitantly, considering the information from both data sources as being responsible for contributing to the weight of evidence, rather than removing individuals (possibly) based on lack of information in the computer record. In fact, two of the studies by García Rodríguez et al (1992 and 1999) [64, 72] do follow this method somewhat, as notes are requested for all individuals identified as having the codes of interest, and all the available information for the individual is considered in order to assign a case status.

Secondly, the reliance on medical notes/completed questionnaires from GP/hospitals for case ascertainment introduces possible limitations in the form of financial and time costs. At present, the approximate cost of obtaining a set of notes for a patient is £70, which could well effect the methodology applied. If there is a tight budget constraint for the study, then a researcher may be less likely to request a large number of sets of notes (e.g. for those individuals returned by an initial codelist search) but could instead use the full computer record to exclude some potential cases before requesting notes, resulting in possible cases being missed due to lack of data as described above. For epidemiological studies performed for publishing in peer-reviewed journals, the additional time required to obtain records from GPs or hospitals is unlikely to represent a serious problem. If the method for the identification of cholestatic liver injury is part of an algorithm that is to be used for the “real-time” identification of cases of (e.g. drug induced) cholestatic liver injury during upload of information into databases of stored electronic health records, however, then it would not be possible to rely on external records in the way that all studies in this review have done.

Thirdly, relying heavily on GP notes to ascertain case status means that there is always a risk of a low % response, which (as discussed in section 2.3.5) could potentially be associated with exposure and therefore introduce a selection bias. Although measures could be taken to minimise or account for this (such as paying practices more for larger patient files or checking the prescription frequency for the drug of interest between participating and non-participating practices), the risk remains, effectively negating

some of the benefits of using stored electronic information in the first place. It is also of note that some GP practices never provide notes, as they have not agreed to take part in this aspect of GPRD research, which could result in a general under-ascertainment of cases. It is possible that participation could be associated with drug exposure, as practices who participate in provision of notes for research purposes may (for example) have a differing level of interest in evidence-based medicine than those who do not provide notes, which could also mean that prescribing practices differ.

Finally, it is also possible that clinicians asked for information may simply refer to the same electronic record already accessed by the researchers. Of note is that one of the studies utilising only the electronic record obtained a similar risk for any (i.e. not just cholestatic) amoxicillin-clavulanate induced liver injury (33.8 per 100 000 people, 95% CI 19.2 – 48.4) [76] as one of the other studies that relied on external note review (22.5 per 100 000 people 95% CI 13.9 – 34.4) [70] (frequency calculated from data presented in this study i.e. 21 cases of any type of liver injury in 93433 exposed people). In future studies, it would be of interest to assess the need for external note review.

The two studies that did not utilise data external to the database relied only on liver test results recorded within the database. Although this handles many of the problems detailed above associated with using external data, other challenges are created. Firstly, not all databases have liver function tests recorded, so this may limit how widely such a method could be applied across different databases. An additional issue relates to whether or not this approach will always be identifying clinically important liver injury – in one of the studies that used liver test results only, 59% of test results reaching the threshold for DILI did not have corresponding liver injury-type codes [76]. In addition to highlighting a challenge with this method, this interesting finding also raises questions about the current method for classification of DILI based on liver function test results.

Eight of the sixteen studies chose to illustrate the suitability of the database in which they are performing their study (GPRD in all cases) by citing three previous studies that have been performed relating to the validity of the database [83-85], which have shown the positive predictive value (PPV) of a GPRD coded diagnoses to be in the region of 90%. As highlighted in a recent review of validation studies within the GPRD [81] and discussed earlier in this section, presenting this figure as a way of supporting the validity of a subsequent study is ambiguous as PPV is only one measure of validity (indicating the likelihood that a person with a particular diagnostic code actually has the disease in question based on the “gold standard” of medical notes obtained from GPs). PPV tells the reader nothing about the sensitivity (the % of true cases that the method will identify as cases), specificity (the % of true non-cases that the method will identify

correctly as non-cases) or negative predictive value (NPV, the likelihood that a person without a particular diagnostic code does not actually have the disease in question). PPV also varies with disease prevalence, which is likely to differ based upon time of the original validation studies (due to actual difference in disease prevalence and knowledge/experience with the disease of GPs at that time, although whether cholestatic liver injury prevalence has actually changed over time is unclear, and further work could be performed in relation to this). In all of these studies, diagnostic codes, information in the computer records and the medical notes were being used as a method of identifying cases. Therefore, the PPV of diagnostic codes is of limited use in assessing the validity of these methods. Preferable would be to choose a completely new "standard" against which to validate the method, sampling those designated as cases but also non cases, so that all of the measures of validity specified above can be assessed, as described elsewhere [81]. Only one of the studies attempted to measure validity in this way, by selecting one reviewer to review all the information on the cases and a sample of potential cases designated as non-cases by the complete method [68]. Where methods for outcome identification are using what is acknowledged elsewhere as the "gold standard" (notes from GPs) as part of the outcome identification method, using one or a number of other data sources that could allow measures of validity to be assessed could be considered.

Although the use of standards and clear definition of criteria can minimise differing results across studies that are due to differing opinions of those reviewing data, in studies where outcome information collected for a purpose other than the study in question is being reviewed, there will always be an element of the decision on outcome that is subjective. One way of handling this variability is to use levels of case designation, where two (or more) categories of case definition are created, indicating (for example) definite and probable case definition status, with the reasoning for the difference in case status clearly identified. The analysis can then be repeated with different scenarios in which, for example, the probable cases are included or excluded. If the reasoning for the difference in case status is clearly defined, then this allows an understanding of some of the clinical uncertainty to be assessed. The impact of decisions on case definition to be clearly assessed by those reading the study and can improve transparency and standardisation across studies. Of the studies assessed in this review, only the three studies by Derby et al ([55, 65, 66]) included levels of case-definition, which included consideration of whether the outcome was drug-induced or not.

A final point relates to reusability of the method used for case identification. All of the studies relied on a review team to assess information held within a variety of sources in

order to assign cholestatic liver injury case status. If another research group wanted to perform a similar study at a later date, their ability to apply the same criteria would be maximised by many of the steps considered in this review, but because the review of information relied ultimately on review teams specific to the original study, application of the same criteria to outcome definition is not guaranteed. Development of a data-driven algorithm, that uses specific characteristics of the database record to assign an individual as having cholestatic liver injury (or to varying levels of evidence for cholestatic liver injury), as was done in a recent study looking at Rheumatoid Arthritis within the GPRD [79], could allow standardised case identification methods to be reused between groups. Database owners could also facilitate collaboration by storing and providing such algorithm program files for reuse.

2.4.1 Limitations of review

In a systematic review of the literature such as this one, there is always the possibility that references have been missed. However, the search was performed on multiple databases, and a number of other sources were searched as were selected reference lists in order to minimise the chances of missing references in this area. The search criteria for the database search were deliberately designed to be sensitive, with specificity increased by manual review, in order not to miss any articles. In addition, setting up a weekly automatic update of the database search allowed the search to be as up-to-date as possible during this project. Publication bias should be considered when performing a systematic review. However as this review was focusing on methodologies for identifying cholestatic liver injury as an outcome and not the results of the studies, the impact on the range of methods found is likely to be minimal. Of note is that the database search did not include any language limitations (although only English-language papers were found). A final limitation that relates to the analysis of methodologies is that the analysis relies on what was described in a scientific paper, and may not reflect actually what was done. Where possible, authors were contacted in order to clarify points of uncertainty, although it remains that issues were picked up as (e.g.) being omitted that were in fact performed but not explained clearly (for example, blinding at one stage of review but not another).

2.4.2 Conclusions

In conclusion, although there was not a single study that might be used as a model for future studies of cholestatic liver injury in databases of stored electronic health records, across all of the studies there were approaches that should certainly be applied, in terms of use of multiple data sources, use of database codelists for identifying potential cases/exclusions, external standards for defining cholestatic liver injury, clear definition

of reviewers, blinding of reviewers, measurement of validity, use of levels of evidence for cases (probable versus possible versus definite) and publication of response rates related to use of external sources.

Important areas for further work include investigating (1) the use of new linkages between databases to improve identification (such as links between primary and secondary care databases) (2) assessing the benefit provided by obtaining notes from health centres to verify case status (3) development of methods that could facilitate "real-time" identification of cases and (4) development of reusable methods that could allow a departure from binary case-designation based on often opaque clinical reasoning.

One novel approach that could be applied to address some of these issues while maintaining the rigour of the studies assessed in this review could be to use a modification of the data-driven algorithm approach used recently in other disease areas [79]. The modified approach could improve upon the range of data sources used by the studies in this review by utilising linkages that now exist between multiple data repositories (such as the UK GPRD and HES), and develop the methodology used by Thomas et al [79] to create an algorithm that could be used entirely within a single database, enabling real-time analysis of data as it is uploaded to a repository (facilitating screening of patients for recruitment in e.g. studies looking at the molecular genetics of cholestatic liver injury as a drug reaction).

One way of approaching this could be to firstly develop a "multisource" algorithm that uses multiple data sources (including multiple databases) to assign a cholestatic liver injury case status to individuals. The case definitions (e.g. possible, probable and definite) from this (multisource) algorithm could then be considered as the outcome, information within the database record could be considered as exposures, and logistic regression could be used to assess the characteristics of the database record that are associated with a case of cholestatic liver injury as defined by the multisource algorithm. If the log Odds Ratios (OR) for exposures in the final model were then used to obtain a cholestatic-liver injury diagnosis score for each patient classified (by the cholestatic liver injury multisource algorithm) as having cholestatic liver injury by assigning the value of the log OR for each of the exposures present in their GPRD record and then summing these values to produce an overall score for the individual, one or more score values could be chosen as case "cut-offs" for defining case status (with the specific score used dependent upon the setting). This would allow real-time identification of cases being uploaded to the database (for example, in relation to a particular drug exposure such as flucloxacillin), and would be reusable in other studies.

Having said this, one should be careful in creating methods that are leaning too far towards reliance on purely algorithmic methods, compared to the input of experienced physicians who can pick up nuances of diagnoses from hospital notes or freetext review that could not be picked up by pre-programmed pattern recognition. This becomes particularly important when estimating absolute effects from population-level data.

A final point should be made in relation to the sensitivity/specificity of a real-time algorithmic method. This could depend on the specific application that the algorithm is being used for. For example, if it were to be used for obtaining individuals that are definitely cases of cholestatic liver injury, for example for further genetic analysis in studying the genetics of drug-induced cholestatic liver injury, then a highly specific non-sensitive method could be appropriate (e.g. only retrieving definite cases). If the algorithm were not specific enough in this case, many individuals could be ascertained incorrectly as having the cholestatic liver injury phenotype, which would make identifying the genotype very difficult. The balance between specificity and sensitivity in this situation is likely to depend on the associated resource available for review of further information to assign case status.

If the algorithm was to be used for identifying cases related to a specific drug for which there were emerging case reports that it was causing cholestatic liver injury, it is likely to be more suitable to use the algorithm with a higher sensitivity but lower specificity, enabling probable as well as definite cases to be obtained for further review with consideration of the drug of interest. Using an algorithm that was too specific in this case, may result in people who have suffered cholestatic liver injury due to this drug being missed, which could mean the reported adverse effect of the drug in relation to cholestatic liver injury was lower than the actual effect.

2.5 Summary

- A systematic review was performed focusing on methods of identifying cholestatic liver injury as an outcome in studies performed within databases of stored electronic health records.

- 16 studies were identified, 13 of which were performed within the GPRD (or its predecessor, VAMP).

- Only one study did not provide a clear description of the diagnostic codes and laboratory test cut-off used, however a completely reproducible case-definition was lacking in the majority of studies

- Strengths of the studies included the use of multiple data sources in order to designate case status, clear definition of database diagnostic codes used, the use of external standards for defining cholestatic liver injury, clear identification of reviewers, and publication of response rates for information requested external to the database.

- Areas identified in this review that would be desirable in a case identification algorithm and will be focused on during algorithm development within this project include: the provision of clear descriptions related to what is considered a case, ensuring that reviewers are blinded to exposure status, a reduction in reliance on non-database sources (such as external notes from GP surgeries), provision of validity measurements for the methods used, the use of more than one database source in a single study, careful consideration of how to apply exclusions in relation to study design, the use of multiple levels of evidence for case status and the creation of methods that can be reused by other research groups.

3 Methods

3.1 Introduction

In this chapter, the data sources used in this study are described (section 3.2), followed by the methods used for the development of the algorithms for detection of cholestatic liver injury (section 3.3) before finally the methods of two epidemiological studies using the CPRD cholestatic liver injury algorithm (section 3.4) are provided.

3.2 Data sources

3.2.1 The Clinical Practice Research Datalink

The main data source utilised was the primary care database of the UK Clinical Practice Research Datalink (CPRD Gold, hereafter referred to as CPRD), which contains anonymised data on patients from over 625 NHS primary care practices from across the UK (approximately 12 million total patients). Information is recorded by general practitioners or other health centre staff as part of routine clinical care, and data quality checks at the database headquarters ensure that each practice contributing data maintains “up-to-standard” data [81]. In addition to the routinely collected data from primary care consultations, information from some secondary sources that has been provided to primary care clinicians (such as major diagnoses made in hospital) may also be recorded. The database has been collecting data since 1987, and has recently been shown to be broadly representative of the UK population [86]. Epidemiological research has been performed using the database for over 20 years (generating over 1000 publications) [87], and the validity of many diagnoses recorded in the database has been shown to be high [81].

Patient records for study cohorts are extracted from the database based upon the presence of standardised diagnostic codes (Read codes) or, if defining a cohort by drug exposure, British National Formulary (BNF) drug product or substance codes. Searchable dictionaries of all diagnostic and drug prescription terms used in the database are provided, with each record including a specific code and the corresponding descriptive term. Based on the diagnostic or prescription codes selected from the dictionaries, electronic health records can be obtained for all patients who have any of the codes of interest during a period of interest. Data are extracted from the database as a number of separate data files, each containing a different type of data, with the information relevant to any particular patient identified via a unique patient identifier (patient id). Table 3-1 provides details of the CPRD data files used in this thesis.

Table 3-1: Description of CPRD extracted data files

Data file	Data organisation	Data description
Index list	One record per patient	Master list for cohort, contains unique patient id[1] and date of clinical diagnosis or drug prescription of interest (the "index date")
Patient	One record per patient	Contains demographic data such as gender, date of birth, death date (if applicable)
Clinical	Multiple records per patient	All clinical diagnoses for the patient, including diagnostic codes, terms and dates
Therapy	Multiple records per patient	All drug prescriptions for the patient, including therapy code, term, dates, strength and formulation
Test	Multiple records per patient	All test records for the patient, including test type, date and result
Referral	Multiple records per patient	All referral records for the patient, including referral date and diagnosis associated with referral
Additional	Multiple records per patient	Contains additional information that can be used to derive smoking, BMI and alcohol status

[1]Unique patient id is present in all data files and allows multiple clinical, therapy, test, referral and additional records to be linked to a single patient

3.2.2 The Hospital Episodes Statistics Database

A secondary data source used was the UK Hospital Episodes Statistics Database (HES). A subset of CPRD-contributing practices (those in England only) have had their patient records linked to HES, which is an administrative data source that contains patient demographics, clinical diagnoses and a record of procedures performed while in hospital for every NHS hospital admission in England. Linked CPRD-HES records exist from April 1997 onwards. The coding system used for diagnoses is a slight modification of the (WHO) ICD-10 standard, while procedures are identified by standard codes for hospital procedures (OPCS4 codes). Lists of all of the HES diagnostic and procedural terms (and codes) are provided as searchable text dictionary files.

HES data is organised into hospitalisations (a single stay in hospital) which can be made up of one or more episodes (the period of care under a single consultant). Within each episode, a patient can have one or multiple diagnoses and one or more procedures. Data from the HES database is extracted as separate files for patients, hospitalisations, episodes, clinical diagnoses and procedures. By linking a CPRD cohort containing patients from practices in England to their HES records (via the unique patient id) and searching on specific dates, it is possible to obtain primary care and secondary care information for a patient in temporal relation to an index diagnosis or prescription of interest.

3.2.3 The UK Office of National Statistics (ONS) mortality data

The final data source used was the Office of National Statistics (ONS) mortality database, which is linked to CPRD (and HES) via the unique patient id. A list of the causes of death (as coded on the individual's death certificate) for people who have died can be obtained from the database by providing CPRD headquarters with a list of patient ids. Cause of death is coded according to the WHO ICD-10 standard.

3.3 Cholestatic liver injury algorithm development

As detailed in section 1.5.3 Objectives, two algorithms were developed for the identification of cholestatic liver injury; one "multisource" algorithm using CPRD, HES and ONS data and a second CPRD algorithm using data only from within the CPRD database. Development of the CPRD algorithm was facilitated by using the multisource algorithm as a validation tool. The multisource algorithm was designed to be a suitable validation tool because it used both primary and secondary health care records. It was considered that the involvement of liver specialists working within specialist liver clinics in secondary care would likely mean that a diagnosis that included information from secondary care would be more accurate and/or reliable than one made using primary care data alone. The results of this algorithm development work are presented in Chapter 4.

3.3.1 The multisource cholestatic liver injury algorithm

Figure 3.1 provides an overview of steps performed by the multisource cholestatic liver injury algorithm in order to assign a cholestatic liver injury case status, which are then described in more detail in the following sections.

Figure 3.2 provides an overview in a diagrammatic form of all the data sources and time periods searched in obtaining data for a multisource algorithm cholestatic liver injury health record.

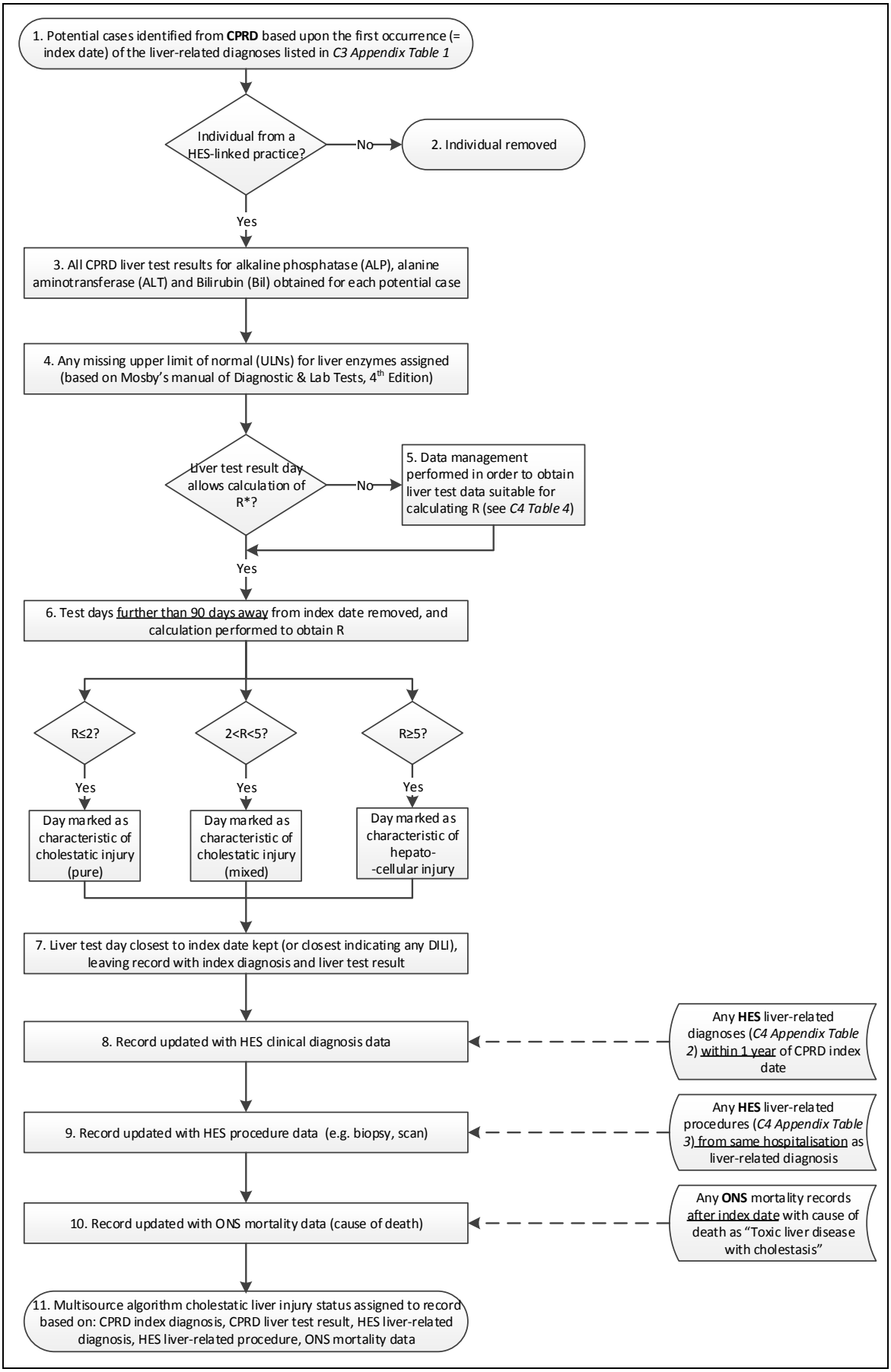


Figure 3.1: Overview of steps performed by the multisource cholestatic liver injury algorithm

3.3.1.1 *Identification of CPRD clinical diagnostic codes indicating possible cholestatic liver injury*

A list of clinical diagnostic terms that could represent cholestatic liver injury was developed following review of the studies included in Chapter 2 of this thesis. These search terms (and additional terms used to increase the specificity of the search) are detailed in Table 3-2, and were used to search the CPRD medical diagnosis dictionaries (clinical, test and referral files).

Table 3-2: Search terms used for the identification of CPRD clinical diagnostic terms that could represent possible cholestatic liver injury

Inclusion terms
Search based on the word "liver" *liver* AND (*biopsy* OR *necrosis* OR *disease* OR * enlarged* OR *disorder*)
Search based on the word "hepatic" *hepatic* AND (*failure* OR *coma* OR encephalopathy*)
Other search terms *cholesta*, *jaundice*, *icterus*, *cholangitis*, *other gall bladder disorders*, *cholaemia*, *yellow atrophy*, *hepatitis*
Terms excluded during search to increase specificity
fetal, *hepatitis a*, *hepatitis b*, *hepatitis c*, *hepatitis e*, *hepatitis g*, *delive*, *pregn*, *neonat*, *perinatal*, *viral*, *virus*, *congenital*, *autoimmune*

Note 1: * represents a wildcard, which means that any text can be present in this position
Note 2: the search was set to look for words after the word "AND" on either side of the main search term (e.g. both "liver biopsy" and "biopsy liver" would be searched for)

Terms identified by the search and considered relevant were included in a final code list, and categorised into three evidence groups as follows (Group 1=strongest evidence for cholestatic liver injury, Group 3=weakest evidence):

- Group 1: included only the term for "Toxic liver disease with cholestasis"
- Group 2: terms for jaundice or unspecified hepatitis
- Group 3: other terms for liver-related diagnoses that could possibly be cholestatic liver injury

Chapter 3 Appendix Table 1 contains the full list of diagnostic terms with their codes and evidence groupings indicated.

3.3.1.2 *Selection of participants from CPRD (primary care) records based on liver diagnosis codes*

The CPRD database was searched for individuals over the age of 18 years with a first occurrence of any of the liver- related codes listed in Chapter 3 Appendix Table 1 between the dates of January 1, 2000 and January 31, 2013. The date of the liver diagnosis was termed the index diagnosis date (or index date), and a restriction that

individuals have to be in the database for at least 12 months before the index date was applied, in order to try and ensure that the index date was the actual date of liver diagnosis (new CPRD registrants often have legacy diagnoses entered within the first few months of their registration [88]).

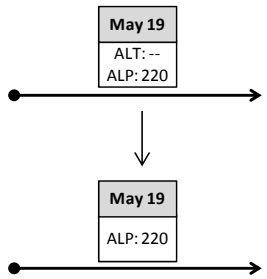
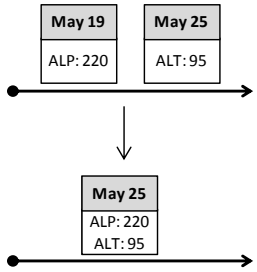
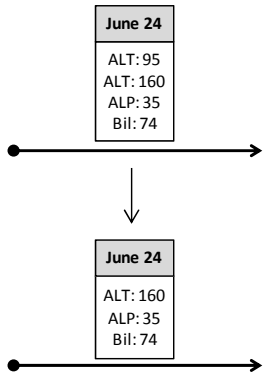
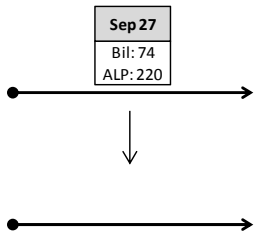
The relevant CPRD data files (see Table 3-1) were extracted for this cohort, and manipulated in order to obtain a dataset with one record per patient, including unique patient ID, index date and relevant medical diagnosis code and term. Any individuals from practices that were not linked to HES were then removed (see section 3.2.2).

3.3.1.3 Management of CPRD liver test data

All test results for the liver enzymes Bilirubin (Bil), Alkaline Phosphatase (ALP) and Alanine Aminotransferase (ALT) were then selected from the extracted test file. Blood levels of these enzymes are standard biochemical parameters for indicating serious liver injury that could be caused by drugs (DILI) and for the classification of that injury based on the R value (=the ratio of (ALT/ULN)/(ALP/ULN), where the ULN is the upper limit of the normal blood level for the enzyme) [46]. Any results with missing ULN's had ULN values inserted based upon standard definitions of these values [89] while any identical repeated results for the same enzyme on the same day were considered as data entry errors and removed .

In order for valid assessment of liver injury based on calculation of the R value, measurement of the enzyme levels should ideally be performed using the same blood sample [46]. Within the CPRD test records, blood enzyme levels recorded on the same day were considered to have been measured using the same blood sample. For days where calculation of a single R value was not possible (due to an arrangement of recorded enzyme levels other than a single ALT level and a single ALP level), data management was performed in order to obtain clean data suitable for calculation of R (see Table 3-3). Any individual whose test records required data management in this way was marked with a "data issue" variable in order to assess the impact on subsequent case status assignment.

Table 3-3: Data management performed to ensure days with liver enzyme levels recorded ("test days") contained sufficient and appropriate data for calculation of R (i.e. exactly one ALT result and exactly one ALP result)

#	Scenario	Problem	Data management performed[1]	Example illustration of change made to an individual's test record
1.	Liver enzyme level record exists but result value is empty (missing)	Cannot calculate R if no result value is present	Liver enzyme level record removed	
2.	Test day only has a single liver enzyme level recorded (e.g. ALT level only)	R cannot be calculated from levels for only a single enzyme type	Enzyme levels recorded <7 days of this day considered to be from the same blood sample, and record date amended accordingly[2]	
3.	Test day has ≥3 enzyme levels recorded on it, 2 of which are for the same enzyme (e.g. (1) Bil, ALP, and ALT x 2 or (2) ALP x 2 and ALT)	If there is (e.g.) 2 x ALP measurements on the same day with different values, then two possible R values can be calculated	Keep only the highest result for any duplicated enzyme level	
4.	Test day has 2 or 3 enzyme level records on it, and all are for the same enzyme (e.g. ALP x 2) or has 2 enzyme level records and one is Bil (e.g. Bil, ALT)	R cannot be calculated from results for a single enzyme, or from results for Bil and one other enzyme	Remove test day	

Note 1: All individuals who had test records that required any of the data management steps described here had a "data issue" variable set to "1", enabling the record to be checked during subsequent analysis if necessary

Note 2: For enzyme levels measured using the same blood sample, 7 days was considered to be the longest time that could elapse between recording the result for the first enzyme and the second enzyme in CPRD. Such a delay could be caused by administrative delay at the general practice or at the laboratory performing the tests.

The resulting dataset was then formatted so that each record represented a single test day, with variables indicating the type of test performed (i.e. ALP or ALT or Bil), its ULN and its result.

Classification of potential liver injury based on pattern of liver test results

The liver test result dates were then classified as not characteristic of drug-induced liver injury, characteristic of hepatic drug-induced liver injury or characteristic of cholestatic drug-induced liver injury (with the difference between pure or mixed cholestatic injury also indicated) by calculating the R value and categorising according to the standard criteria presented in Table 3-4 [46].

Table 3-4: Classification of type of liver injury using liver test results

#	Type of liver injury	Liver test result
1.	Characteristic of any DILI	ALT ≥ 5 x ULN or ALP ≥ 2 x ULN or ALT ≥ 3 x ULN and Bil > 2 x ULN
2.	Characteristic of hepatocellular type of DILI	R* ≥ 5
3.	Characteristic of mixed type of DILI (=cholestatic hepatitis)	R > 2 and < 5
4.	Characteristic of pure cholestatic type of DILI	R ≤ 2

*R=(ALT/ULN)/(ALP/ULN), where ALT=alanine aminotransferase, ALP=alkaline phosphatase, Bil=bilirubin and ULN=upper limit of normal

In order to only consider those test results that had been performed either as a result of (or as part of the steps leading up to) the index diagnosis, only test dates that occurred within 90 days before or after the index date were considered relevant. Liver test dates falling outside of this period were removed, and the liver test results dataset was then finalised by restricting to either (1) the test date closest to the index date (for those people who only had test results that were not characteristic of any DILI) or (2) the closest test date indicating potential injury of any type (for those people who had at least one liver test date with results characteristic of DILI).

3.3.1.4 Management of hospital diagnostic and liver-related procedure information

Identification of HES diagnostic codes indicating possible cholestatic liver injury

The same search terms used to search the CPRD diagnostic terms dictionary were applied to the HES diagnostic terms dictionary (see Table 3-2). Only two that were considered relatively specific were selected (Chapter 3 Appendix Table 2) because in a hospital setting the diagnoses made are likely to be more accurate than in primary care, as outcome assessment is being performed in a liver clinic with specialised clinicians and additional procedures available.

Identification of HES codes for liver-related procedures

Liver-related procedures occurring in hospital were considered relevant because data from a liver-related procedure in hospital (such as a biopsy or a scan) can support the classification of the type of liver injury [46]. Terms used to search the HES procedural dictionary were developed based upon review of an international meeting on case definition and phenotype standardisation of drug-induced liver injury [46] and are provided in Table 3-5. The final codelist created after reviewing the terms returned by the search is provided in Chapter 3 Appendix Table 3.

Table 3-5: Search terms used for the identification of HES procedure codes that are likely to assist in the identification of cholestatic liver injury

Inclusion terms
liver OR *abdomen* AND (*biopsy* OR *endoscopic* OR *imaging* OR *tomography* OR *ultrasound*)

Note 1: * represents a wildcard, which means that any text can be present in this position

Selection of HES diagnostic and procedure information for the cohort

The raw HES data for the cohort was obtained by searching the HES database for the patient ids of those in the cohort. The HES episode diagnoses file was then searched for the liver-related codes of interest (Chapter 3 Appendix Table 2), in order to identify people with a liver-related diagnosis of interest during any hospitalisation within one year before or after the CPRD index diagnosis date or test result indicating cholestatic liver injury (if this was recorded first and within 90 days of the index diagnosis in the patient's CPRD record). Including the prior 365 days (in addition to the subsequent 365 days) covers situations where the individual may have presented initially at hospital with (e.g.) jaundice, with the CPRD record being updated based upon the hospital report. If a person had a code for Jaundice and a code for Toxic Liver Disease with Cholestasis in the period of interest, the Toxic Liver Disease code was considered as the diagnosis of interest. The procedure codelist (Chapter 3 Appendix Table 3) was then used to identify patients who had had a liver-related procedure of interest performed during the same hospitalisation as any of the liver-related diagnoses of interest.

3.3.1.5 Management of ONS mortality data

The ONS mortality data for the cohort was searched for the presence of the code "Toxic Liver Disease with Cholestasis" (=a Group 1 code) as any cause at any time after the index date (or test result indicating cholestatic liver injury, if this preceded the index date).

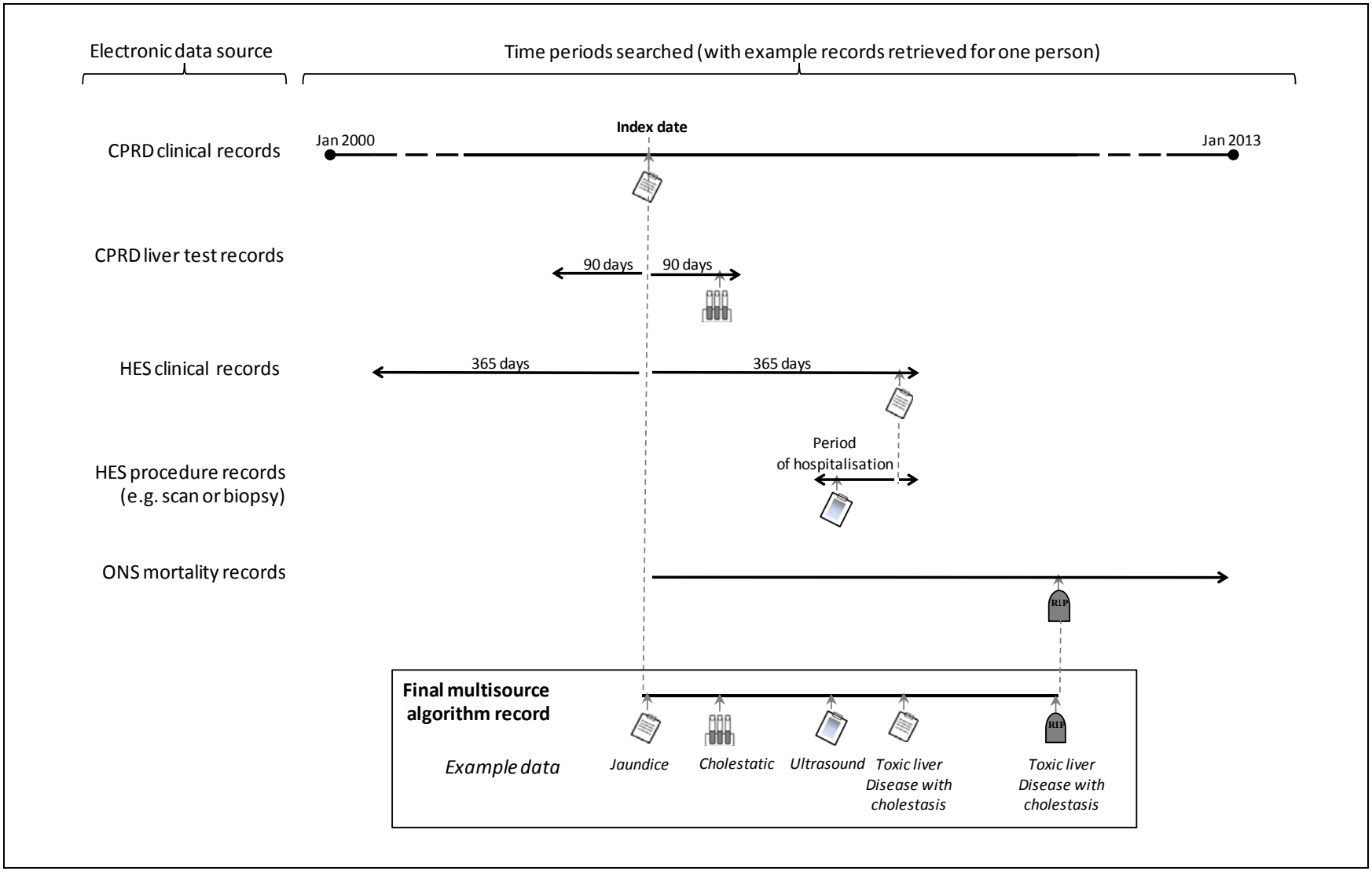


Figure 3.2: Data sources and time-periods searched in obtaining data for a multisource algorithm cholestatic liver injury health record

3.3.1.6 Multisource algorithm status assignment

The multisource cholestatic liver injury algorithm case status was then assigned based upon the following:

1. the CPRD index diagnosis group (group 1, 2 or 3)
2. the diagnostic code group for any HES liver diagnosis of interest within one year either side of the CPRD index diagnosis date (group 1 or 2 or "no HES diagnoses of interest" i.e. person attended hospital within one year of CPRD index diagnosis date but no liver-related diagnoses of interest were recorded or "no HES record" i.e. person did not attend hospital within one year of CPRD index diagnosis date)
3. the presence of any HES liver procedure (e.g. a liver-related biopsy or a scan) in the same hospitalisation as a liver HES code
4. whether any death was recorded within ONS mortality data as "Toxic liver disease with cholestasis".

Table 3-6 shows how each of these elements contributed to case assignment. Anyone with a CPRD liver test result that qualified as cholestatic was considered to be a "definite" case, based on internationally agreed consensus of the importance of biochemical criteria in the classification of liver injury [45, 46]. Individuals who had died and had an ONS ICD code that indicated a death certificate coded with "Toxic liver disease with cholestasis" (group 1) were also considered to be definite cases, in addition to individuals who were assigned this code in hospital after a biopsy or scan.

Subsequent case statuses (from very likely through probable, possible, least likely and non-case) were then assigned. Very likely cases were those who did not have any liver test results (so no biochemical data to show that they either were or were not a definite case), but had a code for "Toxic liver disease with cholestasis" (i.e. group 1) in both their CPRD and HES records. Probable cases also had no liver test result data, but had a code for "Toxic liver disease with cholestasis" in either their CPRD or their HES record. Possible cases were those who had a liver test result that showed no cholestatic injury or had no liver injury test results, but who had "Toxic liver disease with cholestasis" (group 1) indicated within CPRD or HES, or those with no liver test results to reference but both CPRD and HES codes indicating some kind of jaundice (group 2 codes).

Those classified as "least likely" were people with either (1) test results that did not indicate cholestasis who had a CPRD diagnosis of "Toxic liver disease with cholestasis" but either no HES record or no liver code in the HES record or (2) a test result not indicating cholestasis with a group 3 CPRD code (i.e. a relatively non-specific liver diagnosis) and a HES code of "Toxic liver disease with cholestasis" or (3) no LFT

results but a CPRD code indicating jaundice (group 2) and either no HES codes of interest or not admitted to hospital. All individuals with other arrangements of data from each of the sources were considered to be non-cases.

Table 3-6: Assignment of the multisource algorithm case status using CPRD, HES, & ONS data

#	**CPRD (READ) diagnostic code**	**HES diagnostic (ICD-10) code** (plus HES procedural code and ONS mortality code, where considered)	**CPRD liver test result**	**Multisource algorithm case status**
1.	Group 1|2|3[1]	*Not considered*	Cholestatic	Definite
2.	Group 1|2|3	ONS (death): Group 1	*Not considered*	Definite
3.	Group 1|2|3	Biopsy/Scan + Group 1	*Not considered*	Definite
4.	Group 1	Group 1	None[2]	Very likely
5.	Group 1	Group 2 or No HES record[3]	None	Probable
6.	Group 2|3	Group 1	None	Probable
7.	Group 1	Group 1|2	Not cholestatic[4]	Possible
8.	Group 1	HES record has no codes of interest[5]	None	Possible
9.	Group 2	Group 1	Not cholestatic	Possible
10.	Group 2	Group 2	None	Possible
11.	Group 1	No HES record | HES record has no codes of interest	Not cholestatic	Least likely
12.	Group 2	No HES record | HES record has no codes of interest	None	Least likely
13.	Group 3	Group 1	Not cholestatic	Least likely
14.	Group 2	Group 2 | No HES record | HES record has no codes of interest	Not cholestatic	Non-case
15.	Group 3	Group 2	None | Not cholestatic	Non-case
16.	Group 3	No HES record	None | Not cholestatic	Non-case
17.	Group 3	HES record has no codes of interest	None | Not cholestatic	Non-case

Note 1: Group 1=highest evidence for cholestatic liver injury, Group 3=lowest evidence (see Chapter 3 Appendix Tables 1 and 2). **Note 2:** No liver test result recorded within 90 days of index diagnosis. **Note 3:** No HES record indicates person did not attend hospital < 1 year either side of index diagnosis. **Note 4:** Liver test result was recorded within 90 days of index diagnosis but results indicate either no injury or pure hepatic injury. **Note 5:** Person attended hospital < 1year from index diagnosis but no liver diagnoses of interest

3.3.2 The CPRD cholestatic liver injury algorithm

This section details how a CPRD algorithm for assigning cholestatic liver injury case status was developed by using the multisource algorithm case status as a validation tool.

An overview of the CPRD algorithm design is provided in Figure 3.3. Potential cases are selected from extracted CPRD data files based upon the presence of specific CPRD diagnostic codes. CPRD characteristic variables required for calculation of a CPRD cholestatic liver injury cut-off score are then created and each patient record has a score assigned to it based on whether they have a "1" assigned for each variable. There is then a two-stage procedure of case-status assignment:

(a) Stage 1 - individuals with a value of "1" for variables that perfectly predict cholestatic liver injury case status are assigned as cases
(b) Stage 2 - individuals who have not been already assigned as cases (during stage 1) with a CPRD algorithm score above a specific cut-off are assigned as cases, with anyone remaining unassigned considered a non-case

The remainder of this chapter describes the cohort that was used to develop this algorithm (section 3.3.2.1), the identification of CPRD characteristic used as potential explanatory variables for the multisource algorithm response variable (3.3.2.2), the multisource algorithm response variable (3.3.2.3), the statistical analysis performed to identify true CPRD explanatory variables (including those that perfectly predicted the multisource response variable) (3.3.2.4), and finally the use of ROC analysis to assess the validity of the CPRD algorithm and allow consideration of appropriate cut-off scores for further studies.

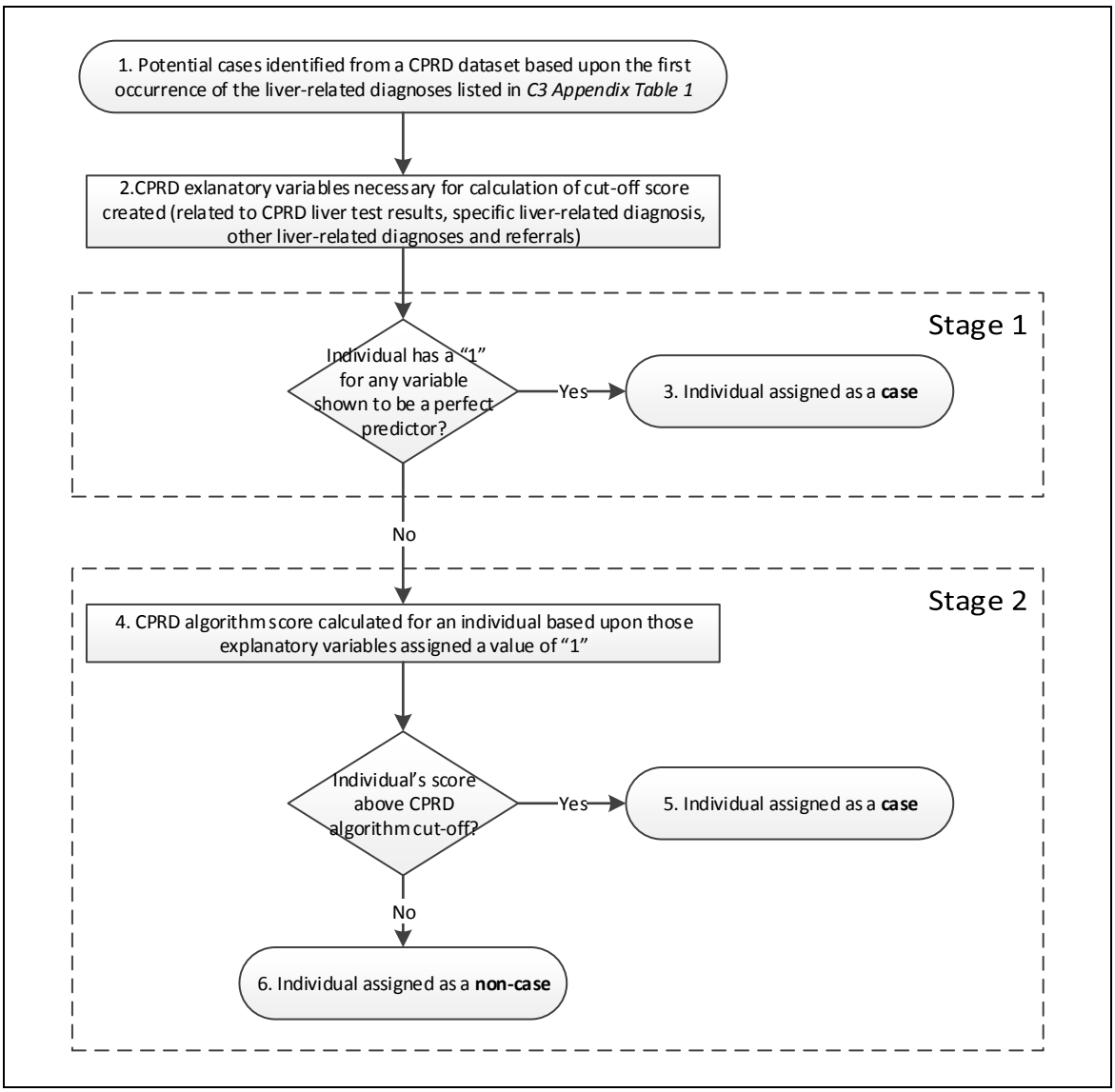


Figure 3.3: Overview of steps performed by the CPRD cholestatic liver injury algorithm

3.3.2.1 Selection of participants from CPRD based on liver diagnosis codes

The CPRD database was searched for individuals over the age of 18 years with a first occurrence of any of the liver-related codes used in the multisource algorithm development (Chapter 3 Appendix Table 1) between the dates of January 1, 2000 and January 31, 2013. The search was performed within the clinical, referral and test records. The date of the liver diagnosis was termed the index diagnosis date (or "index date"), and a restriction that individuals had to be in the database for at least 12 months before the index date was applied.

3.3.2.2 CPRD algorithm exposure: potential explanatory variables

For the individuals selected from CPRD based upon the presence of one of the liver codes of interest (Chapter 3 Appendix Table 1) there were four main characteristics considered to be potential predictors of the multisource algorithm cholestatic liver injury case status: liver test result information, referral information around the index date, the

type of liver-related index diagnosis and information on any other liver-related diagnosis apart from the index diagnosis. Binary variables were created for characteristics of interest, with 0 meaning that the person did not have the characteristic and 1 meaning that they did have the characteristic. Variables created in relation to the index diagnosis terms coded for whether the person had a specific type of diagnosis or not, and the type of diagnosis was defined by grouping together clinically similar diagnoses (for example “hepatic failure related” or “jaundice or similar terms”). A full list of the explanatory variables considered is provided in Chapter 3 Appendix Table 4.

3.3.2.3 CPRD algorithm outcome: multisource case status

The outcome (response variable) used in development of the CPRD cholestatic liver injury algorithm was the multisource case status, categorised so that a value of 1 was a multi-source case status of definite through to possible, while 0 was a multi-source case status of least likely or non-case.

3.3.2.4 Statistical analysis

Prior to statistical analysis, the cohort was randomly split into two separate datasets of equal size, one for statistical model building (the training dataset) and the other for testing of the model (the validation dataset).

Using the training dataset, each of the potential CPRD explanatory variables was tabulated against case status, before univariable analysis was performed in order to obtain a crude odds ratio for the association between the potential explanatory variable (CPRD characteristic) and the response variable (multisource algorithm case status). Any potential explanatory variables that perfectly predicted multisource definite-possible case status (i.e. 100% of the individuals in one of the binary categories of the potential explanatory variable were cases) were noted and removed from consideration as potential explanatory variables.

Multivariable analysis was then performed on those potential explanatory variables that had shown an association with the outcome in univariable analysis (with association in univariable analysis indicated by an odds ratio of association less than or greater than 1.0, and a 95% confidence interval that did not include 1.0). A multivariable logistic regression model was constructed, and used to assess if each variable of interest was associated with the outcome after adjustment for all the other variables in the model. In order to be able to cope with strata containing a lack of data in the multivariable model, Firth’s logistic regression methodology was used for this multivariable analysis, which can handle strata with sparse data by using penalised maximum likelihood estimation

[90]. Evidence for an association was assessed by performing likelihood ratio tests (LRT). Variables with LRT p-values of less than 0.05 were considered to be associated with the outcome and kept in the model. Variables considered not to be associated after adjustments were removed from the model, and the analysis for the other variables repeated as necessary with the reduced model.

3.3.2.5 CPRD algorithm score generation and assignment

Variables for storing explanatory variable "scores" were added to the validation dataset, and if an individual had a value of 1 for any of the CPRD explanatory variables, the corresponding score variable for that variable was populated with the log odds value obtained from the multivariable regression analysis. A total score variable was created and this was populated with the sum of all the explanatory variable scores, in order to generate a final CPRD cholestatic liver injury score for the individual equal to the overall predicted log odds for the individual, given their covariate values.

3.3.2.6 ROC analysis of CPRD cholestatic liver injury algorithm and consideration of cut-off scores

The ability of the CPRD cholestatic liver injury algorithm to discriminate between the two multisource cases statuses (definite to possible vs. least likely to non) was assessed by plotting a Receiver Operator Characteristic (ROC) graph (sensitivity vs. 1-specificity) across the range of CPRD algorithm cut-off scores. As detailed in Figure 3.3, the CPRD algorithm follows a two stage case identification procedure, where firstly those who have a "1" for any variables that perfectly predict multisource case status are assigned as cases, before the cut-off score is applied to the remainder of the cohort in order to identify any remaining cases. The following ROC analyses were therefore performed:

(1) **The complete CPRD algorithm** i.e. assessing the ability of the complete algorithm (both stage 1 and stage 2 from Figure 3.3) to discriminate between the two multisource case statuses. For this analysis, those individuals who had a "1" for any of the variables shown to be perfect predictors of multisource case status were included in the cohort, and were assigned a "perfect prediction" CPRD algorithm score (a score that was manually inputted as higher than the highest combined CPRD algorithm score of all those individuals in the cohort who did not have a "1" for any perfect predictor variables). This allowed the performance of the complete algorithm to be assessed.

(2) **The CPRD algorithm cut-off score** i.e. assessing the ability of the cut-off score only (stage 2 from Figure 3.3) to discriminate between the two multisource case statuses. For this analysis, those individuals who had a "1" for

any of the variables shown to be perfect predictors of multisource case status were removed from the cohort, meaning the performance of the algorithm score on its own could be assessed in the conditions that it would be applied (i.e. identifying cases in the subset of people who did not have a “1” for variables shown to be perfect predictors of multisource case status)

For each ROC analysis, the sensitivity and specificity for a range of CPRD algorithm score cut-offs was considered, using the ROC graph and tabulations of the full range of CPRD algorithm cut-off scores versus sensitivity and specificity.

3.4 Pharmacoepidemiological studies of drug-induced cholestatic liver injury

3.4.1 Applying the algorithm to a well established association: a cohort study of the association between flucloxacillin and cholestatic liver injury

This section describes a study that compares different CPRD cholestatic liver injury algorithm cut-off scores with varying specificity and sensitivity with (1) each other and (2) the multisource algorithm for outcome identification in a pharmacoepidemiological study.

3.4.1.1 Study design

A cohort analysis of the association between flucloxacillin and cholestatic liver injury, with oxytetracyline as a comparator drug.

3.4.1.2 Setting and participants (incl. exclusions)

Setting

The study was performed within the UK CPRD (see section 3.2.1) and included patients actively registered in the database between the dates of 1st January 2000 and 1st January 2012. Additional information required for applying the multisource algorithm was obtained from information within HES that is linked to patients within the CPRD (see section 3.2.2).

Exposed group – inclusion criteria

People over the age of 18 with at least one prescription for flucloxacillin and at least 12 months of computerised prescription history in CPRD prior to their first prescription. The 12 month period was used to ensure that the date of flucloxacillin or oxytetracycline prescription (index date) was as accurate as possible (people who have recently registered may have all legacy diagnoses and prescriptions entered into the system on one date, that represents only the date at which the person was first registered into the system).

Exposed group – exclusion criteria

In order to remove patients with diseases or conditions that were likely to cause cholestatic liver injury, patients who had any of the following in their CPRD record 12 months prior to their flucloxacillin prescription were excluded: any documented liver disease, alcoholism, malignant neoplasm of the liver/gallbladder/pancreas, cholelithiasis, viral hepatitis, chronic liver disease, cirrhosis, congestive heart failure,

hepatitis following blood transfusion, HIV, rheumatoid arthritis, sarcoidosis, systemic lupus or inflammatory bowel disease (see Chapter 3 Appendix Table 5 for full list of diagnostic codes) and any liver test results that met the criteria for drug-induced-liver injury (see Table 3-4). In addition, any woman with a code indicating pregnancy within 40 weeks prior to their index date who did not have a subsequent code indicating delivery (or termination of pregnancy in any other way) was excluded, due to the fact that the incidence of cholestasis in pregnant women is approximately 1 in 100 women [91]. Individuals with any previous prescription for flucloxacillin were also excluded.

Comparison group – inclusion criteria

The comparison group was chosen to be people prescribed another antibiotic with a similar range of indications to flucloxacillin but that was not considered to be a cause of cholestatic liver injury. Users of such antibiotics are likely to be more comparable to the exposed group with respect to exposures (in terms of e.g. health-seeking behaviour, illnesses) than non-users but not the outcome. Oxytetracycline was selected as one such comparator, as it is an antibiotic with a similar range of indications that has been used by researchers performing comparable studies in the past [53]. Figure 3.4 provides details of the exposed and comparator groups.

Comparison group – exclusion criteria

Same criteria as for the exposed group, in relation to oxytetracycline.

3.4.1.3 Sample size/power calculations

A recent CPRD cohort study estimated the risk of cholestatic liver disease to be 8.5 per 100 000 first time users for flucloxacillin (95% CI 5.4 – 12.6) and 0.8 per 100 000 first time users for oxytetracycline (95% CI 0.02 – 4.3), a risk ratio of 8.5/0.8 = 10.63 [53] . A power calculation using STATA version 13 was performed and in order to detect a difference of this size with 95% precision and 90% power, calculated that a sample size of 164,806 in each exposure group would be required.

A feasibility count in CPRD was performed by obtaining the number of people who had a first time prescription for flucloxacillin and for oxytetracycline in a sample of 1 million people (extracted from the database October 2011) between the start and end of the study period (01/01/2000 – 01/01/2012) and multiplying this appropriately to reflect the total number of patient records in CPRD in October 2011 (11.6 million). There were 1,793,267 people with a first time prescription for flucloxacillin with records in the database in October 2011 and 297,795 people with a first time prescription for oxytetracycline, meaning that the study would be adequately powered to detect the likely difference, even after applying exclusion criteria.

3.4.1.4 Standard Protocol Approvals, Registrations, or Patient Consents

The study was approved by the Independent Scientific Advisory Committee for MHRA database research and the internal ethics committee of the London School of Hygiene and Tropical Medicine (see Chapter 3 Appendix Table 7).

3.4.1.5 Exposures, outcomes and co-variates

Exposures

Exposures were determined from prescription records within the CPRD therapy data files for these antibiotics. A person was considered exposed up to 45 days after the start of a first prescription for flucloxacillin or oxytetracycline, as flucloxacillin-induced cholestatic liver injury may occur up to approximately 6 weeks after first administration of the drug (see Chapter 1, Section 1.4.1.1 and Figure 3.4). The date of prescription of the drug under study was termed the index date, and people who were prescribed flucloxacillin on the same date as oxytetracycline were included in the flucloxacillin group only. Any person who received oxytetracycline on their index date but then received a prescription for flucloxacillin (before their end of follow-up) was assigned to the flucloxacillin group, and their index date updated appropriately (see section 3.4.1.6 for a description of end of follow-up).

Whether a person was prescribed the flucloxacillin plus ampicillin (co-fluamp) formulation or flucloxacillin alone was also recorded, as it has previously been shown that there are differing risks associated with pure flucloxacillin vs. the formulation which includes ampicillin. Duration of flucloxacillin use was considered in order to assess any duration-response effects, and was assessed by using prescriptions recorded in CPRD therapy records. The dates of the first, second and third flucloxacillin prescriptions within 90 days after the index date (but before the end of follow-up) were identified and used to create a time-updated variable called *number of flucloxacillin prescriptions*. This allowed events and follow-up time after each person's first, second (if present) and third (if present) prescriptions to be identified and a corresponding rate and rate ratios to be calculated. A time *between first flucloxacillin prescription and case assignment date* was also created.

Outcomes

The main outcome under study was the CPRD cholestatic liver injury algorithm (see section 3.3.2 for details). CPRD algorithm scores of "High" specificity (100% specificity, 81% sensitivity, against a gold standard of the multisource algorithm probable to definite case - cut off score of 5.0), "Medium" specificity: 86% specificity, 87%

sensitivity (cut of score 2.29) and “Low” specificity: 48% specificity, 100% sensitivity (cut off score 1.63) were compared with each other and the following:

1. Each other (within the full dataset)
2. The multisource algorithm as described in section 3.3.1.6 (within the dataset restricted to HES-linked participants only)
3. Published studies on the association between flucloxacillin and cholestatic liver injury [53, 55, 67, 75]

Details on how the CPRD algorithm outcome definitions were compared with each other and the multisource and published outcome definitions are provided in section 3.4.1.6.

The CPRD and multisource algorithms were used to identify cases of cholestatic liver injury as described in sections 3.3.1 and 3.3.2, with an additional check applied to ensure none of the clinical, test or referral dates used by the algorithms occurred before the index date of this study (i.e. before the prescription for either of the drugs of interest). A further slight amendment to the multisource algorithm was made to avoid potential time-related bias in this cohort setting: in contrast to the method for selecting HES diagnostic information described in section 3.3.1.4, if a code for Jaundice occurred before a code for Toxic Liver Disease with Cholestasis, the code for Jaundice was considered as the diagnosis of interest.

All cases of cholestatic liver injury identified by each outcome definition were considered as potentially caused by one of the drugs under study. The full electronic record within 12 months prior to the outcome event was extracted for all these cholestatic liver injury cases and reviewed in order to make a final case status assignment (with consideration as to whether there was any other more likely pathological/therapeutic cause for the detected injury). This review was performed by two reviewers (blinded to drug exposure) one of whom was clinically trained (Dr Adrian Root, LSHTM and General Practitioner). In case of disagreements, the final decision was made by the clinically trained reviewer.

Co-variates

Results of previous studies, a-priori knowledge and causal diagrams were used to assist with the identification of co-variates. A causal diagram was prepared using the DAGitty graphical tool for analyzing causal diagrams [92], and is included in Chapter 3 Appendix Table 6a. Age, gender, smoking, ethnicity, BMI, alcohol intake, SES, use of other drugs known to cause cholestatic liver injury and calendar period were all considered as potential measurable confounders or effect modifiers.

Age and gender have been suggested as risk factors for flucloxacillin-induced liver injury [53, 55, 56], and are included as part of the CPRD patient (demographic) data file. A categorical age variable was created with ten year categories.

The role of ethnicity has not been previously studied, and was considered important due to a likely genetic susceptibility to flucloxacillin-induced liver injury [57, 58]. Ethnicity codes based on the 2001 UK census data were used to search the CPRD additional files [93], in order to assign ethnicity to the cohort, as a 5-category variable. If no ethnicity records were found in CPRD for a patient, the HES records of any patients that were HES-linked were also searched for ethnicity information. Any patients who did not have ethnicity information in CPRD or HES were assigned to an “Unknown” category.

BMI, alcohol intake and SES are likely to be associated with indications requiring flucloxacillin (such as cellulitis) and with susceptibility to liver-related conditions. CPRD smoking status is recorded as non-, ex-, current and unknown. CPRD alcohol status is recorded as never, ex-, current (not-otherwise-specified), ≤2 or less units/day, 3-6 units/day, >6 units per day, and unknown. A categorical BMI status variable was created with categories of <20, 20-25, 25+, and unknown. Smoking, alcohol and BMI status were assigned according to the classification of the nearest date prior to the index date (if no prior status, the status from the nearest post-index date was used). SES information is not part of the standard CPRD database, and was obtained separately as linked data. This was provided by CPRD as an Index of Multiple Deprivation score based upon individual patient postcode, and as a practice level score based upon practice postcode (both variables consisting of 5 categories representing quintiles of score). A dedicated SES variable was created for the study and populated with the patient-level score, unless this was missing, in which case the practice-level score was used. This linked SES data was only available for practices in England.

Calendar period was included in order to assess if changes in prescribing habits or recording of outcomes occurred over time (for example, improved automation of the system for capturing liver test results within CPRD over time). A calendar time-period variable was created with categories spanning 3-year periods.

The possible impact of the use of other drugs associated with cholestatic liver injury was assessed by looking for prescriptions for other drugs that occurred before the end of follow-up for the patient and up to 1 month before the index date. A variable was created with three categories: 0=no use of other drugs, 1=use of drugs thought to cause cholestatic liver injury at a frequency lower than flucloxacillin, 2=use of drugs thought to cause cholestatic liver injury at a frequency higher than flucloxacillin. The list of drugs and categorisation was based upon review of results of studies included in

Chapter 2 of this thesis, combined with a number of additional sources [48, 49, 94, 95], and included non-steroidal anti-inflammatory drugs, other antibiotics, antidepressants and antifungals. A full list of therapies included is provided in Chapter 3 Appendix Table 7.

Patients exposed to both oxytetracycline and flucloxacillin during their follow-up were included in the flucloxacillin group only, and a sensitivity analysis performed to assess the impact of excluding these patients.

3.4.1.6 Data management, statistical analysis and bias

CPRD data file extraction

Drug substance names and BNF codes were used to identify a list of codes used to search prescription records in CPRD. Patient ids and first prescription dates from CPRD for people over 18 years of age receiving a first prescription for flucloxacillin or oxytetracycline between 1st January 2000 and 1st January 2012 (latest prescription date 22 December 2011) were extracted as a single (index) file from CPRD, along with additional separate data files containing information on all patient demographic information, clinical diagnoses, therapies and test results (all linked by unique patient id). Any patient with clinical codes for the exclusions listed in Chapter 3 Appendix Table 5 within 12 months prior to the date of their first prescription for one of the study drugs was excluded. For the comparison of the CPRD algorithm with the multisource algorithm, any individuals from practices that were not linked to HES were then removed (as described previously - see section 3.3.1.2).

A list of potential cases was then prepared by searching patient clinical records for the diagnostic codes applicable to the outcome being assessed, and either the specific CPRD algorithm cut-off score or multisource algorithm was used to assign a cholestatic liver injury case status for each of the outcome definitions (performed blinded to drug exposure status). Due to the different criteria applied by each of the outcomes, it was possible for one individual to be classified as a cholestatic liver injury case according to one outcome definition but not another. The cholestatic liver injury case status information was then applied to the cohort, and the resulting dataset was searched for the presence (and date) of the exclusions listed in Chapter 3 Appendix Table 5. All patients with an exclusion event during their follow-up had the date of that event added to their record, their case status changed to non-case. The co-variate information was then added to the dataset before the record review described in section 3.4.1.5 (Outcomes section) was performed. This allowed case status to be finalised based upon the presence or absence of any more likely cause of cholestatic liver injury than a

prescription with either of the drugs under study. The final step was the addition of the drug exposure status to the cohort.

Statistical analysis

The initial step was to perform a descriptive analysis of the cohort, including tabulation of characteristics of participants by exposure to the drug of interest. A comparison of the 3 CPRD algorithm outcomes and the multisource algorithm outcome was then performed by performing the analyses detailed below, which were considered to be typical of pharmacoepidemiological studies.

1. Number and characteristics of identified cases

The number of cases of cholestatic liver injury identified by each of the outcome definitions was tabulated, along with the number and % that were considered as final cases of cholestatic liver injury caused by one of the drugs under study. The time between first prescription and injury and characteristics of the cases were also tabulated.

2. Risk of cholestatic liver injury

The risk of cholestatic liver injury for each drug was calculated by dividing the total number of events by the number of patients in each exposure group (see Figure 3.4). 95% confidence intervals were calculated on the basis of a Poisson distribution of injury events within each exposure group and the risk of cholestatic liver injury occurring per 100,000 users within each of the exposure groups was tabulated. The risk of cholestatic liver injury in the 46 – 90 day period after exposure to flucloxacillin was also calculated, in order to assess whether this differed from the 1 – 45 day period after an exposure to flucloxacillin.

3. Association between flucloxacillin and cholestatic liver injury

For the analysis of the association between flucloxacillin and cholestatic liver injury, all relative effects were calculated using rates of injury (to obtain rate ratios). The rate of injury was calculated by dividing the number of events by the total person-time at risk. For all rate calculations, individuals were followed up until the first of death, end of the study period (45 days after the index date), drug-induced cholestatic liver injury as defined by the outcome being tested and clinical expert review, any of the exclusion criteria, or transfer out date.

The three CPRD algorithm cut-off scores required multiple sequential pieces of information from the CPRD record (e.g. an index diagnosis followed by an associated liver function test or a hospital referral), and follow-up was until the latest date of the entries that qualified that person as a case for the particular outcome definition. So, for example, if the case cut-off score was 2.29, and the individual was considered a case

due to having a score of 2.29 that was the total of an index diagnosis of obstructive jaundice (contributing a score of 1.89) and another liver-related diagnosis within one month (contributing a score of 0.40), then the person was followed up until the latest of either of these events. Note that it was possible for the same person to have a different case date if they also qualified as a case according to one of the other cut-off score definitions. For example, the patient described here could also have had a cholestatic liver test result, meaning they would qualify as a case according to the CPRD algorithm cut-off score definition of 5. If the cholestatic result appeared later in their record than their additional liver-related diagnosis, they would have a different case date for the algorithm cut-off score of 5. For the multisource algorithm, follow-up was until the earliest date at which they had accumulated the multisource (possible to definite) cut-off score.

Associations between each co-variate and drug exposure were assessed by tabulating and looking for differences in % for exposed vs. unexposed groups. Associations between co-variates and the outcome under test were assessed by looking at rate ratios, their 95% confidence intervals, and Likelihood Ratio Tests for the overall association of the co-variate. Classical (Mantel Haenzel) analysis was performed on each co-variate considered a potential confounder or effect modifier.

Crude rate ratios for the association between flucloxacillin and cholestatic liver injury were then obtained by comparing the rate of cholestatic liver injury during the 1 - 45 day period after a first prescription of flucloxacillin to the rate during the 1 - 45 day after a first prescription of oxytetracycline.

Figure 3.4 provides an example of the exposure groups and follow-up timelines applied in this study.

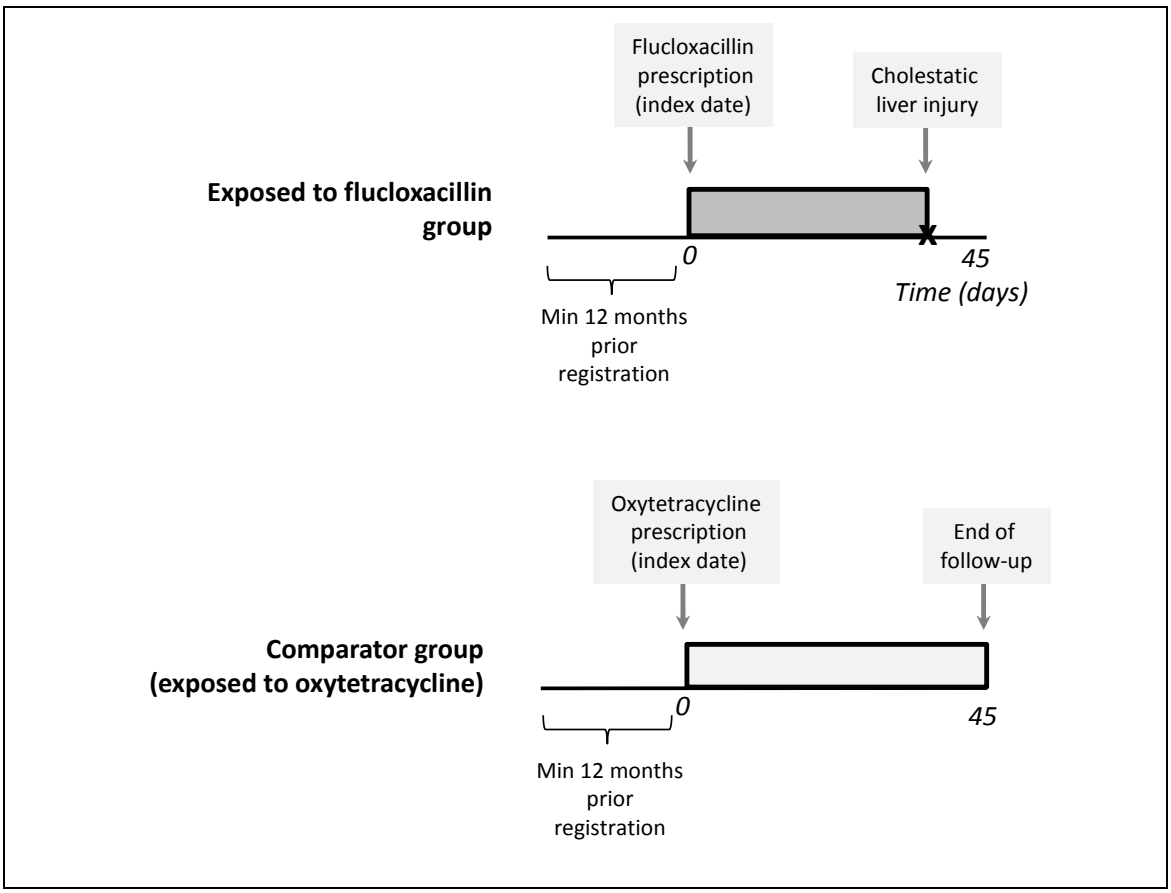


Figure 3.4: Exposed and comparator groups of the flucloxacillin and cholestatic liver injury cohort study

Risk calculations were performed within the following two exposure groups (1) Exposed to flucloxacillin = all people who are prescribed flucloxacillin at day 0 (2) Comparator group = all people who are prescribed oxytetracycline at day 0 (and not also flucloxacillin before their end of follow-up) The risk of injury was calculated by dividing the total number of events that occur within 45 days of the first day of each group by the total number of people in each group.

For **rate** calculations, patients in the exposed to flucloxacillin group were followed up from their first prescription for flucloxacillin (the index date) for a maximum of 45 days. Patients in the comparator group (exposed to oxytetracycline) were followed up from their first prescription of oxytetracycline (the index date), also for a maximum of 45 days. The rate of cholestatic liver injury in the exposed to flucloxacillin group was compared to the rate in the oxytetracycline group to obtain the **rate ratios** used in this study. In this example, the person exposed to flucloxacillin experiences an event within the 45 day period, and is therefore not followed up beyond this point. The person exposed to oxytetracycline does not experience a cholestatic liver injury event (or exclusion event or death), so follow-up for this patient ends after 45 days.

A Poisson regression model was then constructed, with potential confounders or effect-modifiers included as informed by the DAG analysis. Statistical tests (Likelihood Ratio Tests or LRT tests) for interaction were used to investigate effect modification for those co-variates identified as potential effect modifiers in classical analysis, and if confirmed, stratum specific rate ratios were presented. If no effect modifiers were identified, a single multivariable adjusted rate ratio was presented. Multivariable analysis was then performed using two separate approaches: (1) a stepwise approach, adding co-variates to the Poisson regression model one-by-one, and keeping those that were associated with a substantial (>5%) relative change in rate ratio and (2) a fully adjusted approach, including all available variables identified by the DAG analysis. Both results

were presented in the results tables (with the fully adjusted figures included as footnotes).

4. Analysis of risk factors for flucloxacillin-induced cholestatic liver injury

A risk-factor based analysis was performed, in order to look at characteristics within those in the exposed to flucloxacillin group only that were associated with an increased rate of cholestatic liver injury (i.e. identifying variables that interacted with the exposure to flucloxacillin). Risk factors were selected based upon (1) evidence of association in univariable analysis (2) identification in published literature as potential risk factors. Risks, rates, and crude rate ratios were calculated and tabulated, along with multivariable adjusted rate ratios (allowing the impact of adjusting for other potential risk factors to be assessed).

Bias

Observer bias was limited by blinding to drug exposure status and to outcome status during preparation of the cohort for analysis, which was achieved by managing this information separately from the main cohort, and combining only immediately prior to analysis. Review of the electronic record was also performed blinded to initial and any subsequent exposure to the drugs of interest. Attempts to reduce measurement error (related to main exposure) were made by ensuring that people prescribed oxytetracycline but then flucloxacillin before their end of follow-up were assigned to the flucloxacillin group. Misclassification of outcome was considered throughout the study, by careful consideration of the results obtained using the case identification algorithms of varying sensitivity and specificity.

Handling low numbers of events and missing values

For numerical-categorical variables that had a lack of events within one or more categories, where possible these were combined in order to allow analysis of the variable to be performed. The design of the study ensured that the final dataset for analyses had no missing values for the main exposure (flucloxacillin or oxytetracycline) or for the outcome (cholestatic liver injury as determined by different algorithms). For co-variates with missing values, the use of three approaches was compared:

(1) including all individuals in the analyses and assigning individuals with missing data for a particular co-variate to an “unknown” category for that co-variate (allowing the extent of the missing data for that variable to be clearly presented)
(2) restricting the analysis only to those individuals with complete records (complete records analysis)

(3) imputing any missing data for co-variates included in the final substantive models of association using multiple imputation (assuming missing at random and performing sensitivity analyses for missing not at random).

Sensitivity analyses

The following sensitivity analyses were performed:

1. An analysis of the effect of excluding patients exposed to both oxytetracycline and flucloxacillin, compared with assigning them to the flucloxacillin group (as done in this study)
2. An analysis of the effect of assigning people identified as cases of cholestatic liver injury who had an exclusion code between their index date and injury date as cases of drug-induced cholestatic liver injury, compared with considering them as non-cases (as done in this study)
3. The effect of excluding cases who were in the heaviest drinking category was assessed (i.e. considering their liver injury to be caused by drinking)
4. The effect of excluding people who had been prescribed co-fluamp was assessed.

3.4.2 Applying the algorithm to putative but unknown associations: a case-control study of the association between five drug exposures and cholestatic liver injury

This section describes a study that uses two of the cholestatic liver injury CPRD algorithm case definitions (1:highest sensitivity, lowest specificity, 2: highest specificity, lowest sensitivity) to analyse the association between cholestatic liver injury and five possible drug causes of cholestatic liver injury. The drugs of interest were selected because they were considered to be prescribed relatively frequently in the UK and had a lack of large epidemiological studies of their effect on cholestatic liver injury. Furthermore, all the drugs had (a) been included in recent or multiple cholestatic liver injury case reports published in the literature and/or (b) had statements in their prescribing information suggesting that they caused liver injury at an unknown frequency (or at a frequency estimated using adverse event reporting figures). An overview of the five drugs is provided in Table 3-7.

Table 3-7: An overview of the five drugs in the cholestatic liver injury case control study

Drug	Type or class	Indications	Current information on drug as a cause of liver injury
Carbamazepine	Anticonvulsant	Epilepsy, pain association with trigeminal neuralgia and prevention of manic-depressive psychosis	Prescribing information: rare cause of jaundice (≥1/10,000 to <1/1,000) [96] Published literature: case reports (examples [97, 98])
Celecoxib	NSAID (selective COX-2 inhibitor)	Relief of symptoms of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis	Prescribing information: hepatitis and jaundice occur at an unknown frequency [99]
Duloxetine	Serotonin-norepinephrine reuptake inhibitor (SNRI)	Depression, anxiety and diabetic neuropathic pain	Prescribing information: jaundice caused at a frequency of ≥1/10,000 to <1/1,000 users based on adverse event reporting from post-marketing surveillance [100] Published literature: case reports (examples [101, 102])
Ramipril	Angiotensin-converting enzyme inhibitor	Hypertension, renal disease, symptomatic heart failure.	Prescribing information: a rare (≥1/10,000 to <1/1,000) cause of jaundice and causes cholestatic hepatitis at an unknown frequency [103] Published literature: case report plus review of adverse event reporting [104]
Risperidone	Antipsychotic	Schizophrenia, manic episodes of bipolar disorders and persistent aggression associated with Alzheimer's dementia and childhood conduct disorder.	Prescribing information: a rare (≥1/10,000 to <1/1,000) cause of jaundice [105] Published literature: case reports (examples [106, 107])

3.4.3 Study design

A case-control analysis of the association between five drug exposures and cholestatic liver injury in the UK CPRD (described in detail in section 3.2.1). The study also included estimation of the incidence of cholestatic liver injury caused by any of the drugs under study that were (1) shown to be strongly associated with cholestatic liver injury by the case control analysis (with strongly associated considered to be a rate ratio >1.5 and 95% CI not crossing the null value) and (2) have consistent results for the high and low specificity algorithms.

3.4.3.1 Setting and participants (incl exclusions)

Setting

The study was performed within the UK CPRD (see section 3.2.1) and included patients actively registered in the database between the dates of 1st January 1992 and 31st July 2014.

Cases – inclusion criteria

People identified as having cholestatic liver injury by the CPRD cholestatic liver injury algorithm (see section 3.3.2) who have at least 12 months of computerised clinical diagnostic history in CPRD prior to their injury date (see section 3.4.1.2). The date at which the person is identified as a case by the CPRD cholestatic liver injury algorithm will be considered the index date.

Cases – exclusion criteria

In order to remove patients with recently diagnosed diseases or conditions that were likely to cause symptoms of cholestatic liver injury, patients who had any of the following in their CPRD record 12 months prior to their case date (or matched case date for controls) were excluded: any documented liver disease, alcoholism, malignant neoplasm of the liver/gallbladder/pancreas, cholelithiasis, viral hepatitis, chronic liver disease, cirrhosis, congestive heart failure, hepatitis following blood transfusion, HIV, rheumatoid arthritis, sarcoidosis, systemic lupus or inflammatory bowel disease (see Chapter 3 Appendix Table 5 for a full list of diagnostic codes) and any liver test results that met the criteria for drug-induced-liver injury (see Table 3-4). In addition, any woman with a code indicating pregnancy within 40 weeks prior to their index date who did not have a subsequent code indicating delivery (or termination of pregnancy in any other way) was excluded, due to the fact that the incidence of cholestasis in pregnant women is approximately 1 in 100 women [91].

Selection of controls

Controls were selected from the total CPRD population over the age of 18 by a density sampling method, meaning that control subjects were selected from the same point in time when each case occurred [108]. Up to 4 controls were selected for each case, matched on age (+ or – 1 year), sex and practice. Controls were people in the database who were being followed on the index date of their matched case (i.e. did not have any exclusion criteria in the preceding 12 months), did not have a designation as a case of cholestatic liver injury on the index date of their matched case, and also had at least 12 months of computerised clinical diagnostic history in CPRD prior to their (matched) injury date. If no matches were identified for a particular case, the reasons for the no matches being found were considered and presented in the results, before dropping the case. The matching program used was an adaptation of a program created by Tim Collier (LSHTM), 2012.

Controls -exclusion criteria

Identical to that applied to the cases.

3.4.3.2 Sample size/power calculations

Power calculations were performed for a matched case control study for each drug exposure as described in the literature, using proportion of exposed controls, likely number of cases, odds ratio to be detected, number of matched cases and required significance as inputs [109]. The proportion of exposed controls was estimated for each drug by obtaining a 1 million random sample of people in the database who had an end of registration period that was after the start of the study period (1st January 1992), searching for the number of people with at least one prescription for the drug of interest, and dividing by 1 million. The minimum number of likely cases identified by the CPRD cholestatic liver injury algorithm was estimated as 6000, based upon the number of (likely to probable) cases identified by the multisource algorithm for a study period that was ten years shorter (5014 likely to probable cases, see Chapter 4 Table 4-2).

The analysis of carbamazepine, celecoxib and ramipril was estimated to have over 80% power to detect an odds ratio of 1.5 or higher, at a significance of 0.05 (see Table 3-8). For risperidone and duloxetine, due to the lower number of (estimated) exposed controls, the analysis was estimated to have over 80% power to detect an odds ratio of 2.0.

Table 3-8: Power of study to detect cholestatic liver injury for each drug exposure

Drug	Detectable odds ratio	Estimated proportion of exposed controls	Estimated number of cases	Number of matched controls	Significance	Calculated power of study
Carbamazepine	1.5	0.01	6000	4	0.05	83%
Celecoxib	1.5	0.01	6000	4	0.05	83%
Duloxetine	1.5	0.003	6000	4	0.05	39%
	2.0	0.003	6000	4	0.05	84%
Ramipril	1.5	0.04	6000	4	0.05	99%
Risperidone	1.5	0.005	6000	4	0.05	57%
	2.0	0.005	6000	4	0.05	96%

3.4.3.3 Outcome, exposure and co-variates

Outcomes

Two of the CPRD cholestatic liver injury algorithm definitions (see section 3.3.2 for details) were used as outcomes in the study: (1) the “High” specificity definition (100% specificity, 81% sensitivity compared against a gold standard of the multisource algorithm probable to definite case - cut off score of 5.0) and (2) the “Low” specificity definition (48% specificity, 100% sensitivity, cut off score 1.63). The two CPRD algorithm definitions were used to identify cases of cholestatic liver injury as described in sections 3.3.1 and 3.3.2.

For estimations of the relative effect via the case-control analysis (looking at the association between the drug exposure and cholestatic liver injury), selection of outcomes was performed entirely using the CPRD cholestatic liver injury algorithm. Detailed/clinician review of the electronic record of potential cases within a set time period prior to the case date (as performed in the previous study in this chapter) was not performed, because this was not feasible for the potential control population. Furthermore, performing detailed review of only the potential cases and not the potential controls would risk biasing the study, because it may have resulted in final controls being selected from a population that was not representative of the source population from which the final cases were selected [80]. For the analysis of the absolute effect of each drug exposure on the incidence of cholestatic liver injury, however, the full electronic record of those identified as cases of cholestatic liver injury by the CPRD algorithm within 12 months prior to the outcome event was extracted and reviewed in order to make a final case status assignment (with consideration as to whether there was any other more likely pathological/therapeutic cause for the

detected injury). This review was performed by two reviewers (blinded to drug exposure) one of whom was clinically trained (Dr Adrian Root, LSHTM and General Practitioner), the other being myself. In case of disagreements, the final decision was made by the clinically trained reviewer.

Exposures

Exposures were determined by searching prescription records within the CPRD therapy data files for the drugs under study prior to the index date (searching based on drug substance keyword). A person was considered to be exposed to the drug for the period that the drug prescription covered (calculated from dosage and pack size information in CPRD) plus an additional "grace period" which was dependent upon the specific drug, and allowed for the possibility of an additional full prescription. This grace period was particularly designed to accommodate drugs that were likely to have been prescribed repeatedly over a long period (such as risperidone), where it would have been possible that the individual continued to use the drug after the end of their last prescription (due to, for example, having a repeat prescription already at home). The exposure variable was split into four categories, defining the following user groups:

1. *current users*: people who are exposed to the drug on their index date (i.e. the dosage and pack size information plus grace period indicate that the person was taking the drug on their index date)
2. *recent users:* people who are not current users, but received a prescription that ends 1 to 30 days prior to the index date (after taking into account the grace period)
3. *past users:* people who are not current or recent users, but received a prescription that ends earlier than 30 days prior to the index date (after taking into account the grace period)
4. *non-users:* people who had no prescriptions for the drug of interest prior to their index date.

Where dosage pack and size information was missing and duration could not be calculated, a median population pack duration was imputed. In addition to the five drugs under study, flucloxacillin was also analysed as an exposure. This was to allow the results obtained in the case control analysis to be verified against those obtained in the previous cohort study,(as described in section 3.4.1).

Co-variates

Results of previous studies, a-priori knowledge and causal diagrams were used to assist with the identification of co-variates. A causal diagram was prepared using the

DAGittty graphical tool for analyzing causal diagrams [92], and is included in Chapter 3 Appendix Table 6b. Age, gender, smoking, ethnicity, BMI, alcohol intake, SES, use of other drugs known to cause cholestatic liver injury and calendar period were all considered as potential measurable confounders or effect modifiers. Existing variables in the CPRD dataset were created or new ones setup as described for the previous study in this chapter (section 3.4.1.5),

3.4.3.4 Data management, statistical analysis and bias

CPRD data file extraction

Patient ids and date of first potential cholestatic liver injury codes (see Chapter 3 Appendix Table 1) from CPRD for people over 18 years of age with one of the liver-injury codes of interest between 1st January 1992 and 31st July 2014 were extracted as a single (index) file from CPRD, along with additional separate data files containing information on all patient demographic information, clinical diagnoses, therapies and test results (all linked by unique patient id). This list comprised potential cases. The necessary variables for running the CPRD algorithm were then created (see section 3.3.2), and the CPRD algorithm was applied in order to select actual cases from the list of potential cases. Two separate potential case files were created: one containing the cases identified by the high specificity version of the algorithm and the other containing cases identified by the low specificity version. In both files, all cases had a case date variable populated with the date at which they qualified as a case for the specific case type (e.g. date of the CPRD record that qualified them as a case according to the specific algorithm definition). Any potential case with clinical codes corresponding to the exclusions described in Chapter 3 Appendix Table 5 or liver test results indicative of drug-induced liver injury within one year prior to the date of their case assignment date was then removed from the two cohorts of cases. Matched controls from anyone in CPRD over the age of 18 with at least 12 months of clinical diagnostic history prior to their matched case date were then identified for the cases, and identical exclusion criteria applied (searching within one year prior to the matched case date). The two case cohorts were then combined with their controls in order to form complete analysis cohorts for the two case definitions. All co-variate information was then added to each cohort. Finally, drug substance name was used to prepare codelists for each of the study drugs (with an additional check using BNF code), which were used to search for prescription records for the 5 drugs under study and allowed the (current, recent, past and non- user) drug exposure variable to be created.

For calculating incidence, the drug codelists were used to identify the total number of people over the age of 18 who had at least one prescription for the drug of interest in CPRD during the study period.

Statistical analysis

Relative effect estimates (rate ratio)

As density sampling was used to select controls (meaning that controls were selected longitudinally through the course of the study as each case was found), the calculated odds ratio in this study estimates the rate ratio [108]. All subsequent effect measures will therefore be described as rate ratios. Initial descriptive analysis involved tabulating demographic characteristics and co-variates by case and control status. Conditional logistic regression was then used to assess the association of the co-variates with the outcome, by looking at the estimated rate ratios, their 95% confidence intervals and Likelihood Ratio Tests for the overall association of the co-variate.

A conditional logistic regression model was then prepared, comparing each use category with having never used the drug of interest, in order to obtain a crude estimated rate ratio for the association between being a current, recent or past user of one of the drugs of interest and experiencing a subsequent cholestatic liver injury.

Figure 3.5 illustrates index dates and drug user classification periods for a sample case and a matched control.

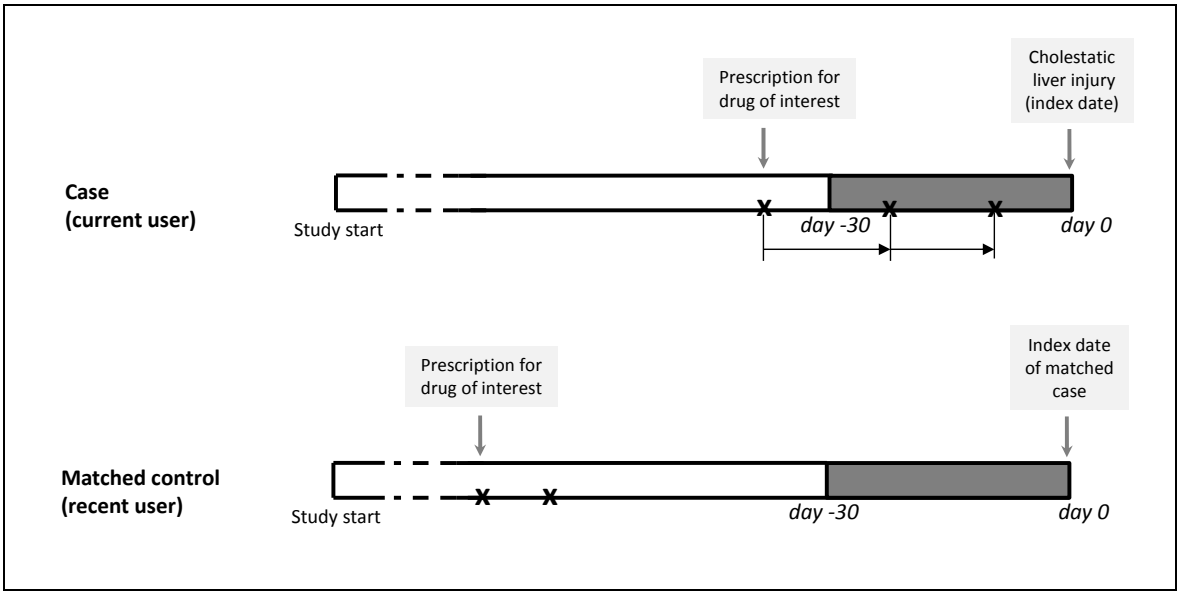


Figure 3.5: Example patient outcome and exposure timelines for the cholestatic liver injury and multiple drug exposures matched cases control study

Index date for the case is the cholestatic liver injury date, while for the control, it is the cholestatic liver injury date for the case it was matched to. In this example, the case has three consecutive prescriptions for the drug under study, with the final prescription occurring within 30 days of the index date. Based on dosage/pack size information and the addition of any "grace period", the prescription is estimated to cover a period that ends after the index date, meaning that this case would be classified as a "current user". In contrast, the matched control has two prescriptions, the second of which begins more than 30 days before the matched index date. The period this prescription is estimated to cover (again based on dosage/pack size and "grace period") ends within 30 days of the matched index date, meaning that this control would be classified as a "recent user".

Multivariable analysis was then performed by creating a model that included the drug exposure of interest and all of the co-variates identified in the causal analysis (age, gender, smoking, ethnicity, BMI, alcohol intake, SES, use of other drugs known to cause cholestatic liver injury and calendar period). Statistical tests (LRT) for interaction were used to investigate effect modification for each co-variate, and if confirmed, stratum specific rate ratios were presented. If no effect modifiers were identified, only a single multivariable adjusted rate ratio was presented.

Absolute effect measures (risk)

Absolute effect measures were estimated for the drugs under study if the results produced by the case control analysis met the following criteria:

1. There was good evidence that being a current or recent user of the drug was shown to be strongly associated with cholestatic liver injury (with a RR of >1.5 selected as representing a strong association and a 95% CI that did not cross the null value of 1.0 selected to indicate good evidence)

2. The results obtained by the low and high specificity algorithms were consistent. This meant that if an association that met the criteria in "1." above

was detected by either the high or low specificity algorithm, the effect estimated by the other algorithm was in the same direction and/or the 95% CI included the original effect estimate.

The risk of cholestatic liver injury was calculated by dividing (1) the total number of cases identified for the drug (by the low and the high specificity algorithms separately) by (2) the total number of people in CPRD with at least one prescription during the study period. 95% confidence intervals were calculated on the basis of a Poisson distribution of injury events and the risk of cholestatic liver injury per 100 000 current and recent users was presented.

Bias

Attempts to minimize selection bias were made by selecting controls from the total underlying source population that the cases were selected from, and applying exactly the same exclusion criteria to controls as cases. Care was taken not to perform review of case information that could not also be applied to control information (for example, detailed review of potential case records that could not also be applied to potential control records was not performed). Observer bias was minimised by defining set periods when people were considered exposed to the drugs of interest and assigning exposure status without consideration of case status. Considering consistency of results across algorithms carefully (as detailed in the section on absolute effect measures above) was performed in order to separate real results from those that may have been caused by (for example) unmeasured confounders.

Handling missing values and low numbers of events

For numerical-categorical variables that had a lack of events within one or more categories, where possible these were combined in order to allow analysis of the variable to be performed. The way of defining exposure ensured that the final dataset for analyses had no missing values for the main exposure (any of the drugs under study) or for the outcome (cholestatic liver injury as determined by different algorithms). For co-variates with missing values, the use of two approaches was compared (see Chapter 6 for full discussion):

(1) including all individuals in the analyses and assigning individuals with missing data for a particular co-variate to an “unknown” category for that co-variate (allowing the extent of the missing data for that variable to be clearly presented)
(2) restricting the analysis only to those individuals with complete records (complete records analysis)

3.5 Chapter 3 Summary

- This chapter described the overall methodology used in the thesis, including data sources, methods for algorithm development and methods for two epidemiological studies

- The methodology for the following steps was described in detail:

    - The use of multiple linked electronic health record sources (CPRD, HES, and ONS mortality databases) to construct a multisource algorithm for detection of cholestatic liver injury

    - The use of the multisource algorithm case status to aid development and validation of a CPRD cholestatic liver injury algorithm, using data only from a standard CPRD record

    - Assessment of the performance of the CPRD algorithm via a cohort study on the association between flucloxacillin and cholestatic liver injury

    - Investigation of a number of putative drug causes of cholestatic liver injury via a case control study

4 Results – multisource and CPRD cholestatic liver injury algorithm development

4.1 Introduction

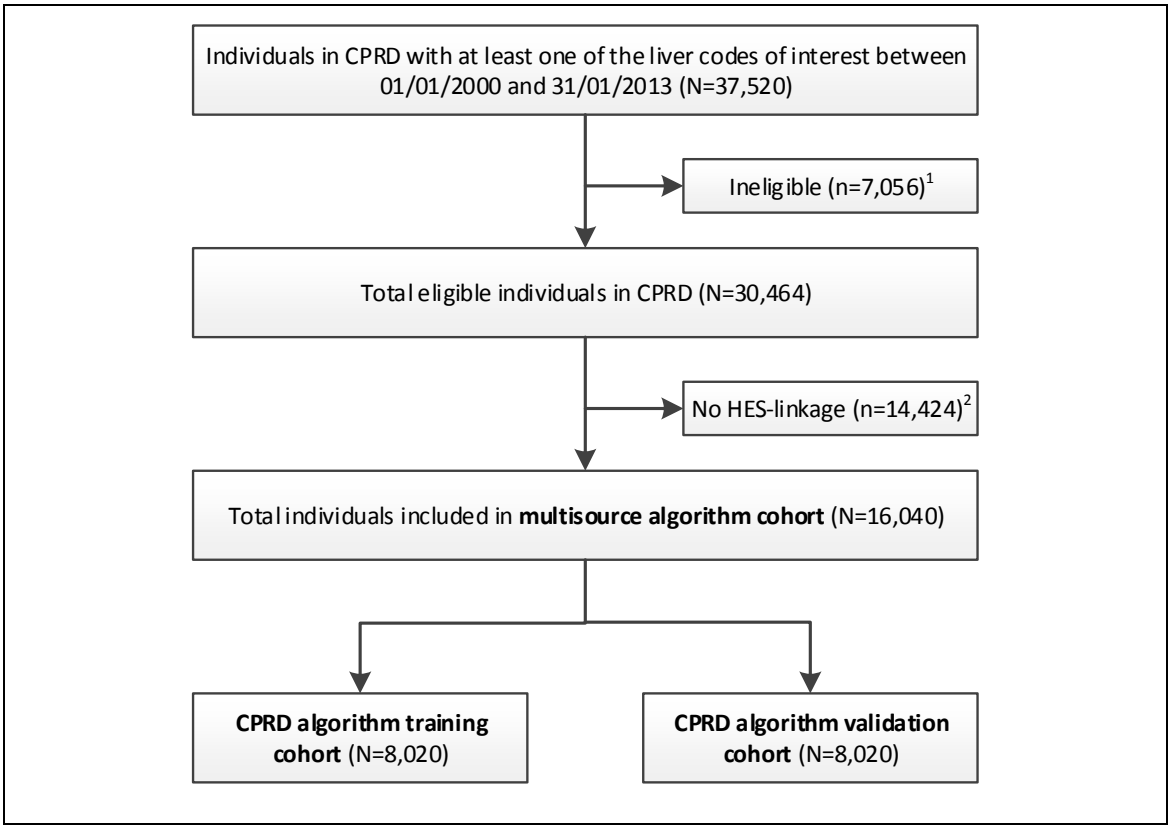
In this chapter, the results of the development of the multisource and CPRD cholestatic liver injury algorithms are presented. Details of the number of participants included and cohort descriptions are provided for the cohorts used to develop both algorithms. For the multisource algorithm, additional results are provided related to the number of individuals assigned to each case status (according to the methods described in Chapter 3 section 3.3.1.6). For the CPRD algorithm regression analyses results for the association between the CPRD explanatory variables and the multisource outcome (according to Chapter 3 section 3.3.2.4) are provided, followed by the results of ROC analyses to assess how well the CPRD algorithm discriminates the multisource case status (according to Chapter 3 section 3.3.2.6).

4.2 Multisource algorithm development

4.2.1 Participants

Between the dates of January 1st 2000 and December 31st 2012 37,520 people were identified in CPRD with any of the codes indicative of possible liver injury (as detailed in Chapter 3, Appendix Table 1). 7,056 people were then removed as they did not meet the necessary eligibility criteria (see Figure 4.1), and removal of a further 14,424 individuals from practices in Scotland, Wales or Northern Ireland (not part of the HES-linkage process, as detailed in Chapter 3 section 3.2.2) left a total of 16,040 individuals in the cohort used for the development of the multisource algorithm.

Figure 4.1: Flow of number of individuals included in the multisource algorithm and the CPRD algorithm cohorts



[1]**Ineligible:** <18 years of age or registered in CPRD for <12 months prior to liver-related diagnosis
[2]**No HES-linkage:** individual was registered with a primary care practice that was not part of the HES-linkage process (i.e. practices in Scotland, Wales or Northern Ireland; English practices that have not agreed to participate and patients within participating practices that have opted out)

4.2.2 Descriptive data

Table 4-1 provides an overview of the characteristics of the people included in the multisource algorithm cohort. The median age of the cohort was 62 years, and 52% were male. There was a slight increase in the number of diagnoses for the codes of interests (see Chapter 3 Appendix Table 1) over the recruitment period of 2000 - 2012 (with 30% of codes diagnosed between 2009 and 2012), but only in accordance with the increase in size of the database between 2009 - 2012 compared to the other (shorter) time periods (Chapter 4 Appendix Table 1). The most common index diagnosis codes in CPRD were jaundice, obstructive jaundice and cholangitis. Over 54% of people had a CPRD liver test result recorded within 90 days of their index diagnosis date, with the majority of these occurring on or after the index diagnosis date. 79% of people had been admitted to hospital for any reason within 1 year either side of the index diagnosis date (or date of liver test indicating any type of liver injury, if the test date was within 90 days prior to the index diagnosis), and for the majority of these, the closest hospital admission date to the index date was either on or after the diagnosis date. 37% of the cohort had any ONS mortality record at any time after index diagnosis.

Table 4-1: Characteristics of people included in the multisource algorithm cohort (data from CPRD record unless otherwise stated)

		(N = 16040) n (%)
Age at index diagnosis date[1]	18 – 29	948 (6)
	30 – 39	1452 (9)
	40 – 49	2164 (13)
	50 – 59	2736 (17)
	60 – 69	2937 (18)
	70 – 79	3127 (20)
	80+	2676 (17)
	median (25 - 75%):	*62 (47 – 75)*
Gender	Male	8406 (52)
	Female	7634 (48)
Date of index diagnosis	2000 – 2002	3336 (21)
	2003 – 2005	3867 (24)
	2006 – 2008	3962 (25)
	2009 – 2012	4875 (30)
Index diagnosis	Jaundice[2]	6951 (43)
	Obstructive jaundice nos	2531 (16)
	Cholangitis	1144 (7)
	Hepatitis unspecified	408 (4)
	Chronic hepatitis	541 (3)
	Other liver disorders	528 (3)
	Biopsy of liver	412 (3)
	Any other code[3]	3223 (20)
Liver test results[4]	No liver test result	7354 (46)
	Test results before index diagnosis	4039 (25)
	Test results on or after index diagnosis	4647 (29)
HES record[5]	No HES record	3392 (21)
	HES record before index diagnosis	923 (6)
	HES record on or after index diagnosis	11725 (73)
ONS mortality record[6]	No ONS mortality record	10157 (63)
	Had ONS mortality record	5883 (37)

Note 1: Date of diagnosis with one of the potential cholestatic liver injury codes listed in Chapter 3 Appendix, Table 1
Note 2: Includes codes "Jaundice – symptom", "[d]jaundice", "O/e – jaundiced", "[d]jaundice (not of newborn)"
Note 3: People in this group had an index diagnosis of any of the other codes listed in Chapter 3 Appendix, Table 1
Note 4: No liver test results=none within 90 days either side of index diagnosis date; test results before/after=closest liver test result was before/after the index and within 90 days
Note 5: No HES record=no HES record ever (n=1080) or no record within 365 days either side of index diagnosis date (n=2312); HES record before/after index diagnosis:=closest HES record was before/after the index & within 365 days
Note 6: ONS mortality record at any time (after index diagnosis)

4.2.3 Results

Applying the algorithm described in Chapter 3 section 3.3.1 resulted in individuals being assigned the case statuses shown in Table 4-2. 4032/16040 (25%) of the cohort were assigned as definite cases, with almost all of these assigned due to the presence of a liver test result recorded in CPRD indicating cholestatic liver injury (pure or mixed, as defined in Chapter 3 section 3.3.1.3). None of the individuals who had ONS mortality records had “toxic liver disease with cholestasis” indicated on their ONS death certificate, and after assignment of definite case status based upon liver test results in CPRD, only one person remained who was classified as a definite case based upon having had a biopsy or scan in hospital and then being diagnosed with a HES diagnosis of toxic liver disease with cholestasis (Group 1).

No individuals were assigned into the very likely case category (being people without a liver test result recorded in CPRD but with both CPRD and HES diagnoses of toxic liver disease with cholestasis), and only four individuals were assigned as probable (people without liver test results but with at least one database having a recorded diagnosis of toxic liver disease with cholestasis). 977/16040 (6%) of the cohort were assigned as possible cases of cholestatic liver injury, with the majority (947/977) assigned this status due to codes related to jaundice (Group 2) in both databases but no liver tests to confirm the presence or absence of a cholestatic pattern of liver injury. The remainder of the cohort were assigned as unlikely or non-cases, with unlikely cases being assigned as such primarily (3468/3492) due to having a code for jaundice in CPRD but no HES records of interest and no liver test results, and non-cases having a less specific injury code in CPRD (Group 3) without any codes of interest recorded in HES.

Table 4-2: Multisource cholestatic liver injury algorithm – results of case status assignment

CPRD (READ) diagnostic code	**HES diagnostic (ICD-10) code** (plus *HES procedural code* or *ONS mortality code*, where considered)	**CPRD liver test result**	**Multisource algorithm case status**	**(N = 16040)** n (%)
Group 1|2|3[1]	Not considered	Cholestatic	Definite	4032 (25)
Group 1|2|3	*ONS (mortality): Group 1*	Not considered	Definite	0 (0)
Group 1|2|3	*HES Biopsy/Scan* + Group 1	Not considered	Definite	1 (0)
			Total definite	***4033 (25)***
Group 1	Group 1	None[2]	Very likely	0 (0)
			Total very likely	***0 (0)***
Group 1	Group 2 or No HES record[3]	None	Probable	0 (0)
Group 2|3	Group 1	None	Probable	4 (0)
			Total probable	***4 (0)***
Group 1	Group 1|2	Not cholestatic[4]	Possible	1 (0)
Group 1	HES no codes of interest[5]	None	Possible	25 (0)
Group 2	Group 1	Not cholestatic	Possible	4 (0)
Group 2	Group 2	None	Possible	947(6)
			Total possible	***977 (6)***
Group 1	No HES record | HES no codes of interest	Not cholestatic	Unlikely	22 (0)
Group 2	No HES record | HES no codes of interest	None	Unlikely	3468 (22)
Group 3	Group 1	Not cholestatic	Unlikely	2 (0)
			Total unlikely	***3492 (22)***
Group 2	Group 2 | No HES record | HES no codes of interest	Not cholestatic	Non-case	2869 (18)
Group 3	Group 2	None | Not cholestatic	Non-case	173 (1)
Group 3	No HES record	None | Not cholestatic	Non-case	340 (2)
Group 3	HES no codes of interest	None | Not cholestatic	Non-case	4152 (26)
			Total non-case	***7534 (47)***

Note 1: Group 1=highest evidence for cholestatic liver injury, Group 3=lowest evidence (see Chapter 3 Appendix Tables 1 & 2)
Note 2: No liver test result recorded within 90 days of index diagnosis
Note 3: No HES record indicates person did not attend hospital < 1 year either side of index diagnosis
Note 4: Liver test result was recorded <90 days from index diagnosis but results indicate either no injury or pure hepatic injury
Note 5: Person attended hospital < 1year from index diagnosis but no liver diagnoses of interest

4.3 CPRD algorithm development

4.3.1 Participants

Randomly splitting the multisource algorithm cohort into equal-sized datasets (as described in Chapter 3 Section 3.3.2.4) left a CPRD algorithm training cohort and a CPRD algorithm validation cohort which each contained 8020 individuals (see Figure 4.1).

4.3.2 Descriptive data

The CPRD algorithm training and validation cohorts had a similar distribution of gender, age and date of diagnosis characteristics to the (parent) multisource algorithm cohort described in section 4.2.2 (see Chapter 4 Appendix Table 2), which was as expected given that patients were divided into the two cohorts at random.

Additional descriptive characteristics for the training cohort are presented in the first results column of Table 4-3. 25% of the individuals had a CPRD cholestatic liver test result, and just under one fifth had at least one liver-related referral recorded in CPRD within 30 days either side of the index date. Almost half of the CPRD index diagnoses (46%) were jaundice-related codes, while only 1% were for a code of toxic liver disease with cholestasis. 23% of people had one or more additional liver-related diagnoses in CPRD within 30 days of the index date, with only 5% having a referral for a liver-related scan (or other hospital-based test) recorded in their CPRD record within the same time period.

4.3.3 Results

4.3.3.1 Univariable and multivariable analysis (training cohort)

As detailed in Chapter 3 Section 3.3.2, the univariable and multivariable results were used to identify those CPRD explanatory variables that were predictors of multisource algorithm definite to possible cases (subsequently referred to as “cases”, with multisource algorithm least-likely to non-case status referred to as “non-cases”) using the training cohort.

Liver test result status was shown to perfectly predict case status i.e. all of those with CPRD cholestatic liver test results were classified as cases, This variable was therefore not considered subsequently in the multivariable model, but was included in the final design of the CPRD algorithm as described in Chapter 3 section 3.3.2 (Figure 3.3).

The two CPRD explanatory variables that were the strongest predictors of being a case in univariable analysis were having an index diagnosis of "Toxic liver disease with cholestasis" (OR 5.53, 95% CI 2.54 – 12.03) or having an index diagnosis of "Obstructive jaundice" (OR 2.29, 95% CI 2.02 – 2.59). For both these index diagnoses, the association with case status was strengthened after adjustments for all other variables, with multivariable odds ratios of 20.59 (95% CI 9.41 – 45.08) for "Toxic liver disease with cholestasis" and 6.64 (95% CI 5.42 - 8.13) for "Obstructive jaundice". Having a code for "Jaundice" (or similar) was also strongly associated with being a case (multivariable OR 5.10, 95% CI 4.25 – 6.11). Multivariable analysis showed weaker positive associations for "Cholangitis" (or similar) (OR 1.89, 95% CI 1.47 – 2.44) and "Liver enlargement" (or related) (OR 1.98, 95% CI 1.12 – 3.49). Of the remaining index-diagnosis related variables, two were demonstrated to be negative predictors for outcome status after multivariable adjustments: "Other or non-specific diagnoses" (OR 0.63, 95% CI 0.42 – 0.95) and "Chronic hepatitis" (OR 0.20, 95% CI 0.09 – 0.45).

Of the CPRD explanatory variables related to referrals or to additional liver-related diagnoses, three showed strong evidence of small associations with multisource algorithm case status. People who had any referral recorded in CPRD within 30 days before or after the index diagnosis date were more likely to be cases (multivariable OR 1.48, 95% CI 1.33 – 1.65), as were people referred for a liver-related scan or test (multivariable OR 1.51, 95% CI 1.18 – 1.94). Having an additional liver-related diagnosis within 30 days of the index diagnosis date was also a weak predictor for being a case (multivariable OR 1.49, 95% CI 1.33 – 1.67).

The univariable and multivariable results therefore meant that the CPRD variables included in the final CPRD cholestatic liver injury algorithm were as listed below. The stages refer to the two case-assignment stages of the algorithm as described in Chapter 3 section 3.3.2, Figure 3.3 (stage 1=variable perfectly predicts outcome status, so anyone with a "1" for this variable is assigned as a case first; stage 2=variable is used when obtaining a score for each individual not assigned as a case by stage 1, so that total score for the individual can be compared against a specified cut-off score in order to assign case status).

1. **CPRD liver test result of cholestatic:** algorithm stage 1
2. **Had any referrals:** algorithm stage 2
3. **Jaundice (or similar) as index diagnosis:** stage 2
4. **Cholangitis-related index diagnosis:** stage 2
5. **Chronic hepatitis index diagnosis:** stage 2
6. **Obstructive jaundice index diagnosis:** stage 2

7. **Toxic liver disease with cholestasis index diagnosis:** stage 2
8. **Liver-enlargement related index diagnosis:** stage 2
9. **Other or non-specific liver-related index diagnosis:** stage 2
10. **Number of liver related diagnoses <30 days from index diagnosis:** stage 2
11. **Referral for liver-related scan <30 days from index diagnosis:** stage 2

Table 4-3: Descriptive, univariable and multivariable analysis of the association between being a multisource algorithm (definite to possible) case and potential CPRD explanatory variables (using the training cohort)

CPRD explanatory variable		Total N = 8020 n (%)	Cases N = 2468 n (%)	Crude OR[1] (95% CI[2])	Multivariable[3] OR (95% CI)	p-value[4]
CPRD liver test result	None |not cholestatic	6044 (75)	492 (20)	-	-	
Had any referrals[5]	None	4650 (58)	1132 (46)	1	1	
	1 or more referrals	3370 (42)	1338 (54)	2.04 (1.86 - 2.25)	1.48 (1.33 - 1.65)	<0.001
Had liver referrals[5]	None	6513 (81)	1812 (73)	1	1	
	1 or more	1507 (19)	658 (27)	2.01 (1.79 - 2.26)	0.99 (0.84 - 1.15)	0.858
Top liver referral code[5]	None | Group 3	6936 (86)	1973 (80)	1	1	
	Group 1 or Group 2	1084 (14)	497 (20)	2.13 (1.87 - 2.43)	0.98 (0.84 - 1.15)	0.840
Hepatitis (or similar) index[6]	No	7442 (93)	2403 (97)	1	1	
	Yes	578 (7)	67 (3)	0.27 (0.21 - 0.36)	1.07 (0.76 - 1.50)	0.701
Jaundice (or similar) index	No	4301 (54)	944 (38)	1	1	
	Yes	3719 (46)	1526 (62)	2.47 (2.24 - 2.73)	5.10 (4.25 - 6.11)	<0.001
Hepatic failure-related index	No	7951 (99)	2464 (100)	1	1	
	Yes	69 (1)	6 (0)	0.21 (0.09 - 0.49)	0.88 (0.38 - 2.01)	0.752
Cholangitis-related index	No	7262 (91)	2337 (95)	1	1	
	Yes	758 (9)	133 (5)	0.45 (0.37 - 0.54)	1.89 (1.47 - 2.44)	<0.001
Chronic hepatitis index	No	7720 (96)	2464 (100)	1	1	
	Yes	300 (4)	6 (0)	0.04 (0.02 - 0.10)	0.20 (0.09 - 0.45)	<0.001
Hepatic enceph or coma index	No	7932 (99)	2462 (100)	1	1	
	Yes	88 (1)	8 (0)	0.22 (0.11 - 0.46)	0.96 (0.46 - 1.98)	0.904
Alcohol-related index	No	7659 (96)	2423 (98)	1	1	

CPRD explanatory variable		Total N = 8020 n (%)	Cases N = 2468 n (%)	Crude OR[1] (95% CI[2])	Multivariable[3] OR (95% CI)	p-value[4]
	Yes	361 (5)	47 (2)	0.32 (0.24 - 0.44)	1.42 (0.99 - 2.05)	0.065
Liver necrosis-related index	No	8014 (100)	2469 (100)	1	1	
	Yes	6 (0)	1 (0)	0.45 (0.05 - 3.85)	2.12 (0.34 - 13.24)	0.455
Obstructive jaundice index	No	6774 (84)	1886 (76)	1	1	
	Yes	1246 (16)	584 (24)	2.29 (2.02 - 2.59)	6.64 (5.42 - 8.13)	<0.001
Toxic liver w cholestasis index	No	7989 (99)	2448 (99)	1	1	
	Yes	31 (1)	22 (1)	5.53 (2.54 - 12.03)	20.59 (9.41 - 45.08)	<0.001
Toxic liver disease (non-chol) index	No	7994 (100)	2468 (100)	1	1	
	Yes	26 (0)	2 (0)	0.19 (0.04 - 0.79)	0.88 (0.24 - 3.31)	0.851
Liver-enlargement related index	No	7939 (99)	2454 (99)	1	1	
	Yes	81 (1)	16 (1)	0.55 (0.32 - 0.95)	1.98 (1.12 - 3.49)	0.027
Liver biopsy-related index	No	7689 (96)	2447 (99)	1	1	
	Yes	331 (4)	23 (1)	0.16 (0.10 - 0.25)	0.66 (0.41 - 1.04)	0.060
Cholaemia index	No	8016 (100)	2470 (100)	-	-	
	Yes	4 (0)	0 (0)	-	-	
Other|non-specific liver-related index	No	7598 (95)	2439 (99)	1	1	
	Yes	422 (5)	29 (1)	0.16 (0.11 - 0.23)	0.63 (0.42 - 0.95)	0.020
No. of liver-related diag on index[5]	One	7734 (96)	2350 (95)	1	1	
	More than one	286 (4)	120 (5)	1.66 (1.30 - 2.10)	1.16 (0.90 - 1.49)	0.256
No. of liver-related diag[5]	None	6165 (77)	1652 (67)	1	1	
	One | more	1855 (23)	818 (33)	2.15 (1.94 - 2.40)	1.49 (1.33 - 1.67)	<0.001
Top-ranked addtl liver diag[5]	None or Group 3	6693 (83)	1842 (75)	1	1	

CPRD explanatory variable		Total N = 8020 n (%)	Cases N = 2468 n (%)	Crude OR[1] (95% CI[2])	Multivariable[3] OR (95% CI)	p-value[4]
	Group 1 | Group 2	1327 (17)	628 (25)	2.15 (1.93 - 2.39)	1.00 (0.82 - 1.22)	0.950
Referral for liver-related scan|test[5]	No referral	7719 (96)	2312 (94)	1	1	
	Had a referral	301 (4)	158 (6)	2.58 (2.05 – 3.26)	1.51 (1.18 - 1.94)	<0.001

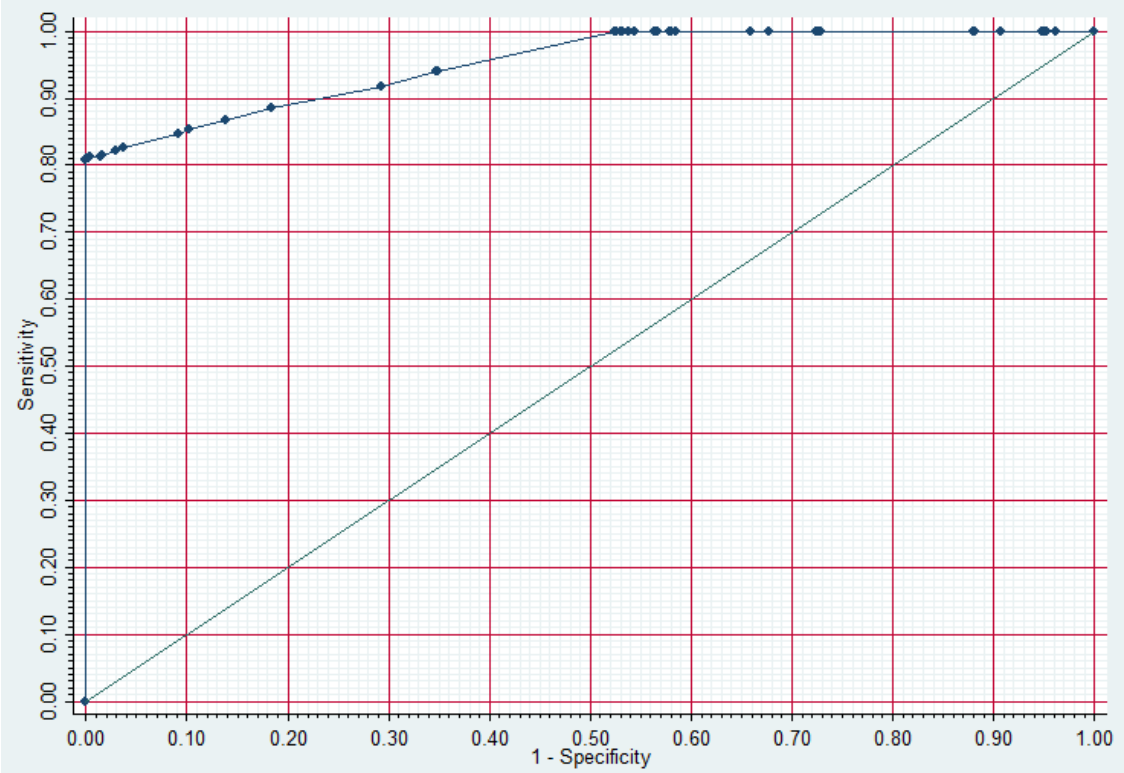
[1]**OR:** Odds ratio, [2]**CI:** Confidence interval, [3]**Multivariable OR:** Frith method (see Chapter 3), adjusted for all other variables in the table, [4]**p-value:** result of the Likelihood Ratio Test of the assoc. of the variable with the outcome after adjustments for all other variables in the table , [5]***multiple variables:***+-30 days from index date (see Chapter 3 Appendix Table 4), [6]**index:** index diagnosis

4.3.3.2 *ROC analyses of cholestatic liver injury algorithm and consideration of cut-off scores (validation cohort)*

ROC analysis of the complete CPRD cholestatic algorithm (stage 1 and stage 2 case assignment)

The variables shown to be predictors of multisource algorithm case status in the training cohort were added to the validation cohort. A CPRD algorithm score was then generated for each person and a "perfect prediction" score was assigned to those individuals with a "1" for any of the variables shown to be perfect predictors of multisource case status (as described in Chapter 3 section 3.3.2.6). The sensitivity and specificity of the full 2-stage algorithm was then calculated by applying the algorithm to the validation cohort data, using a range of cut-off scores to define case status. A ROC (receiver operating graph) of these results is provided in Figure 4.2, with the full tabulation of results provided in Chapter 4 Appendix Table 3.

Figure 4.2: ROC (Receiver Operating Characteristics) graph of Sensitivity against 1-Specificity for a range of CPRD algorithm cut-off scores, comparing the complete CPRD cholestatic liver injury algorithm against a multisource algorithm case status of probable to definite



Area under ROC curve: 0.95

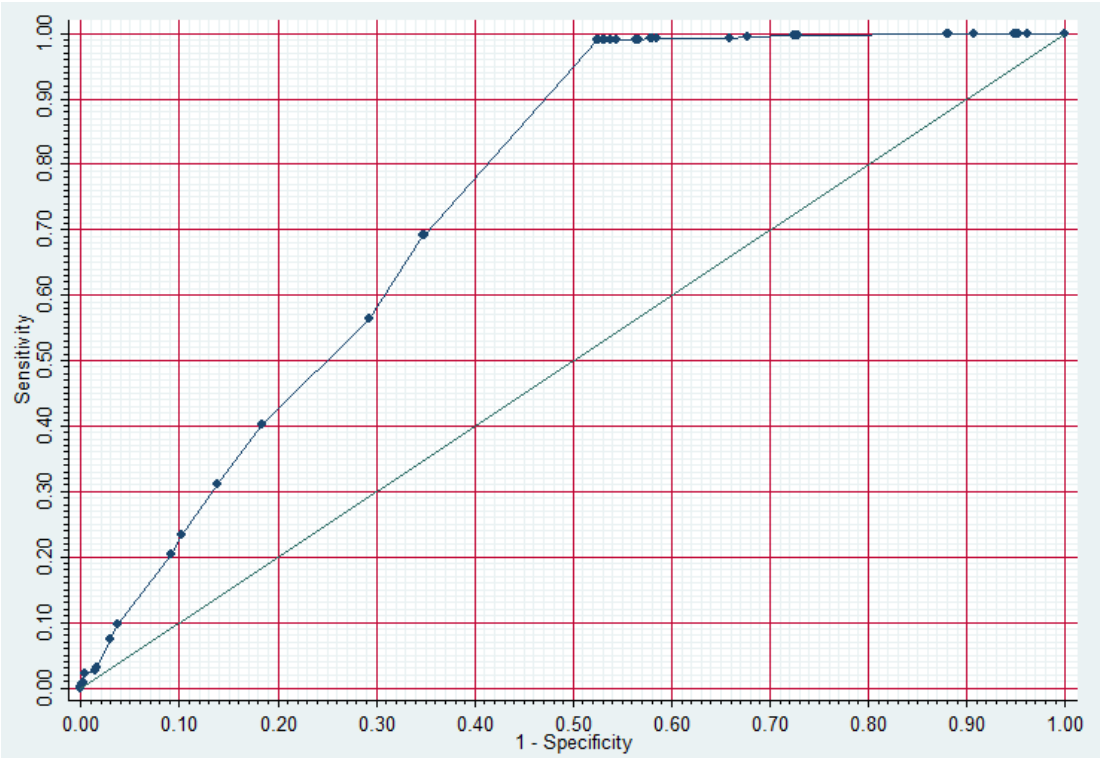
The area under the ROC curve (AUC) may be used to indicate the ability of a measure (in this case the complete CPRD algorithm) to discriminate between individuals with and without a particular condition [110] (in this case discrimination is between individuals with a multisource algorithm case status of “definite” to “possible” and individuals with a multisource algorithm case status of “unlikely” to “non-“). An AUC of 1.0 indicates perfect discrimination, while 0.5 indicates that the measure under test does not discriminate at all. The value of 0.95 obtained indicates that overall the complete CPRD algorithm has an excellent ability to discriminate between individuals with a multisource algorithm case status of “definite” to “possible” and individuals with a multisource algorithm case status of “unlikely” to “non-“.

Chapter 4 Appendix Table 3 provides detail on how the sensitivity and specificity are related to the total CPRD algorithm (stage 1 and stage 2 case assignment) score, this shows that with increasing specificity, sensitivity remains high (for a specificity of 100.0%, sensitivity is over 80.0%).

ROC analysis of the algorithm cut-off score (stage 2 case assignment only)

As described in Chapter 3 section 3.3.2.6, in order to assess how well the CPRD algorithm cut-off score can discriminate between multisource case status within the population that it would be applied to (i.e. all those people who do not have a cholestatic liver test result), the ROC analysis was repeated having removed all the individuals from the cohort who had a cholestatic liver enzyme test result (i.e. all people who would already have been assigned as cases by stage 1 of the CPRD cholestatic liver test result algorithm). A ROC graph of these results is provided in Figure 4.3 with the full tabulation of results provided in Chapter 4 Appendix Table 4.

Figure 4.3: ROC (Receiver Operating Characteristics) graph of Sensitivity against 1-Specificity for a range of CPRD algorithm cut-off scores, comparing the CPRD cholestatic liver injury algorithm score (i.e. stage 2 case assignment only) against a multisource algorithm case status of probable to definite



Area under ROC curve: 0.75

The area under the ROC curve of 0.75 obtained indicates that overall, within the population that it will be applied to (i.e. people who do not have a cholestatic liver test result), the CPRD algorithm score has quite good ability to discriminate between individuals with a multisource algorithm case status of “definite” to “possible” and individuals with a multisource algorithm case status of “unlikely” to “non-“.

Chapter 4 Appendix Table 4 provides detail on how the sensitivity and specificity are related to the CPRD algorithm score generated for stage 2 of the algorithm. For a score that would achieve a specificity of over 90%, sensitivity would be less than 25% (meaning that although one could be sure that 90% of the non-cases would be identified as such, less than 25% of all cases would be correctly identified = a high number of false negatives). Conversely, a moderately high specificity (65%) could be achieved while maintaining a very high sensitivity (94%), meaning that one could be sure that 94% of all cases and 65% of all non-cases would be correctly identified (very nearly all cases would be correctly classified and the majority of non-cases would be correctly classified).

4.4 Discussion

4.4.1 Multisource algorithm

The results for development of the multisource algorithm showed that definite case status is heavily influenced by the CPRD record, with almost all of the definite cases assigned as such based on liver enzyme level test results recorded in CPRD (Table 4-2). HES or ONS information only allowed for the identification of one additional definite cases among the people not identified as definite cases by their liver test results in CPRD. The lack of people with an ONS cause of death of “Toxic liver disease with cholestasis” could be because it is a rare occurrence, or also because the actual cause of death is likely to be recorded as a less specific term such as acute liver failure (which would not be specific enough to aid with the identification of cholestatic injury as the cause of death).

Table 4-1 showed that nearly half of the cohort (46%) did not have a liver test result recorded in CPRD within 90 days either side of their index diagnosis. Given the fact that the members of the cohort were identified by the presence of (mainly acute) liver-related codes, one could expect that standard clinical procedure would be to have performed a test of liver enzyme levels within the period 90 days before or after the index diagnosis. Many of the individuals who did not have liver tests recorded in CPRD are therefore likely to have had tests performed elsewhere, for example in hospital. An important limiting factor of HES data is that while liver enzyme level tests are performed in UK hospitals, their results are not recorded in the HES database. If results from hospital liver tests were available in HES, this could result in individuals within the cohort being promoted from any other multisource case status to “definite”. The lack of liver test data within HES is a major deficiency when comparing HES to CPRD, and for the ability of HES to facilitate detection of patients with liver injury.

A second reason why the proportion of people with liver tests is not higher could be because the standardisation and automation of transfer of laboratory test results between pathology labs and primary care centres was only implemented around 2007 [111], and therefore although the tests were being performed in primary care, they were not being entered into the database. An analysis of the proportion of people who had liver test results recorded in CPRD for those with liver injury dates before 2007 compared with after 2007 (Chapter 4 Appendix Table 5) supports this reasoning, as 65% of people with potential liver injury after 2007 had liver test results recorded in CPRD (compared to 45% of those with potential liver injury dates before 2007).

In addition to the definite case status, HES data do not help identify any very likely cases and only four probably cases. Apart from the lack of liver test result information,

this could be attributed to the fact that there are very few people without liver test results recorded in primary care who have diagnoses in hospital of toxic liver disease with cholestasis (with or without a biopsy or related procedure). Searching for these people within the subset who had already been identified as definite cases based upon liver enzyme level results retrieved an additional three people only, showing that a hospital diagnosis of toxic liver disease with cholestasis is rare, even within those people who have liver enzyme levels indicating a cholestatic pattern of liver injury.

The HES data in this algorithm did allow possible cases to be distinguished from unlikely cases, within people who had a code for “Jaundice” in CPRD but did not have any liver test results recorded. These people make up 28% of the cohort, with around a fifth of these people (6% of the cohort) identified as possible cases (rather than unlikely cases) due to the presence of a code for “Jaundice” in the HES data.

4.4.2 CPRD algorithm

A liver enzyme test result of cholestatic was a perfect predictor of multisource case status, which is not surprising given the weight applied to this in the multisource algorithm (based upon international consensus on the importance of liver enzyme levels for classification of liver injury [46]). Strong predictors were diagnostic terms that clinically would be expected to be describing a cholestatic type of liver injury (toxic liver disease with cholestasis, obstructive jaundice and jaundice). Having other referrals was associated with being a multisource case, while the association between having a liver-related referral and being a multisource case observed in univariable analysis was removed after multivariable adjustments. The people with one or more liver-related referrals are likely to also have liver diagnoses that are strong predictors for cholestatic injury (such as jaundice), so adjusting for these diagnoses removes the observed association.

In the complete CPRD algorithm, the first stage assigns all those with liver enzyme test results of cholestatic as cases, before the second stage assigns a case status to the remaining individuals based upon the algorithm score obtained from the regression analysis. The ROC analysis showed that this complete CPRD algorithm had very good ability to discriminate between the two multisource algorithm case statuses (definite to probable compared to unlikely to non-). This is not an unexpected result, because the CPRD liver test results included in stage 1 of the CPRD liver test results are a strong driver of the multisource case status (81% of the definite to possible multisource cases have a cholestatic liver test result). Individuals without cholestatic liver test results who are multisource cases may then be assigned as cases by the CPRD algorithm score developed by the regression analysis. The second ROC analysis showed that within

those people who did not have cholestatic liver test results, the regression score had quite good ability to identify the remaining multisource cases.

As discussed in the results section, there is a trade-off of sensitivity and specificity. A very specific application of the algorithm and one that is comparable with legacy approaches (although it is difficult to accurately compare with legacy approaches due to generally vague descriptions of exactly how cases have been identified in the past - see Chapter 2) would be to use the score of 5 from Chapter 4 Appendix Table 3 (100% specificity, 81% sensitivity), which corresponds to only assigning those people who have cholestatic liver test results as cases. A more sensitive and less specific method could use a cut-off score set to 2.29 (86% sensitivity and 86% specificity), which would still include all those people with cholestatic liver test results, but in addition would include people who (for example) did not have cholestatic liver test results but had index diagnosis of “obstructive jaundice” and multiple liver-related diagnoses within one month, or people who had an index diagnosis of “jaundice” (or similar), multiple liver-related diagnoses within one month and one or more referrals to hospital within a month.

In terms of applying the score for pharmacoepidemiological studies, it is important to note that the CPRD algorithm (and multisource algorithm) developed here only detects people who have biochemical, clinical and administrative characteristics that are suggestive of a cholestatic type of liver injury. For estimating absolute effects, further review of the records of people selected by the algorithm by medically trained professionals would be needed in order to rule out non-drug causes, and assist in the decision as to whether the injury is caused by the drug under study. Nevertheless, it is worthwhile to consider what type of score could be used for different scenarios. The score used to identify people is likely to depend on the type of study being performed, financial resources available, time that the drug has been on the market, and frequency of liver injury events associated with the drug. For performing a pharmacoepidemiological study to quantify frequency and risk of cholestatic liver injury for a drug that has been marketed for some time and/or is associated with a relatively high rate of event, a highly specific method could be applied (for example, based only on liver test results), because power is unlikely to be an issue. In contrast, if the study is being performed shortly after drug launch, when exposure has been limited and/or the drug does not cause many events, a more sensitive score could be needed in order to increase numbers. When considering the results obtained, one might expect that if performing a cohort study, using a more sensitive less-specific score may not be expected to influence the relative risk or rate ratios (assuming that the loss of specificity

is non-differential with respect to exposure status), but would influence the absolute rate estimates, which could be critical for making risk/benefit decisions about a drug.

For active recruitment to studies required for developing predictive genetic tests, the proximity to market launch, severity of injury and how much of the reaction can be attributed to genetic factors are all aspects that could influence which score to use. For drugs that have been marketed for some time and are not at risk of being withdrawn from the market due to the adverse reaction, a specific score could be used. In contrast, if fast recruitment is required in order to try and develop a predictive genetic test to ensure that a drug causing more serious liver injury can be used as an effective therapy and not removed from the market, a more sensitive method could be adopted, but really only if additional resources are available for thorough clinical review of records and/or obtaining data that could confirm the individual as a definite cases (e.g. liver test results that were performed but not recorded in CPRD, possibly via new linkages to liver clinics).

4.4.3 Comparison with previous work

A systematic review of the studies that included a number of algorithms for identifying cholestatic liver injury was performed as Chapter 2 of this thesis. None of these studies used multiple databases in the development of their algorithms. One study looking at acute liver injury published in 2014 did develop algorithms involving more than one database (one in the UK and one in Spain), but in contrast to the work performed here, was looking at the application of separate algorithms to each database, rather than using multiple database sources to validate a single-source database algorithm [112]. A validation step was performed, but this was not part of the development of the algorithm, but instead was to assess performance of the computer algorithm against expert review of the records. A similar exercise is described within a U.S. study involving the application of 4 different algorithms across 7 databases [113]. While this study does discuss the fact that the recording of liver test results was found to be variable across databases, liver test results were available in each database, and differences in measurement are discussed as being due to differences in the evaluation and handling of acute liver injury within each database population.

4.4.4 Limitations

In the development of the CPRD algorithm, the response variable “case” included multisource case statuses of “definite” to “possible”, with possible cases including people who had been diagnosed with jaundice in both CPRD and HES, but with no additional supporting information to guide case status assignment (such as liver enzyme level test results or biopsies/scans). There is therefore a potential for people to

have been incorrectly classified as cases of cholestatic liver injury in this scenario, and development of a CPRD algorithm based upon this potentially non-specific case definition could negatively affect the ability of the resultant CPRD algorithm to detect true cases of cholestatic liver injury. Including these people in the response variable "case" was considered preferable to not including them, however, because it is likely that many of them did have liver tests performed that indicated cholestasis, but this information was not available. The uncertainty is due to the (lack of) availability of data, therefore. Including them as cases means that they at the very least have the opportunity to be considered as cases subsequently, and ensures that the CPRD algorithm developed using the multisource algorithm also has the ability to identify these people as cases. Their inclusion in epidemiological studies can then be considered based upon some of the factors detailed previously in this section.

A second limitation that should be considered relates to the similarity of data that the multisource and CPRD algorithms relied on for assigning case status, meaning that the comparison of the two algorithms performed in this chapter was a slightly circular exercise. As discussed earlier, this is partly due to the lack of liver test result records in HES data, and also because the number of liver-related procedures recorded in HES was low. Despite this effective negative result, the finding that combined (linked) CPRD and HES data does not increase the ability to detect acute liver injury when compared to CPRD alone is an important one. Publication of the results of this method development will allow other researchers considering similar approaches to benefit. Furthermore, publication will allow emphasis to be placed on the fact that while new linkages are being created between databases that could facilitate pharmacoepidemiology, the quality of the underlying data needs to be improved in order to realise the full potential of such linkages.

4.5 Chapter 4 Summary

- In this chapter, the results of the development of the multisource and CPRD cholestatic liver injury algorithms were presented.

- Within the multisource algorithm, data from CPRD (particularly liver test results) enabled definite cases of cholestatic liver injury to be identified.

- HES data allowed possible cases to be discriminated from unlikely cases (6% of total cases were considered to be possible due to the presence of HES data), but a lack of liver test result data limited the use of HES data for the identification of definite cases of cholestatic liver injury.

- CPRD variables that were shown to be predictors of multisource algorithm case status during development of the CPRD algorithm included liver test results, diagnostic terms related to cholestasis and jaundice, and being referred to hospital.

- ROC analysis showed that the CPRD algorithm had a very good ability to discriminate between multisource case statuses of definite to possible vs. unlikely to non- (area under the curve 0.95), and that highly specific CPRD algorithm cut-off scores had a lower sensitivity than those with lower specificity

- The use of either a highly specific or highly sensitive CPRD algorithm score for identifying cases of cholestatic liver injury could depend on the setting (pharmacoepidemiological studies versus recruitment for genetic association studies; newly marketed drug versus older drug; poorly resourced study versus well-resourced study)

5 Results: applying the algorithm to a well-established association – a cohort study of the association between flucloxacillin and cholestatic liver injury

5.1 Introduction

In this chapter, the results are presented for the cohort study of the association between flucloxacillin (compared with oxytetracycline) and cholestatic liver injury, performed in order to compare CPRD cholestatic liver injury algorithm cut-off scores with varying specificity and sensitivity with (1) each other (2) the multisource algorithm and (3) published literature. Details of the number of participants included and a cohort description are provided, before the results of the following analyses are presented for each outcome definition:

(1) Number and characteristics of identified cases
(2) Frequency (risk) of cholestatic liver injury
(3) Association between flucloxacillin (compared with oxytetracycline) and cholestatic liver injury
(4) Risk factors for flucloxacillin-induced liver injury

Finally, a discussion of the results is provided, in which the outcome definitions are compared with each other and with previously published results.

Throughout this chapter, the CPRD algorithm cut-off scores will be described in terms of their specificity (as compared to the multisource case status of definite to probable), with cut-off score 5 being “high” specificity (specificity 100%, sensitivity 81%), score 2.29 “medium” specificity (86%, 87%) and score 1.63 “low” specificity (48%, 100%).

5.2 Participants

Between the dates of 1st January 2000 and 1st January 2012 1 073 894 people aged 18 years and over were identified in CPRD who received a first prescription for either flucloxacillin or oxytetracycline and had been registered in the database for at least 12 months. 27 156 people were subsequently removed as they did not meet the necessary eligibility criteria, leaving 1 046 738 patients in the cohort. An additional 42 were found to have reasons for exclusion during case review (i.e. assessment of whether one of the study drugs had caused the detected injury), leaving a final cohort of 1 046 696 people for analysis. Of these, 861 959 were in the flucloxacillin exposure group. For analyses performed with the multisource algorithm outcome, restricting the cohort to only those from HES-linked practices left 621 476, 517 803 of whom were in

the exposed to flucloxacillin group. Full details of exclusions, numbers of individuals in each analysis and their exposure status are provided in Figure 5.1.

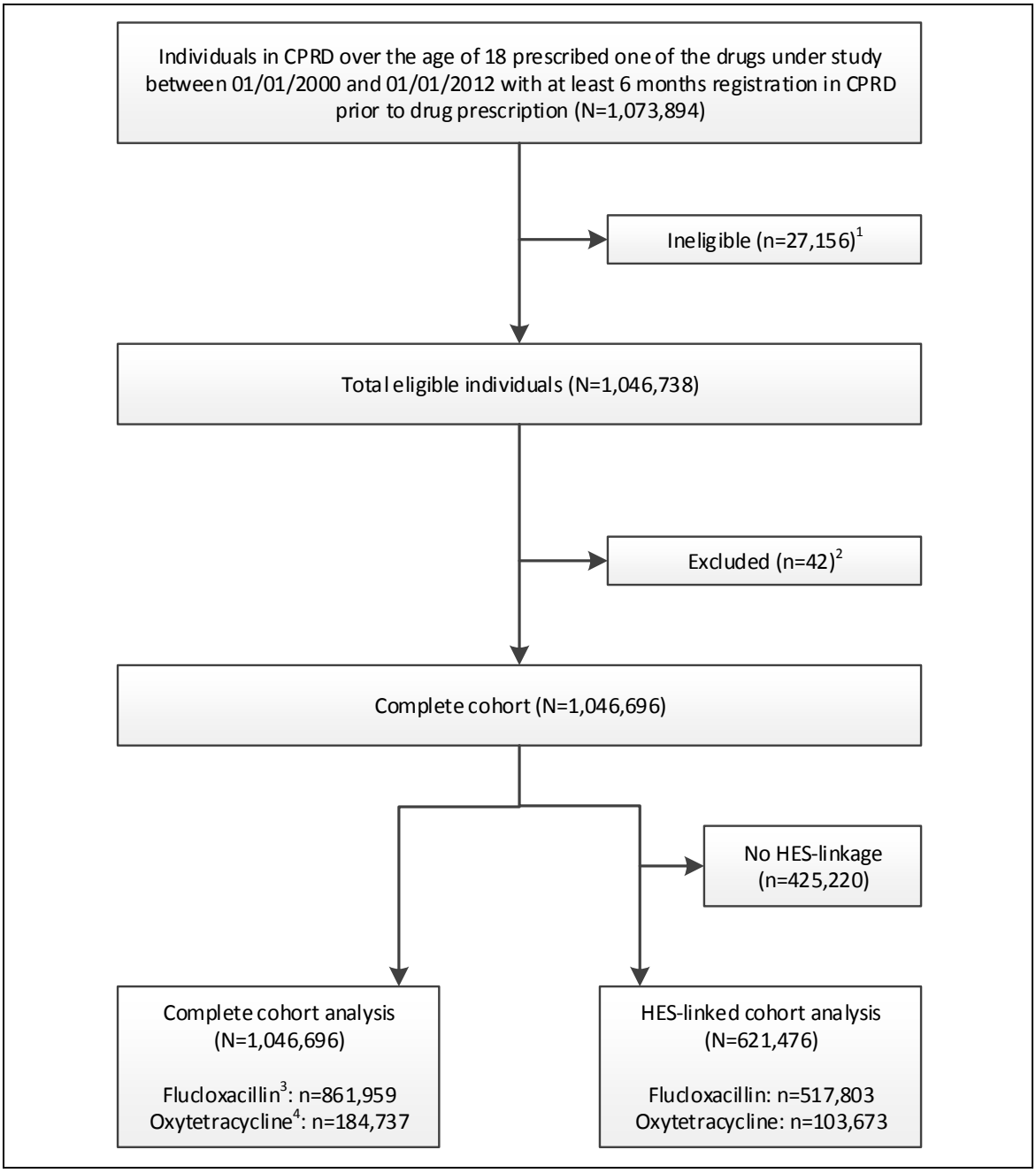


Figure 5.1: Flow of number of individuals included in the cohort study of the association between flucloxacillin (compared with oxytetracycline) and cholestatic liver injury

[1]**Ineligible:** had a diagnostic exclusion code or test result within 1 year prior to their index date, made up of: (i) 11089 individuals with pregnancy codes but no subsequent end of pregnancy code before index date (ii) 13139 individuals with liver pathology codes as defined in Chapter 3 Appendix Table 5 and (iii) 2928 individuals with liver test results that qualified as DILI as defined in Chapter 3 Table 5
[2]**Excluded:** individuals identified as cases of cholestatic liver injury, but on clinician review of record from 2 years prior to index date, an underlying cause other than a prescription with either of the drugs of interest was identified (and the date was prior to the index date)
[3]**Flucloxacillin:** Number of people prescribed flucloxacillin on their index date. 47370/861959 were prescribed the flucloxacillin-ampicillin combination (co-fluampicil).
[4]**Oxytetracycline:** Number of individuals prescribed oxytetracycline on their index date who were not also prescribed flucloxacillin before their end of follow-up. Individuals who were also prescribed flucloxacillin before their end of follow-up were assigned to the flucloxacillin group.

5.3 Descriptive data

The cohort consisted of 1 046 696 people, 861 959 first-time users of flucloxacillin and 184 737 first time users of oxytetracycline (who were not also prescribed flucloxacillin during follow-up). Between the earliest prescription for a drug of interest (1st January 2000) and the latest end of follow-up (22 December 2011), the cohort contributed a total of 3 026 080 person months at risk (2 490 320 person months of exposure to flucloxacillin). The median follow-up was 90 days. Table 5-1 provides an overview of the characteristics of the people included in the cohort, by therapy exposure group. There was a suggestion that people prescribed flucloxacillin were slightly younger than those prescribed oxytetracycline (median age of 48 compared to median age 50). 56% of those prescribed oxytetracycline were female, compared with 54% of those prescribed flucloxacillin, and a higher proportion of those in the oxytetracycline group (55%) had an index date prior to 2006 than in the flucloxacillin group (48%). Oxytetracycline included a higher proportion of people on other drugs likely to cause cholestatic liver injury than flucloxacillin users (81% vs. 52%). There was no difference in recorded ethnicity between the groups, with 51% of people recorded as “White”, and there were minimal differences in alcohol intake, SES, smoking and BMI between exposure groups.

For the HES-linked cohort used for all analyses involving the multisource algorithm, there was a total of 621 476 people (517 803 of whom were in the exposed to flucloxacillin group), contributing a total of 1 796 270 person months at risk. Distribution of co-variates by exposure status was comparable to the main cohort (Chapter 5 Appendix Table 1).

Table 5-1: Characteristics of participants included in the cohort analysis of the association between flucloxacillin (compared with oxytetracycline) and cholestatic liver injury, by exposure status

		Oxytetracycline (N = 184,737) n (%)	Flucloxacillin (N = 861,959) n (%)
Age at index date	18 – 29	31067 (17)	147656 (17)
	30 – 39	30594 (17)	159177 (18)
	40 – 49	30678 (17)	146803 (17)
	50 – 59	30269 (16)	129179 (15)
	60 – 69	28229 (15)	111368 (13)
	70 – 79	21214 (11)	91441 (11)
	80+	12686 (7)	76335 (9)
	median (25 - 75%):	*50 (35 – 65)*	*48 (34 – 65)*
Gender	Male	81316 (44)	394125 (46)
	Female	103421 (56)	467834 (54)
Date of index prescription	2000 – 2001	32439 (17)	112188 (13)
	2002 - 2003	34830 (19)	143752 (17)
	2004 - 2005	32615 (18)	156808 (18)
	2006 - 2007	30090 (16)	159304 (18)
	2008 - 2009	29217 (16)	153679 (18)
	2010 - 2011	25546 (14)	136228 (16)
Prescriptions for other causes of cholestatic injury[1]	None	34529 (19)	415687 (48)
	Less common cause	143164 (77)	399846 (47)
	More common cause	7044 (4)	46426 (5)
Smoking status	Non-smoker	84864 (46)	382320 (44)
	Ex-smoker	40979 (22)	219122 (25)
	Current smoker	55343 (30)	242314 (29)
	Unknown	3551 (2)	18203 (2)
BMI	<20	10923 (6)	48451 (6)
	20 - 25	55689 (30)	247583 (29)
	25+	95215 (52)	447203 (52)
	Unknown	22910 (12)	118722 (13)
Alcohol intake	Non-drinker	20831 (11)	97065 (11)
	Ex-drinker	5581 (3)	28277 (3)
	Current NOS	5852 (3)	27452 (3)
	2 or less u/d	30424 (16)	139300 (16)
	3/6 u/d	84057 (46)	381539 (44)
	>6 u/d	13232 (7)	66576 (8)
	Unknown	24760 (14)	121750 (15)
Socioeconomic status[2]	1 (Highest SES)	33239 (18)	153552 (18)
	2	29919 (16)	145586 (17)
	3	27753 (15)	140223 (16)
	4	27541 (15)	131425 (15)
	5 (Lowest SES)	19122 (10)	102723 (12)
	Unknown	47163 (26)	188450 (22)

		Oxytetracycline (N = 184,737) n (%)	Flucloxacillin (N = 861,959) n (%)
Ethnicity[3]	White	93400 (51)	440740 (51)
	South Asian	3010 (2)	14487 (2)
	Black	1445 (1)	8566 (1)
	Other	1470 (1)	6202 (1)
	Mixed	392 (0)	2238 (0)
	Not Stated	14390 (8)	70946 (8)
	Unknown	70630 (37)	318780 (37)

Note 1: Prescription counted if it occurred anytime from 1 month prior to index date or between index date and before end of follow-up (see Chapter 3 section 3.4.1.5). Less or more common in relation to flucloxacillin, as reported in the literature (see Chapter 3 section 3.4.1.5)

Note 2: Linked data, only available for practices in England, based on index of Multiple Deprivation (individual patient postcode) or otherwise practice level score based upon practice postcode (if no individual-level data) (see Chapter 3 section 3.4.1.5).

Note 3: Obtained from CPRD, unless none found, in which case from HES if patient from a linked practice (see Chapter 3 section 3.4.1.5).

5.4 Number and characteristics of identified cases

393 people had one of the liver-related codes used as the first identification step in all of the algorithms (as detailed in Chapter 3 Appendix Table 1). Of these, 277 (70%) were identified as being cholestatic liver injury cases by any of the CPRD algorithms (3 cut-off scores of high to low specificity) within 90 days of the index prescription. The % of cholestatic liver injury cases identified as being due to flucloxacillin or oxytetracycline was similar for the high and low specificity CPRD algorithms (72% and 68% respectively), and slightly higher for the medium specificity CPRD algorithm (78%) (Table 5-2). When the CPRD algorithms were applied to the HES-linked cohort, the proportions of cholestatic liver injury cases considered to be caused by flucloxacillin or oxytetracycline following record review were higher than in the complete cohort. The proportion detected by the multisource algorithm in the HES-linked cohort was 74%, which was lower than any of the CPRD algorithms when applied to this population (lowest figure: 82%).

The median time between the index prescription and date of liver injury was between 32 and 36 days across all four algorithms. Cases selected by the high specificity CPRD algorithm were characterised by the broadest range of diagnoses, but all had cholestatic liver injury results. The medium-specificity algorithm included all 75 of the individuals identified by the high specificity algorithm, along with an additional 43 people identified due to having a diagnoses for jaundice/obstructive jaundice or toxic liver disease with cholestasis and either a hospital referral or another liver-related diagnoses. An additional 71 people identified by the low specificity algorithm were included due to having had one of the afore-mentioned diagnoses only. All of the 70 individuals identified as cases of flucloxacillin or oxytetracycline-induced liver injury by review of the multisource algorithm individuals had these diagnoses, with 40 also having cholestatic liver test results and the remainder having a HES diagnosis of jaundice or toxic liver disease with cholestasis.

Table 5-2: Number of cholestatic liver injury cases and number subsequently identified as flucloxacillin or oxytetracycline-induced, time between prescription and case assignment date, and characteristics of cases for each of the algorithms under test

Algorithm (specificity[1])	Specificity, sensitivity	Number identified as cholestatic liver injury cases: Number subsequently identified as flucl- or oxyt-induced (%)[2]		Time from first prescription until case assignment	Characteristics of cases[3]
		Complete cohort	HES-linked cohort	Median in days (25 - 75%)	
CPRD (High)	100%, 81%	104:75 (72%)	68:56 (82%)	36 (24 – 40)	**From CPRD:** an index diagnosis of jaundice, obstructive jaundice, toxic liver disease with cholestasis or liver disorder nos AND (2) a cholestatic liver test result (within 90 days of index diagnosis).
CPRD (Medium)	86%, 87%	151:118 (78%)	96:84 (88%)	35 (25 – 40)	**From CPRD:** an index diagnosis of jaundice, obstructive jaundice or toxic liver disease with cholestasis AND EITHER a hospital referral OR an additional liver-related diagnosis (both within 30 days of index diagnosis).
CPRD (Low)	48%, 100%	277:189(68%)	149:123 (83%)	32 (22 – 38)	**From CPRD:** an index diagnosis of jaundice, obstructive jaundice or toxic liver disease with cholestasis.
Multisource (probable - definite)	-	NP[4]	95:70 (74%)	36 (24 - 40)	**From CPRD:** an index diagnosis of jaundice, obstructive jaundice or toxic liver disease with cholestasis AND EITHER a cholestatic liver test result OR **FROM HES:** a diagnoses of jaundice or toxic liver disease with cholestasis (within 90 days of the index diagnosis).

Note 1: Compared against a gold standard of the multisource algorithm (probable – definite case). High=CPRD algorithm cut-off score of 5, medium=CPRD algorithm cut-off score of 2.29, low=CPRD algorithm cut-off score of 1.63.
Note 2: The multisource results were obtained from a dataset that was (necessarily) smaller than that used to obtain the CPRD algorithm results, because only the subset of patients in English practices have HES data (which is required for the multisource algorithm). For ease of comparability with the multisource figures, the CPRD algorithm figures are presented for the HES-linked proportion of the CPRD dataset (in addition to those for the full dataset).
Note 3: CPRD algorithms 2.29 and 1.63 include patients selected with the characteristics described, in addition to all of those selected by the higher algorithm cut-off score(s)
Note 4: NP = analysis not performed, as multisource algorithm can only be applied to a cohort of HES-linked CPRD patients.

5.5 Frequency (risk) of cholestatic liver injury

Within the complete cohort, there were 53 out of 861959 users of flucloxacillin who experienced cholestatic liver injury as defined by the high specificity algorithm in the 45-days after prescription. This gave a 45-day risk of flucloxacillin-induced cholestatic liver injury of 6.15 (95% confidence interval (CI) 4.61 – 8.04) cases per 100 000 patients prescribed the drug (Table 5-3). The risk increased with increasing algorithm sensitivity, up to 14.15 (95% CI 11.95 – 16.90) cases per 100 000 patients for the least specific algorithm. The oxytetracycline risk for the comparable period was 1.62 (95% CI 0.33 – 4.75) for the algorithms with medium and high specificity, increasing to 3.79 (95% CI 1.52 – 7.81) for the most sensitive algorithm (low specificity). The 45-day risk starting from the 46th day from first prescription with flucloxacillin was 2.02 (95% CI 1.18 – 3.24) for the high specificity CPRD algorithm, and increased slightly by increasing algorithm sensitivity.

The estimated 45-day risk from the day of first prescription with flucloxacillin using the multisource algorithm in the cohort restricted to HES-linked patients was 8.69 (95% CI 6.34 – 11.63) per 100 000 users. The risk estimates for the CPRD algorithms for flucloxacillin in the restricted cohort were all higher than (but generally similar to) those obtained in the complete cohort analyses.

Table 5-3: Risk of cholestatic liver injury identified using the CPRD algorithm (3 different cut-off scores) and the multisource algorithm by (1) exposure to flucloxacillin or oxytetracycline (for the 1-45 day period after exposure) and (2) flucloxacillin exposure period (1-45 days compared to 46-90 days after exposure)

			Complete cohort			Cohort restricted to HES-linked patients only		
Algorithm (specificity)	**Specificity, sensitivity[1]**	**Exposure group**	**# with outcome**	**Patients[2]**	**45-day risk (CI[3])** (per 100 000 patients prescribed the drug)	**# with outcome**	**Patients**	**45-day risk (CI)**
CPRD (High)	100%, 81%	Oxytetracycline 1 - 45 days	<5[4]	184737	1.62 (0.33 – 4.75)	0	103673	-
		Flucloxacillin 1 - 45 days	53	861959	6.15 (4.61 – 8.04)	40	517803	7.72 (5.52 – 10.52)
		Flucloxacillin 46 - 90 days	17	840910	2.02 (1.18 – 3.24)	15	505020	2.97 (1.66 – 4.90)
CPRD (Medium)	86%, 87%	Oxytetracycline 1 - 45 days	<5	184737	1.62 (0.33 – 4.75)	0	103673	-
		Flucloxacillin 1 - 45 days	77	861959	8.93 (7.05 – 11.16)	57	517803	11.01 (8.34 – 14.26)
		Flucloxacillin 46 - 90 days	32	840888	3.81 (2.60 – 5.37)	24	505020	4.75 (3.04 – 7.07)
CPRD (Low)	48%, 100%	Oxytetracycline 1 - 45 days	7	184737	3.79 (1.52 – 7.81)	<5	103673	1.93 (0.23 – 6.97)
		Flucloxacillin 1 - 45 days	122	861959	14.15 (11.75 – 16.90)	83	517803	16.03 (12.77 – 19.87)
		Flucloxacillin 46 - 90 days	48	840847	5.71 (4.21 – 7.57)	32	504997	6.34 (4.33 – 8.95)
Multisource	-	Oxytetracycline 1 - 45 days	NP[5]	NP	NP	<5	103673	0.96 (0.02 – 5.37)
		Flucloxacillin 1 - 45 days	NP	NP	NP	45	517803	8.69 (6.34 – 11.63)
		Flucloxacillin 46 - 90 days	NP	NP	NP	22	505035	4.36 (2.73 – 6.59)

Note 1: Compared against a gold standard of the multisource algorithm (probable – definite case). High=CPRD algorithm cut-off score of 5, medium=CPRD algorithm cut-off score of 2.29, low=CPRD algorithm cut-off score of 1.63.
Note 2: Number of patients prescribed the drug at day 1 of the specific analysis.
Note 3: 95% confidence interval.
Note 4: CPRD guidance stipulates that cells with under 5 events should be reported this way
Note 5: NP = analysis not performed, as multisource algorithm can only be applied to a cohort of HES-linked CPRD patients.

5.6 Association between flucloxacillin and cholestatic liver injury

5.6.1 Associations between cholestatic liver injury and co-variates

Chapter 5 Appendix Table 2 shows the association between each of the co-variates in the study and flucloxacillin-induced cholestatic liver injury as identified by the four algorithm outcomes (and subsequent record review). For each of the algorithm definitions, there was very good evidence that the rate of injury increased with increasing age (result of likelihood ratio test (LRT) of an association with age over all categories of the variable: $p<0.001$), with the association increasing in strength substantially over the age of 50 and over the age of 70 for each outcome definition. The rate ratio (RR) comparing the oldest age group (80+ yrs) to the youngest (18-49 yrs) was estimated at over 50 for both the CPRD medium specificity algorithm and the multisource algorithm, and over 100 for the high specificity CPRD algorithm (although with wide confidence intervals in all cases).

Being prescribed another drug that was a possible cause of cholestatic liver injury was also associated with drug-induced liver injury (LRT p-values <=0.002 for all outcome definitions), with a maximum RR of 3.69 (95% CI 1.53 – 8.89) for the medium specificity CPRD algorithm (comparing those people prescribed a relatively common cause of injury to those not prescribed any cause). Smoking status also demonstrated good evidence of an association across all outcome definitions (with the highest rate for ex-smokers) while for gender, RRs were consistent with an increased rate in females for all outcomes, although there was a lack of evidence to support this association for all outcome definitions except the least specific CPRD algorithm. There was a suggestion across all outcome definitions that those with a moderate BMI had a higher rate than those at lower or higher weights. There was a lack of evidence of an association between the date of index prescription and cholestatic liver injury.

5.6.2 Stratified (classical) analysis

Chapter 5 Appendix Table 3 shows stratified and Mantel Haenzel pooled rate ratios for co-variates considered to be potential confounders or effect modifiers. For all outcomes, the mantel haenzel pooled rate ratio suggested that having had a prescription for another cause of cholestatic liver injury was a strong negative confounder, as rate ratios increased from crude for all algorithms. Date of index prescription did not seem to confound the association for any of the outcome definitions, and there was a lack of evidence for effect modification (although only the

lowest specificity CPRD algorithm had sufficient numbers to allow analysis across the majority of categories for this variable).

5.6.3 Crude and multivariable adjusted result of exposure to flucloxacillin compared to oxytetracycline analysis

Table 5-4 shows the crude and multivariable adjusted results of the association between flucloxacillin and cholestatic liver injury (compared with oxytetracycline), for the varying specificity CPRD algorithms and the multisource algorithm. The crude rate ratio for the association between flucloxacillin and cholestatic liver injury for the high specificity CPRD algorithm was 3.79 (95% CI 1.19 – 12.13). After adjusting for date of index prescription and concomitant prescriptions for other causes of cholestatic liver injury, the rate ratio decreased slightly to 3.72 (95% CI 1.16 – 11.96). This was very similar to the age-adjusted rate ratio estimate for the most sensitive (low specificity) CPRD algorithm, which had a slightly narrower confidence interval (RR 3.70, 95% CI 1.73 – 7.94). The estimated multivariable (age-adjusted) rate ratio for the medium specificity CPRD algorithm was higher than for the other two CPRD algorithms, and with wider confidence intervals (RR 5.46, 95% CI 1.72 – 17.30).

For the analysis performed within the HES-linked cohort (in order to test the multisource algorithm), only the multisource algorithm and the most sensitive (low specificity) CPRD algorithm identified sufficient numbers of oxytetracycline – exposed cases for a rate ratio to be estimated. For the multisource algorithm, the crude rate ratio was 9.02 (95% CI 1.24 – 65.7), which increased to 9.26 (95% CI 1.27 – 67.35) after adjustment for age and concomitant prescriptions. The (age-adjusted) multivariable rate ratio for the low specificity CPRD algorithm in the HES-linked dataset was over two times more than the rate ratio estimated using the same algorithm in the unrestricted cohort (RR 8.86, 95% CI 2.17 – 36.11), but similar to the multisource estimated rate ratio (although with narrower confidence intervals), suggesting that the difference is due to differences in the underlying populations within each dataset.

Classical (Mantel Haenszel) did not provide evidence that any of the co-variates were effect modifiers of the association between flucloxacillin and cholestatic liver injury, when compared with oxytetracycline (Chapter 5 Appendix Table 3). For the multivariable adjusted models, the RRs obtained using the fully adjusted models based on the DAG analysis were almost identical to the RRs obtained from models prepared using a stepwise approach (maximum change in relative effect <2%).

Table 5-4: Rates and crude/multivariable adjusted rate ratios (RR) of cholestatic liver injury identified using the CPRD algorithm (3 different cut-off scores) and the multisource algorithm by exposure to flucloxacillin or oxytetracycline (for the 1-45 day period after exposure)

			Complete cohort					Cohort restricted to HES-linked patients only				
Algorithm	**Sp, Sns[2]**	**Drug**	**Cases**	**PM[5]**	**Rate[6](CI[7])**	**Crude RR (CI)**	**MV RR (CI)**	**Cases**	**PM**	**Rate (CI)**	**Crude RR (CI)**	**MV RR (CI)**
CPRD (High)	100%, 81%	Oxyt[3]	<5	2.71	1.11 (0.36 – 3.44)	1	1	0	1.52	-[9]	-	-
		Flucl	53	12.61	4.20 (3.21 – 5.50)	3.79 (1.19 – 12.13)	3.72 (1.16 – 11.96)[8]	40	7.57	5.28 (3.87 – 7.20)	-	-
CPRD (Med)	86%, 87%	Oxyt	<5	2.71	1.11 (0.36 – 3.44)	1	1	0	1.52	-	-	-
		Flucl	77	12.61	6.11 (4.88 – 7.63)	5.51 (1.74 – 17.46)	5.46 (1.72 – 17.30)[10]	57	7.57	7.53 (5.80 – 9.76)	-	-
CPRD (Low)	48%, 100%	Oxyt	7	2.71	2.59 (1.23 – 5.3)	1	1	<5	1.52	1.32 (0.33 – 5.26)	1	1
		Flucl	122	12.61	9.67 (8.10 – 11.55)	3.74 (1.75 – 8.01)	3.70 (1.73 – 7.94)[11]	83	7.57	10.96 (8.84 – 13.59)	8.32 (2.05 – 33.83)	8.86 (2.17 – 36.11)
MS[1]	-	Oxyt	NP[4]	NP	NP	NP	NP	<5	1.52	0.65 (0.09 – 4.67)	1	1
		Flucl	NP	NP	NP	NP	NP	45	7.57	5.81 (4.32 – 7.81)	9.02 (1.24 – 65.47)	9.26 (1.27 – 67.35)[12]

Note 1: Multisource.
Note 2: Specificity and sensitivity compared against a gold standard of the multisource algorithm (probable – definite case). High=CPRD algorithm cut-off score of 5, med=CPRD algorithm cut-off score of 2.29, low=CPRD algorithm cut-off score of 1.63.
Note 3: Oxyt=Oxytetracycline, Flucl=Flucloxacillin.
Note 4: NP = analysis not performed, as multisource algorithm can only be applied to a cohort of HES-linked CPRD patients.
Note 5: 100000 person-months at risk.
Note 6: Per 100000 person months.
Note 7: 95% confidence interval.
Note 8: Adjusted for age, date of index prescr and concomitant prescriptions for other causes of cholestatic liver injury. Fully adjusted model: 3.71 (1.15 – 11.94).
Note 9: All blanks ("-") are where there were insufficient numbers to perform the analysis in question.
Note 10: Adjusted for age. Fully adjusted model: 5.56 (1.75 – 17.70).
Note 11: Adjusted for age. Fully adjusted model: 3.74 (1.74 – 8.04).
Note 12: Adjusted for age and concomitant prescriptions. Fully adjusted model: 9.12 (1.25 - 64.42).

5.7 Risk factors for flucloxacillin-induced liver injury

Table 5-5 shows the rate and multivariable rate ratios for cholestatic liver injury for a number of characteristics previously reported as being possible predictors for flucloxacillin-induced cholestatic liver injury (age, gender, number of prescriptions and concomitant therapy with other causes of cholestatic liver injury) [56]. All multivariable rate ratios are presented adjusted for all other variables in the table and date of index prescription.

Age was the strongest predictor for flucloxacillin-induced cholestatic liver injury, after adjustments, particularly as measured by the most specific CPRD algorithm and the multisource algorithm. For all algorithms, the increase was particularly marked over the age of 50 and over the age of 70 (e.g. CPRD algorithm cut-off 5 RR for aged 70 – 79 compared to aged 18 – 49: 80.80, 95% CI 10.66 – 612.20). For gender, there was a suggestion across all outcomes except the multisource that females had a slightly higher rate of injury, although the 95% confidence intervals did not rule out a decreased rate (e.g. CPRD algorithm 1.63 RR: 1.38, 95% CI 0.92 – 2.00). Number of flucloxacillin prescriptions did seem to be a risk factor, with increasing numbers of prescriptions associated with a higher rate of injury. This was particularly marked for both the high specificity CPRD algorithm (RR comparing those with 3+ prescriptions to those with 1: 5.62, 95% 2.48 – 12.74) and the multisource algorithm (RR 5.66, 95% CI 2.33 – 13.74). Being prescribed other drugs considered to be causes of cholestatic liver injury did not seem to be a strong predictor of flucloxacillin-induced cholestatic liver injury, although wide 95% confidence intervals meant that moderate increased or decreased rates associated with their use could not be ruled out.

Table 5-5: Rate ratio for cholestatic liver injury within those exposed to flucloxacillin (for the 1-45 day period after exposure) using the three CPRD algorithm cut-off scores and the multisource algorithm by age, gender, no. of prescriptions and concomitant therapies

Algorithm (specificity[1])	Specificity, sensitivity	Risk factor		# with outcome	PMAR[2]	Rate[3] (CI[4])	Multivariable RR[5] (CI)
CPRD (High)	100%, 81%	Age[6]	18 – 49	1	6.64	0.15 (0.02 – 1.07)	1
			50 – 59	9	1.90	4.75 (2.47 – 9.12)	29.55 (3.73 – 233.88)
			60 – 69	5	1.63	3.06 (1.27 – 7.36)	17.49 (2.02 – 151.21)
			70 – 79	20	1.34	14.94 (9.64 – 23.18)	80.80 (10.66 – 612.20)
			80+	18	1.11	16.28 (10.37 – 26.96)	82.47 (10.79 – 630.16)
		Gender	Male	21	5.77	3.64 (2.37 – 5.58)	1
			Female	32	6.84	4.68 (3.31 – 6.62)	1.16 (0.67 – 2.03)
		No. of prescrs	1	34	11.02	3.08 (2.20 - 4.32)	1
			2	12	1.32	9.10 (5.17 - 16.02)	2.33 (1.20 – 4.50)
			3+	7	0.28	26.06 (12.42 - 54.65)	5.62 (2.48 – 12.74)
		Concomitant[7]	None	11	6.08	1.81 (1.00 – 3.27)	1
			Common	38	5.85	6.49 (4.72 – 8.92)	1.32 (0.66 – 2.64)
			Less common	4	0.68	5.89 (2.21 – 15.70)	1.27 (0.40 – 4.03)
CPRD (Med)	86%, 87%	Age	18 – 49	3	6.64	0.45 (0.15 - 1.40)	1
			50 – 59	12	1.90	6.33 (3.59 - 11.14)	13.25 (3.73 – 47.09)
			60 – 69	13	1.63	7.96 (4.62 - 13.71)	15.55 (4.38 – 55.14)
			70 – 79	24	1.34	17.93 (12.02 - 26.75)	33.06 (9.78 – 111.75)
			80+	25	1.11	22.61 (15.28 - 33.46)	39.01 (11.53 – 132.00)
		Gender	Male	29	5.77	5.02 (3.49 - 7.23)	1
			Female	48	6.84	7.02 (5.29 - 9.31)	1.28 (0.80 – 2.04)
		No. of prescrs	1	52	11.02	4.72 (3.60 - 6.19)	1
			2	18	1.32	13.64 (8.60 - 1.65)	2.32 (1.35 – 3.97)
			3+	7	0.27	26.06 (12.42 - 54.65)	3.75 (1.70 – 8.29)
		Concomitant	None	17	6.08	2.80 (1.74 – 4.50)	1
			Common	53	5.85	9.05 (6.92 – 11.85)	1.30 (0.74 – 2.30)
			Less common	7	0.68	10.31 (4.92 – 21.63)	1.59 (0.65 – 3.89)
CPRD (Low)	100%, 81%	Age	18 – 49	13	6.64	1.96 (1.14 - 3.37)	1
			50 – 59	19	1.90	10.02 (6.39 - 15.71)	5.04 (2.48 – 10.24)

Algorithm (specificity[1])	Specificity, sensitivity	Risk factor		# with outcome	PMAR[2]	Rate[3] (CI[4])	Multivariable RR[5] (CI)
			60 – 69	16	1.63	9.80 (6.00 - 16.00)	4.80 (2.29 – 10.09)
			70 – 79	40	1.34	29.88 (21.92 - 40.74)	14.09 (7.38 – 26.90)
			80+	34	1.11	30.75 (21.97 - 43.03)	13.60 (7.00 – 26.40)
		Gender	Male	44	5.77	7.62 (5.67 - 10.24)	1
			Female	78	6.84	11.41 (9.14 - 14.24)	1.38 (0.92 – 2.00)
		No. of prescrs	1	87	11.02	7.89 (6.40 - 9.74)	1
			2	27	1.32	20.46 (14.03 - 29.84)	2.16 (1.40 – 3.33)
			3+	8	0.27	29.78 (14.89 - 59.54)	2.74 (1.33 – 5.68)
		Concomitant	None	35	6.08	5.76 (4.13 – 8.02)	1
			Common	77	5.85	13.15 (10.52 – 16.45)	1.04 (0.68 – 1.58)
			Less common	10	0.68	14.73 (7.93 – 27.38)	1.25 (0.61 – 2.55)
Multisource	-	Age	18 – 49	1	3.99	0.25 (0.04 - 1.78)	1
			50 – 59	6	1.13	5.30 (2.38 – 12.56)	19.45 (2.33 – 162.20)
			60 – 69	4	0.97	4.12 (1.55 – 11.63)	13.85 (1.53 – 125.50)
			70 – 79	16	0.80	20.68 (12.52 – 32.22)	63.31 (8.23 – 487.76)
			80+	18	0.68	26.57 (17.01 – 42.29)	81.81 (10.65 – 628.38)
		Gender	Male	20	3.45	5.79 (3.74 - 8.98)	1
			Female	25	4.12	6.06 (4.09 - 8.98)	0.91 (0.50 – 1.65)
		No. of prescrs	1	28	6.62	4.23 (2.92 – 6.13)	1
			2	11	0.79	13.87 (7.68 – 25.05)	2.55 (1.27 – 5.13)
			3+	6	0.16	37.03 (16.64 – 82.43)	5.66 (2.33 – 13.74)
		Concomitant	None	9	3.72	2.42 (1.26 – 4.66)	1
			Common	33	3.47	9.50 (6.75 – 13.36)	1.37 (0.64 – 2.93)
			Less common	3	0.39	7.79 (2.51 – 24.15)	1.20 (0.32 – 4.49)

Note 1: Compared against a gold standard of the multisource algorithm (probable – definite case). High=CPRD algorithm cut-off score of 5, med=CPRD algorithm cut-off score of 2.29, low=CPRD algorithm cut-off score of 1.63.
Note 2: 100000 person-months at risk.
Note 3: Per 100000 person months.
Note 4: 95% confidence interval.
Note 5: Adjusted for date of index prescription and all other variables in this table.
Note 6: Age categorised into 5 groups due to no events in the 18-29 yr olds for CPRD algorithm score 5 and the multisource algorithm.
Note 7: Concomitant prescription for drugs considered (common or less common) causes of cholestatic liver injury, within 1 month prior to end of follow-up.

5.8 Sensitivity analyses

Removing people who had a prescription for co-fluampicil from the exposed to flucloxacillin group increased all estimated risks and rate ratios, although by a negligible amount. For example the 1-45 day risk estimate for the high specificity CPRD algorithm for flucloxacillin increased from 6.15 per 100 000 users (95% CI 4.61 – 8.04) to 6.26 per 100 000 users (95% CI 4.66 – 8.23), while the multivariable rate ratio for the same algorithm increased from 3.72 (1.16 – 11.96) to 3.75 (95% CI 1.16 - 12.06).

Removing people who were classified as the heaviest drinkers from the analysis (i.e. in the over 6 units per day category) resulted in a very small increase in the flucloxacillin 45-day risk estimate, with a (larger) reduction in the multivariable rate ratio for all algorithms. For example, the 1-45 day risk estimate for the low specificity algorithm for flucloxacillin increased from 14.15 per 100 000 users (95% CI 11.75 – 16.90) to 14.21 (95% CI 11.71 – 17.08), while the multivariable rate ratio decreased from 3.70 (95% CI 1.73 – 7.94) to 3.43 (95% CI 1.60 – 7.36).

If people were prescribed both oxytetracycline and flucloxacillin in the cohort (with either both (i) drugs prescribed on the index date or (ii) oxytetracycline prescribed on the index and then flucloxacillin prescribed subsequently before the end of follow-up) this was handled by considering these people as exposed to flucloxacillin. Removing these people completely did not change the 45-day risk estimates or the relative effect estimates.

In this study, people were excluded during detailed review of those with cholestatic liver injury if they had an exclusion code between their date of drug prescription and their case assignment date. It is possible that these people could still have been cases of cholestatic liver injury caused by one of the study drugs. A sensitivity analysis in which these people were considered as cases and not exclusions, resulted in an increased 45-day risk estimate and a decreased relative effect. For example, the flucloxacillin 45-day risk estimate for the most specific CPRD algorithm increased from 6.15 per 100 000 users (95% CI 4.61 – 8.04) to 7.19 per 100 000 users (95% CI 5.51 – 9.22), while the multivariable rate ratio decreased from 3.72 (95% CI 1.16 – 11.96) to 3.04 (95% CI 1.10 - 8.40).

In summary, there was little differences observed with any of the sensitivity analyses, and the number of outcomes is so low that small changes to the number of outcomes will inevitably lead to changes to the relative effect (to some extent).

5.9 Discussion

5.9.1 Key results

In this study, the use of the CPRD cholestatic liver injury algorithm (three cut-off scores of low to high specificity) and the multisource cholestatic liver injury algorithm as outcomes in a pharmacoepidemiological study were assessed by performing a cohort study on the association between cholestatic liver injury and flucloxacillin (compared to oxytetracycline).

The number of cases of cholestatic liver injury identified increased with increasing algorithm sensitivity, and the proportion considered to be caused by one of the drugs under study was generally higher for the CPRD algorithm(s) than for the multisource algorithm. Flucloxacillin risk estimates were 6.15 (95% CI 4.61 – 8.04) per 100 000 users and increased with increasing CPRD algorithm sensitivity (up to 14.15, 95% CI 11.75 – 16.90). The multisource algorithm obtained an estimate of cholestatic liver injury in the exposed to flucloxacillin group of 8.69 (95% CI 6.34 – 11.63). Multivariable adjusted rate ratios for the association (comparing flucloxacillin to oxytetracycline exposure) were very similar for the most specific (RR 3.72, 95% CI 1.16 – 11.96) and the most sensitive (RR 3.70, 95% CI 1.73 – 7.94) CPRD algorithms. In HES-linked practices only, the rate ratio using the most sensitive CPRD algorithm was 8.86 (95% CI 2.17 – 36.11), similar to the estimate obtained using the multisource algorithm (RR 9.26, 95% CI 1.27 – 67.35), although more precise. In risk factor analysis, all algorithms demonstrated age to be the most important risk factor after multivariable adjustments, with sharp increases in risk over the age of 50 and over the age of 70. Number of prescriptions of flucloxacillin was another important risk factor.

5.9.2 Number of cases identified and case characteristics

When considering the number of people initially identified as cholestatic liver injury cases who were subsequently confirmed as flucloxacillin or oxytetracycline cases based upon detailed record review, of interest was the higher proportion of cholestatic liver injury cases identified as drug-induced in the HES-linked data for each of the CPRD algorithms. This suggests that the prevalence of conditions leading to exclusions may be higher in the complete cohort than in the HES-linked cohort, due to a higher prevalence in the underlying populations from which the samples are taken.

Chapter 5 Appendix Table 4 provides an overview of the reasons why individuals who were considered as cholestatic liver injury cases were subsequently excluded based upon detailed clinician review of records for (i) the complete cohort (ii) the HES-linked cohort (used in this study) and (iii) individuals from the complete cohort who were not

linked to HES. Of note is the fact that while the HES-linked cohort contained of 60% of the individuals from the complete cohort, the proportion of those people excluded was only 30% of the total excluded from the complete cohort. Exclusions related to alcoholism, cancer and heart failure made up the largest proportion of exclusions in the complete cohort (33% of all exclusions). In the subset of practices not linked to HES there was a much higher proportion of people excluded due to these three conditions (50%) than in the HES-linked subset (19%, with no alcoholics). This comparison is effectively comparing practices in England (HES-linked) with those in Scotland, Wales and Northern Ireland (not linked to HES), and the differing prevalence of these conditions by region (for example, higher alcoholism and ischaemic heart disease rates in Scotland [114, 115]) may explain the variation seen here.

In Chapter 2 of this thesis, a comparison of the proportion (%) of those with a liver-related diagnostic code of interest who were eventually considered to be cases of (drug-induced) cholestatic liver injury in previously published studies is presented (Chapter 2 Table 2-6). Proportions range from 4% to 83% (median 8%), with two flucloxacillin studies that reported numbers having figures of 7% (for a study that used a relatively sensitive initial codelist) and 83% (for the study that used the most specific codelist) [53, 55]. In this study, 393 people had a liver-related code in the complete cohort, and the comparable % for the CPRD algorithms tested in this study were therefore 26%, 38% and 70% (by increasing algorithm specificity, and using numerator figures obtained from Table 5-2). For the multisource algorithm 220 people had any of the liver-related codes and 43% of these ended up as cases, showing that the sensitivity of the codelists used in this study was midway between the two previous flucloxacillin studies. In Chapter 2, the suggestion that a sensitive codelist could lead to increased manual review of electronic health records was discussed. If applying the algorithms used in this study, this becomes less of an issue, because a validated score is used to identify non-cases of cholestatic liver injury from potential cases of cholestatic liver injury based on an automated search of their electronic health record. Subsequent record review is then needed to attribute the cause of the injury to the medication under study, but a reduction in the amount of manual review is still likely to be conferred. This means that use of a codelist of moderate to high sensitivity becomes possible, and it is not necessary to use a restricted codelist (which could potentially result in cases being missed).

A previous cohort study performed in the GPRD (predecessor to the CPRD) between 1992 – 2002 found that the time from first administration of flucloxacillin until injury was 25.5 days [53]. In another Swedish analysis of 43 spontaneous reports considered to be flucloxacillin, dicloxacillin or cloxacillin-induced cholestatic liver injury between 1981

and 1990, it was reported that 72% of patients suffered reactions within 30 days [116]. In this study, the median times were slightly longer, ranging from 32 - 36 days depending on algorithm (see Table 5-2, median times when considering flucloxacillin on its own were no different to those presented in this table).

It is possible that the latency result in the study performed here is a more precise estimate of the median time from flucloxacillin prescription until injury than the referenced 1992 - 2002 GPRD cohort, as the study performed here was three times larger. Calculation of the 95% CI around the median using a binomial method (for the highly specific CPRD algorithm) provides an estimate of 36 days (95% CI 33 day – 37 days), which would exclude the median of 25.5 from the earlier cohort study. An alternative explanation is that the measurement has been performed differently in the previous studies. Generally, for all algorithms in this study, the earliest possible event used as a case date is a diagnostic code for jaundice (or similar). In both the other studies mentioned here, it is not specified what clinical information is used to determine the onset of injury, but this could well have been other less severe symptoms that were identified earlier than the onset of (e.g.) jaundice, such as pruritus, or feeling unwell. This potentially provides more accurate clinical information, but not specifying clearly the information used and timing of case assignment risks introducing bias into cohort studies. In any case, the median latency for flucloxacillin-induced liver injury found here is consistent with injury occurring up to 45 days from first prescription.

5.9.3 Absolute and relative effect measures

5.9.3.1 Comparison across the algorithms used in this study

The increase in flucloxacillin 45-day risk estimates with increasing CPRD algorithm sensitivity is as expected, given the fact that as algorithm specificity decreases the sensitivity increases and more individuals are considered to be cases. Of note was the fact that while the absolute risk for both drugs increased in a similar way between the least and most sensitive CPRD algorithms, the CPRD algorithm with moderate sensitivity/specificity estimated a higher flucloxacillin risk than the most specific algorithm, but the same oxytetracycline risk. Given the low numbers of oxytetracycline users with cholestatic liver injury identified by any of the algorithms (minimum of 1 for the multisource algorithm, maximum of 7 for the most sensitive CPRD algorithm), this could well be explained by chance, as if one more case had been identified by the algorithm with medium specificity, the oxytetracycline and flucloxacillin trends would have been similar. Furthermore, the 95% confidence intervals for all of the oxytetracycline risk estimates do not exclude the possibility that the true values in the underlying population are no different from each other.

The slightly higher 45-day risk point estimates obtained for all CPRD algorithms in the HES-linked cohort (Table 5-3) are likely to be due to the lower prevalence of exclusion conditions in the HES-linked cohort, as described previously (see previous section and Chapter 5 Appendix Table 4). Despite this slight difference, the 95% confidence intervals for each algorithm do not exclude the point estimates for the corresponding algorithm in the HES-linked subset. The fact that the similarity between the multisource algorithm measurement for flucloxacillin risk (8.69, 95% CI 6.34 – 11.63) is very similar to the most specific CPRD algorithm within the HES-linked subset (7.72, 95% CI 5.52 – 10.52), shows that the specific CPRD algorithm can be used as a reliable way of calculating risk within only the CPRD, and provides estimates with similar precision.

Despite the difference in the absolute effect estimates (45-day risk), the multivariable rate ratios were comparable for each of the CPRD algorithms. The estimates were particularly similar when comparing the least sensitive (RR 3.72, 95% CI 1.16 – 11.96) with the most sensitive CPRD algorithm (RR 3.70, 95% CI 1.73 – 7.94) and of note was that the more sensitive algorithm estimated with greater precision. This suggests that although the least specific CPRD algorithm is identifying more false positives, the measurement error occurs randomly across the exposure groups and therefore a valid rate ratio estimation can be obtained. This could be particularly useful when considering situations when low-power is an issue. This point is emphasised by the fact that rate ratios could not be estimated within the HES-linked cohort for the two more specific CPRD algorithms due to low numbers. In contrast, the most sensitive algorithm was able to identify sufficient cases to allow a rate ratio to be calculated (RR 8.86, 95% CI 2.17 – 36.11), and the similarity to the multisource algorithm result in this setting (RR 9.26, 95% CI 1.27 – 67.35) suggests that a sensitive algorithm with low specificity could provide reliable rate ratios when studying drug-induced cholestatic liver injury in a pharmacoepidemiological study.

The different results obtained for the most sensitive algorithm in the two settings (complete dataset compare to HES-linked only) could well be explained by chance, as the 95% confidence intervals overlap the point estimates, and if only 2 more cases of oxytetracycline-induced cholestatic liver injury had been identified in the HES-linked cohort, the rate ratios would have been similar.

5.9.3.2 Comparison with results in the literature

Table 5-6 shows the risk and risk ratios for the three CPRD algorithm cut-offs and for two published studies within CPRD (or its predecessor databases) looking at flucloxacillin, oxytetracycline and cholestatic liver injury. Figure 5.2 presents the risk ratios as a forest plot. As time-to-event information was not available in the referenced

studies [53, 55], risk ratios rather than rate ratios have been presented here to aid the comparison of relative effects. The risk ratios for each of the CPRD algorithms are very similar to the multivariable rate ratios calculated and discussed previously (see Table 5-4).

The risk estimate obtained using the most specific algorithm (6.15, 95% CI 4.61 – 8.04) is very similar to the risk estimated in the study by Derby et al (7.57, 95% CI 3.63 – 13.92), while the slightly less specific CPRD algorithm estimate (8.93, 95% CI 7.05 – 11.16) is similar to the risk estimated in the study by Russman et al (8.48, 95% CI 5.43 – 12.61). In terms of relative risk, the crude risk ratio for the most specific CPRD algorithm (3.79, 95% CI 1.18 – 12.12) and the least specific CPRD algorithm (3.74, 95% CI 1.74 – 8.00) are similar to the estimate obtained in the study by Derby et al (3.68, 95% CI 1.01 – 13.37). All of these are lower than the estimate from the study by Russman et al (RR 11.12, 95% CI 1.50 – 82.23), although this does have wide confidence intervals.

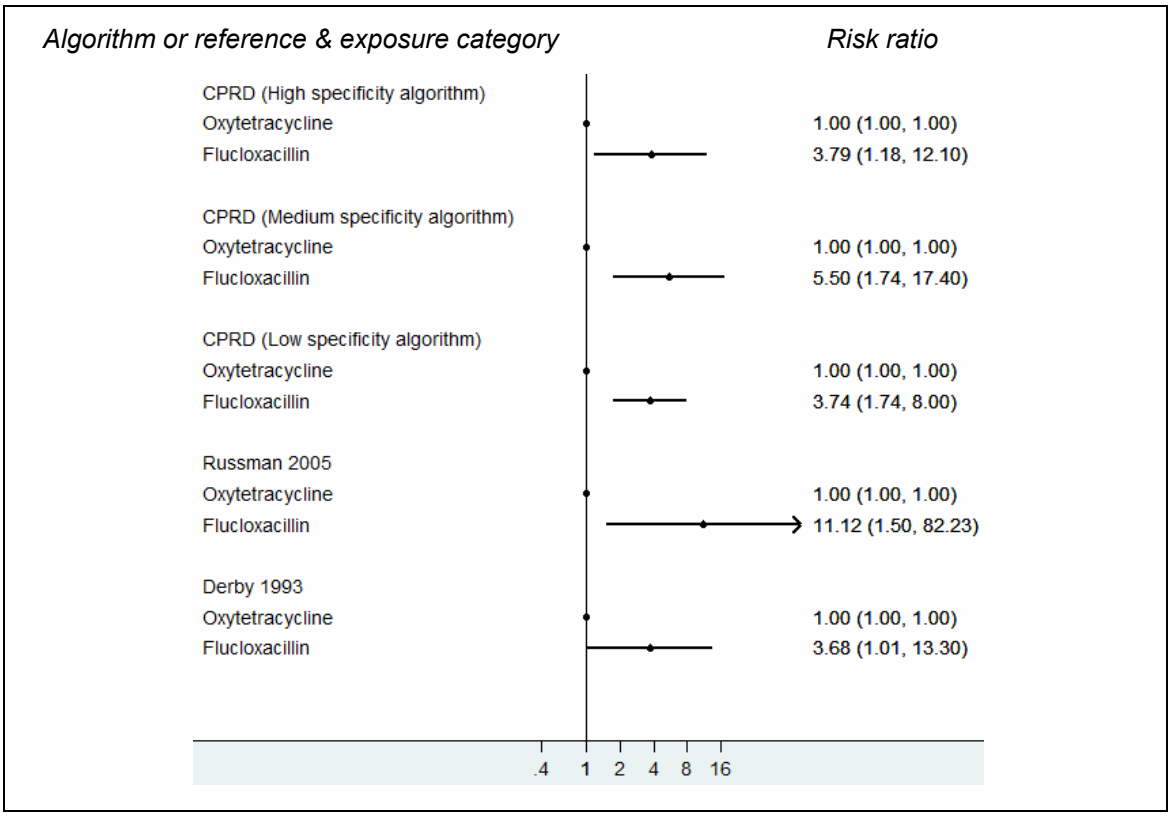
The risk ratio obtained from the Russman study is driven by fewer oxytetracycline cases having been identified than might have been expected (based upon the study performed here and that performed by Derby et al). As discussed previously, this could have been a chance result, given the low numbers that are being dealt with. Another possible factor is blinding to exposure status: while the Derby paper specifies that one of the reviewers was blinded to exposure status, and all reviewers were blinded in this work, there is no mention that reviewers were blinded to exposure status in the Russman study. Where the absolute numbers in an exposure group are very small, the inclusion or omission of even one case can have a large impact on the relative effect measure. When reviewing routinely collected primary care records of potentially drug-induced liver injury, the experience in this study was that there are likely to be numerous instances where it is difficult to decide whether an acute liver symptom or test result is due to a drug or to another cause. Knowing that the person was or was not exposed to a known cause of liver injury may well tip the case assignment decision one way or another.

Table 5-6: Comparison of risk and crude risk ratios estimated by the three CPRD algorithm cut-off scores and those obtained from previous studies in CPRD (or its preceding database VAMP and GPRD)

Algorithm or Reference (specificity[1])	Specificity, sensitivity	Exposure	# exposed[cases]	45-day risk[2] (CI[3])	Crude Risk Ratio[4]
Algorithm					
CPRD (High)	100%, 81%	Oxytetracycline	184737[3]	1.62 (0.33 – 4.75)	1
		Flucloxacillin	861959[53]	6.15 (4.61 – 8.04)	3.79 (1.18 – 12.12)
CPRD (Med)	86%, 87%	Oxytetracycline	184737[3]	1.62 (0.33 – 4.75)	1
		Flucloxacillin	861959[77]	8.93 (7.05 – 11.16)	5.50 (1.74 – 17.43)
CPRD (Low)	48%, 100%	Oxytetracycline	184737[7]	3.79 (1.52 – 7.81)	1
		Flucloxacillin	861959[122]	14.15 (11.75 – 16.90)	3.74 (1.74 – 8.00)
Reference[5]					
Russman 2005 [53]	N/A	Oxytetracycline	131189[1]	0.76 (0.02 – 4.25)	1
		Flucloxacillin	283097[24]	8.48 (5.43 – 12.61)	11.12 (1.50 – 82.23)
Derby 1993 [55]	N/A	Oxytetracycline	145844[3]	2.06 (0.42 – 6.01)	1
		Flucloxacillin	132087[10]	7.57 (3.63 – 13.92)	3.68 (1.01 – 13.37)

Note 1: Compared against a gold standard of the multisource algorithm (probable – definite case). High=CPRD algorithm cut-off score of 5, med=CPRD algorithm cut-off score of 2.29, low=CPRD algorithm cut-off score of 1.63.
Note 2: Per 100000 patients prescribed the drug.
Note 3: 95% confidence interval.
Note 4: Risk ratios presented (rather than rate ratios) as follow-up time was not available in published articles. Risk ratios calculated here for the three CPRD algorithm cut-offs are very similar to the multivariable rate ratios presented in Table 5-4.
Note 5: Risk ratios for references calculated from number of exposed and number of cases presented in articles.

Figure 5.2: Forest plot of the crude risk ratios estimated by the three CPRD algorithm cut-off scores and those obtained from previous studies in CPRD (or its preceding database VAMP and GPRD)



5.9.4 Risk factor analysis

The results for all algorithms demonstrated increasing age to be the most important risk factor for flucloxacillin-induced liver injury, with number of prescriptions another important risk factor. Unlike for the comparative analysis with oxytetracycline, power was not a particular issue for the most specific algorithm or the multisource algorithm, although the age estimations for both of these algorithms did have wide confidence intervals. Risk increased sharply over the age of 50, and increased substantially again over the age of 70 when measured by all algorithms.

Previous studies also showed age to be the most important risk factor. One case-control study reported that people over the age of 55 experienced 18.61 times the odds of cholestatic liver injury than those in the under 30 age group (95% CI 5.16 – 67.17 [56]), while a more recent cohort study reported that people over the age of 60 were 6.1 times more likely to develop flucloxacillin-induced cholestatic liver injury than those under the age of 60 (95% CI 2.9 – 13.0). Neither study provide any absolute effect measures stratified by age, as presented here. One of the studies also identified use of flucloxacillin for greater than 14 days as a risk factor, with people using the drug for more than 14 days having 7.13 times the risk of injury than those using for less than this period (95% CI 2.90 – 17.58) [56]. This is comparable with the relative effect estimates for number of prescriptions obtained by all of the algorithms in this study (see Table 5-5), considering that one prescription for flucloxacillin typically lasts 7 days.

The results of this study provide further detail of age and duration related effects, and include absolute estimates of effect as well as relative. Both the most specific and multisource algorithms suggest that the increased risk is very high over the age of 70 (e.g. multivariable rate ratio of 80.80, 95% CI 10.66 – 612.20 when comparing the 70 – 79 year olds with those under the age of 50). For the most specific CPRD algorithm (requiring that all cases had biochemically confirmed cholestatic liver injury), the absolute 45-day risk of cholestatic liver injury in those prescribed flucloxacillin under the age of 70 is estimated at 2.16 per 100 000 users (95% CI 1.21 – 3.56), while for those over the age of 70, this rises to 2.26 per 10 000 users (95% CI 1.60 – 3.11). Within the highest risk group (those over the age of 70 who received three or more prescriptions), this risk approaches 1 in every 1000 users (7.27 per 10 000 users, 95% CI 1.98 – 18.59).

5.9.5 Limitations

5.9.5.1 Chance

The study used 95% confidence intervals throughout to assess the role of chance. Some of the analyses were underpowered, but these were discussed appropriately where they occurred, considering the full range of possible values (and not just whether the confidence interval spanned the null). Test of significance were used to test for interactions.

5.9.5.2 Bias

Misclassification of main exposures

This study based drug exposure on prescription records from CPRD, which are automatically generated and recorded for automated uploading to the database as the general practitioner selects the drug for prescription. Therefore, the group assignments are very accurate representations of who was prescribed each of the drugs. This does not account for whether or not the individual actually took the drug, however, which is a problem for all studies that use CPRD prescription records for assigning drug exposure. As the two drugs are used for similar indications, and the characteristics of patients were shown to be broadly similar (Table 5-1), it is unlikely that one exposure group had improved adherence to the drugs prescribed than another, unless one of the drugs has a markedly better or worse side-effect profile than the other. A review of the prescribing information for each drug suggests comparable side-effect profiles (with the exception of serious liver injury) [117, 118], so this non-differential misclassification would likely bias results towards the null. Care was taken to ensure that that patients initially prescribed oxytetracycline but then prescribed flucloxacillin before their end of follow-up were assigned to the flucloxacillin group. Sensitivity analyses testing the impact of this decision and that of the decision to include co-fluampicil in the flucloxacillin group showed minimal impact on results.

Misclassification of outcomes

Clearly there will be patients identified as cases of flucloxacillin or oxytetracycline induced liver injury by all of the algorithms who then go on to be diagnosed with conditions that were more likely to have been responsible for the detected injury. Considering events occurring after the case assignment date was considered to be problematic, however, because it is not possible to apply a time window within which one could be sure that all study participants would remain available in the database. This is in contrast to the application of exclusions for such conditions based on information prior to the case date, where all participants had to be included in the

database for the same amount of time prior to the case (or matched case) date and exclusion conditions were searched for within this time period. Furthermore, there is a possibility that case and or exposure status to the drugs under study could influence the likelihood of being tested for the conditions in question, which risks biasing the study. A count of the number of people identified as cases who then went on to have an exclusion code at any time after their case date identified 66 people out of the 277 cases (24%), 32 of whom were within a 90 day period (12%). It should be noted, however, that having a subsequent exclusion code would not necessarily mean that the individual's injury was not due to one of the study drugs.

As the aim of this study was to assess the performance of a number of outcome definitions of varying specificity and sensitivity, there was very likely to be misclassification of outcome status for the less specific, more sensitive outcome definitions, which assign case status based upon relatively sparse information (for example, a CPRD diagnostic code of jaundice only, with no other information). Therefore, a degree of non-differential measurement error here is inevitable, which would likely increase absolute measures of effect and reduce relative measure of effect. This was observed and commented on throughout this study (for example the increasing risk measurements with decreasing sensitivity and the reduction in rate ratios for the risk factor analysis when comparing the less specific algorithms with the more specific ones). Furthermore, where external standards exist (e.g. for liver test results), these have been utilised and referenced throughout, and a careful and detailed exclusions list was prepared and followed to try and ensure those with other underlying causes of cholestatic liver injury would not be included in the study. Finally, a systematic and methodological approach to case definition was applied, using multiple database sources, and then validating carefully when developing other algorithms.

Differential misclassification of outcome has the potential to bias the results much more strongly, and could reduce or increase rate ratios observed. This study is very unlikely to have been susceptible to this, due to the fact that drug exposure status was only added to the cohort at the last possible time point, and that all review was completely blinded to drug exposure status (including the exposure on the index date, and any subsequent exposures during follow-up). One point that should be noted is that those identified as potential cases by the presence of diagnostic codes had a detailed (clinician) review of the electronic record performed in order to identify underlying causes for the injury. This review was not performed on the individuals not identified as potential cases (due to resource). Given the rarity of the underlying conditions in those without liver symptoms, not performing this is unlikely to have biased the results

obtained. In the future, application of automated algorithms to this part of the process (as has recently been tested elsewhere [77]) might allow a greater proportion of records to be reviewed in this way.

Observer/ascertainment bias

Ascertainment bias has the potential to impact this study in 2 ways. Firstly, at the level of the general practitioners who are responsible for diagnosing patients in the source population. All CPRD health records used in this study were routinely collected during primary care consultations before the design of this study, meaning that prescribing/diagnosing clinicians would not be aware which group particular exposure group of this study patients had been assigned to. Clinicians would be aware of flucloxacillin as a potential cause of cholestatic liver injury, however. Given this, there is a possibility that GPs could be more likely to identify these people as suffering drug-induced liver injury, which could increase both the absolute and relative effect measures obtained. This is unlikely to have affected the study results because the endpoints used were clinical symptoms/laboratory test results, and not a diagnosis of drug-induced liver injury by the attending clinician. Drug exposure status could have made the clinician more or less likely to have requested liver tests for a patient. The impact within this study would have been to make those on flucloxacillin with cholestatic liver injury more likely to be identified as definite cases than those on oxytetracycline with cholestatic liver injury. As diagnostic codes were also used as part of the case definition, oxytetracyline users with cholestatic liver injury would still have a high likelihood of being identified as (at least probable) cases by their symptoms. At the stage of review of electronic record for this study, the use of two exposure groups and blinding to exposure status would have greatly minimised the likelihood of observer bias contributing to the results.

Selection bias

Selection bias would occur if the relationship between exposure (flucl or oxyt) and outcome (cholestatic liver injury) is different within those people included in the study, compared to those people in the underlying study population. This is unlikely to have affected this study, because everybody who was ever prescribed flucl or oxyt in the total CPRD database was initially included in the study.

5.9.5.3 Co-variates, confounding and missing data

Age, gender, date of index prescription, prescriptions for other causes of liver injury, smoking status, BMI, alcohol intake, socioeconomic status and ethnicity were all considered as potential confounders, based upon a-priori knowledge and causal

modelling. All categories for age, gender, and date of prescription were complete and accurate, as these were obtained directly from CPRD. Prescriptions for other causes of cholestatic liver injury for use in the *concomitant prescriptions* variable were obtained from a variety of sources, but there are likely to be as yet unknown causes of cholestatic liver injury that people were taking. Furthermore, those drugs that are known to cause injury are poorly characterised, so the categorisation used here could only be a rough approximation. Of note is that in this study it was not possible to monitor the use of over-the-counter or hospital-prescribed drugs, although this is unlikely to have had a substantial impact on results.

The primary method for handling missing data was by including "missing" or "unknown" categories, allowing the extent of missing data to be observed for each variable. Smoking status, BMI and alcohol intake had missing values (all under 15% of records in all cases), as did socioeconomic status (>20% missing) and ethnicity (>30% missing). Creating missing categories in this way can be problematic, however, because it risks individuals with very dissimilar true values for the variable being placed in the same category, and adjustments with the variable may not adjust for confounding. When considering the association of flucloxacillin with cholestatic liver injury using each of the algorithms, none of the final multivariable models included variables with any missing data. Multiple imputation was therefore not performed. Restricting the cohort to only those individuals with complete records for all co-variates (not just those included in the final model) left a cohort of 489 367 people (46% of the original cohort), within which only the lowest specificity CPRD algorithm was able to identify any oxytetracycline cases. The multivariable rate ratio for the CPRD low specificity algorithm using only individuals with complete records (analysing the final multivariable model) was 7.76 (95% CI 1.91 - 31.62), stronger (but consistent with) that obtained in the main analysis (RR 3.70, 95% CI 1.73 – 7.94).

5.9.6 Generalisability

The CPRD is a good representation of the geographic distribution, age and sex structure of the UK population [119] and has a very large coverage (around 8% of the population [120]). In this study we used a total sample of over 1 000 000 people from this database, so our results are likely to be as generalisable to the UK population as the CPRD database itself. UK population groups not covered might include those not registered with a primary care practice which for example may include people who have immigrated to the UK and do not trust the health system or the homeless/prisoners. Given the rarity of the outcome under study, it is unlikely that omission of such groups would occur at a high enough level to impact the results of this

study. Overall, it is likely that our results could be generalised to the UK as a whole and other countries with a similar demography to the UK.

5.9.7 Conclusions and recommendations

This study has demonstrated that an algorithm for the detection of cholestatic liver injury using CPRD data alone can perform similarly to a (multiple source) algorithm using CPRD, HES and ONS data in measuring the frequency of drug-induced cholestatic liver injury. Furthermore, although increasing the sensitivity and decreasing the specificity of the CPRD algorithm (when compared to the multisource algorithm) results in an increase in the measured frequency of drug-induced cholestatic liver injury, analyses of association (relative effects) between a drug exposure and injury produce very similar estimates. When performing a risk-factor type analyses, the conclusions that can be drawn from the data are again likely to be similar across varying specificity of CPRD algorithm, with the less specific algorithms generating more conservative estimates with narrower confidence intervals.

For studies that are being performed to assess absolute risk (e.g. possibly to inform decisions about drug licence suspension or withdrawal), the algorithm versions with high sensitivity and high specificity could both play a role. For example, the lower specificity more sensitive algorithm could be used initially, possibly run in an automated fashion on a database (or databases) in order to obtain early signals of issues related to cholestatic liver injury. If cases were detected and further investigation warranted, the more specific version of the algorithm could then be run to obtain an accurate estimation of absolute risk.

When considering the results and methods that have been applied in the literature, frequency estimates for the two higher specificity CPRD algorithms were comparable with those obtained in the literature. Furthermore, relative estimates of effect were comparable with published data. It is of particular note that the two previous studies (and in fact, all of the studies with cholestatic liver injury as an outcome discussed in Chapter 2 of this thesis), performed retrieval and review of actual hard copies of notes from health centres in order to assign a case status. In this study, only electronic data was used, and the results obtained were very similar. This suggests that for pharmacoepidemiological studies of cholestatic liver injury within the databases used, hard copies of notes are unlikely to add any additional information above and beyond the information provided in the electronic record. The CPRD algorithm tested in this study could be used to facilitate the monitoring of CPRD for new case recruitment to genetic association studies, or as part of pharmacovigilance activities (as discussed in Chapter 4, section 4.4 and in reference [36]). If subsequent verification of case status

from a patient's responsible clinician was required (for example, in the situation where recruitment to a genetic association study was being performed using the low specificity CPRD algorithm as described in Chapter 4, section 4.4), a targeted, brief questionnaire similar to the one contained in Chapter 5, Appendix Table 5 could be utilised to verify status.

Finally, the results of this study have confirmed that the risk of flucloxacillin-induced liver injury increases dramatically over the age of 50, but also suggests that those over the age of 70 who receive more than one prescription are particularly susceptible. Current prescribing information only specifies an increased risk over the age of 50 [117] and could be updated accordingly with absolute risk figures for different age groups based on the results found here, in the largest study performed to date on the association between flucloxacillin and cholestatic liver injury.

5.10 Chapter 5 Summary

- In this chapter, the results were presented for the cohort study of the association between flucloxacillin (compared with oxytetracycline) and cholestatic liver injury

- Three versions of the CPRD cholestatic liver algorithm (low, medium and high specificity) were used

- Although absolute effects were different across the algorithms (low specificity algorithm crude rate 9.67 per 100 000 person months 95% CI 8.10 – 11.55, high specificity 4.20, 3.21 – 5.50), the lowest specificity algorithm estimated a rate ratio that was slightly lower but similar to the highest specificity algorithm but with narrower confidence intervals (multivariable adjusted rate ratio low specificity 3.70, 1.73 – 7.94; high specificity 3.72, 1.16 – 11.96)

- The 45-day risk estimate for the most specific algorithm (6.15 per 100 000 users, 95% CI 4.61 – 8.04) was very similar to estimates obtained by previous studies in the literature, which in contrast to this study relied on information requested from clinicians (external to the database)

- Risk of cholestatic liver injury is highest within people aged over 70 who receive three or more prescriptions (i.e. 45-day risk for those over the age of 70 who receive three or more prescriptions was 7.27 per 10 000 users 95% CI 1.98 – 18.59, compared to a risk of 2.16 per 100 000 users 95% CI 1.21 – 3.56 for people under the age of 70)

6 Results: applying the algorithm to putative but unknown associations - a case control study of the association between five drug exposures and cholestatic liver injury

6.1 Introduction

In this chapter, the results are presented for the case-control study of the association between cholestatic liver injury and the following five drug exposures:

1. Carbamazepine
2. Celecoxib
3. Duloxetine
4. Ramipril
5. Risperidone

As discussed in Section 3.4.2, these drugs were selected because they were (1) considered to be prescribed relatively frequently in the UK (2) had a lack of large epidemiological studies of their effect on cholestatic liver injury and (3) had been included in recent or multiple cholestatic liver injury case reports published in the literature and/or had statements in their prescribing information suggesting that they caused liver injury at an unknown frequency.

Details of the number of participants and a description of those included in the final case-control analysis are provided, before the following results are presented.

(5) The association of the study co-variates with cholestatic liver injury
(6) Crude estimated rate ratios for the association between being a current, recent or past user of one of the drugs of interest and experiencing a subsequent cholestatic liver injury
(7) Multivariable adjusted rate ratios of the associations described in (2)
(8) Frequency (risk) of cholestatic liver injury for those drug exposures shown to be strongly associated with cholestatic liver injury by the analysis in (3)
(9) Adjusted rate ratios presented stratified by important risk factors

Finally, a discussion of the results is provided, in which the results obtained for each drug exposure are discussed (including a comparison with flucloxacillin, included in the analysis in order to illustrate validity of the results).

Throughout this chapter, the CPRD algorithm cut-off scores will be described in terms of their specificity (as compared to the multisource case status of definite to probable), with cut-off score 5 being “high” specificity (specificity 100%, sensitivity 81%) and score 1.63 “low” specificity (48%, 100%).

6.2 Participants

Between the dates of 1st January 1992 and 31 January 2014 38 529 people aged 18 years and over were identified in CPRD with a diagnostic code indicating potential liver injury (see Chapter 3 Appendix Table 1) who had been registered in the database for at least 6 months. Applying the low specificity CPRD cholestatic liver injury algorithm to this cohort resulted in 13 693 individuals being identified as non-cases, while 30 967 individuals were identified as non-cases by the high specificity CPRD cholestatic liver injury algorithm. After applying exclusions to the low specificity cases, matching them to controls (by age, gender and practice) and applying the same exclusions to the controls, the low specificity algorithm case-control cohort consisted of 19 891 cases and 77 476 matched controls. The equivalent final figures for the high specificity algorithm were 5 681 cases matched to 22 176 controls. Full details are provided in Figure 6.1.

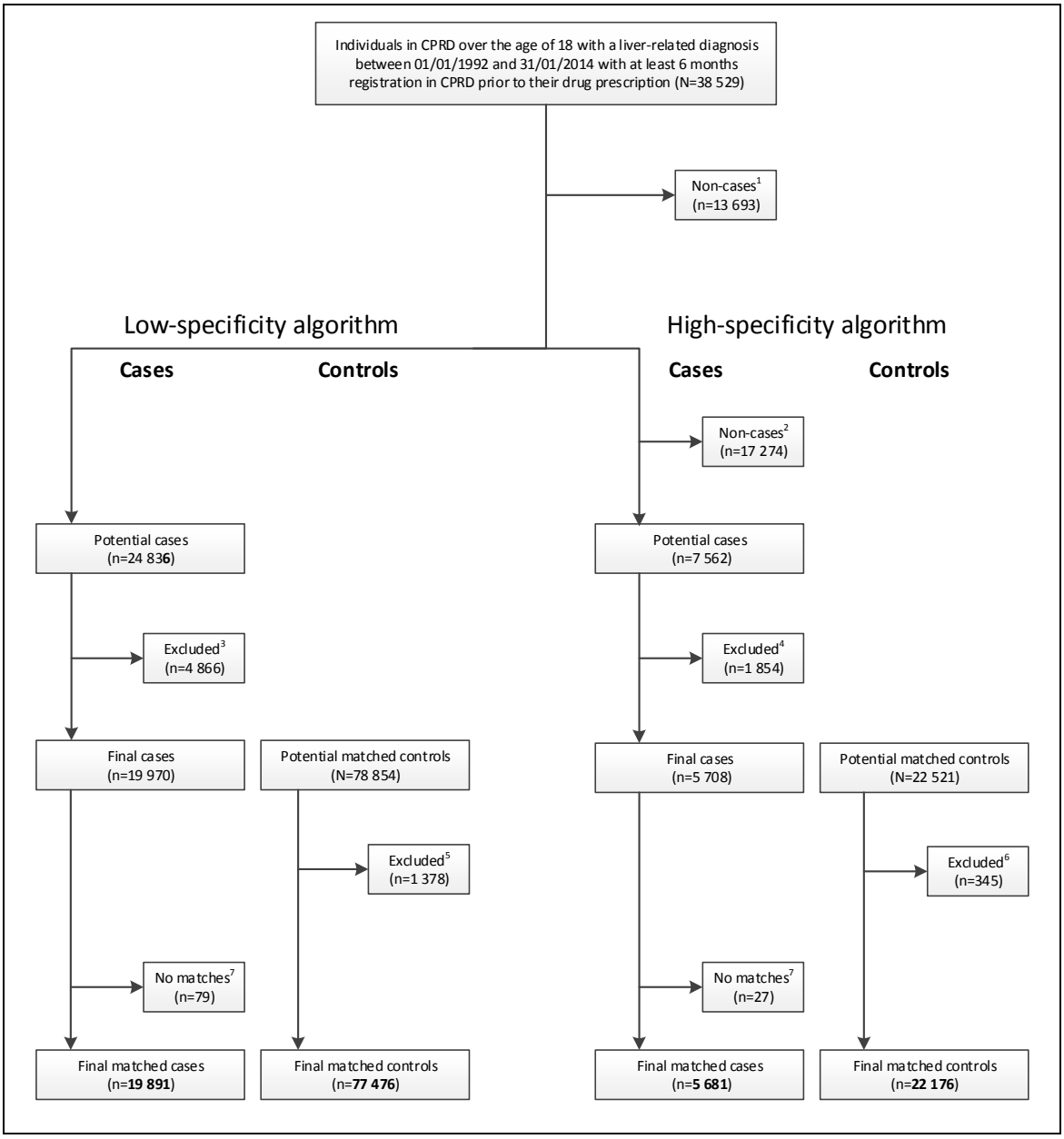


Figure 6.1: Flow of number of individuals included in the case-control study of the association between five drug exposures and cholestatic liver injury (with cases identified using a low-specificity and a high-specificity case identification algorithm)

[1]**Non-cases:** individuals who were not identified as cases by the high or the low specificity CPRD algorithm
[2]**Non-cases (high-specificity algorithm):** individuals who were not identified as cases by the high specificity CPRD algorithm only
[3]**Excluded potential cases (low-specificity algorithm):** made up of people who (1) had an underlying liver-related condition (n=4 095) (2) were pregnant (n=109) (3) had a pre-follow-up DILI liver test result (n=662)
[4]**Excluded potential cases (high-specificity algorithm):** (1) underlying liver-related condition (n=1 385) (2) pregnant (n=23) (3) pre-follow-up DILI liver test result (n=446)
[5]**Excluded potential matched controls (low-specificity algorithm):** (1) underlying liver-related condition (n=955) (2) pregnant (n=298) (3) pre-follow-up DILI liver test result (n=125)
[6]**Excluded potential matched controls (high-specificity algorithm):** (1) underlying liver-related condition (n=257) (2) pregnant (n=38) (3) pre-follow-up DILI liver test result (n=50)
[7]**No matches:** no potential controls identified as suitable matches, cases not included in final analysis

6.3 Descriptive data

There were 19 891 cases identified by the low-specificity algorithm in the database between 1st January 1992 and 31 January 2014 that were matched to 77 476 controls (by age, gender and practice). 5 681 of the low-specificity algorithm cases were also classified as cases by the high-specificity algorithm, and these were matched to 22 176 controls.

Table 6-1 provides an overview of the characteristics of the people included in the study, presented by algorithm and case-control status, including percentage distributions and odds of association across categories of each co-variate. The distribution of cases and controls for the matched variables (age, gender and date of cholestatic liver injury) were comparable within each algorithm. Comparing between the algorithms, 63% of the cases identified by the low-specificity algorithm were aged 60 or over, compared to 75% of those identified by the high-specificity algorithm, and a greater proportion of cases were identified after 2005 by the high-specificity algorithm (65%) than by the low-specificity algorithm (45%).

Receiving prescriptions for drugs considered to be causes of cholestatic liver injury (not including any of the drugs under study) was the variable associated most strongly with the outcome as defined by both algorithms, with an odds ratio of 8.28 (95% CI 7.62 – 9.00) for prescriptions considered to be the most common causes of liver injury for the low specificity algorithm. The estimate of the association for drugs considered to be causes of cholestatic liver injury when applying the high specificity algorithm was approximately 8-times higher than the low-specificity for both categories (e.g. 68.41, 52.52 – 89.11 for the more common causes).

Smoking status was associated with cholestatic liver injury as defined by both algorithms, with current smokers 1.48 (1.36 – 1.61) times more likely to experience cholestatic liver injury than non-smokers (high-specificity algorithm). BMI was also associated with cholestatic liver injury as defined by both algorithms, with people with a BMI of less than 20 more likely to suffer the injury when compared to those over this weight. People with alcohol status recorded as ex- drinkers and those who reported drinking over 6 units per day experienced an increased risk of cholestatic liver injury according to both the low and high-specificity algorithms, and both algorithms also detected a trend of increasing risk of injury with decreasing socioeconomic status. There was a suggestion that people who were any other ethnicity than white had a reduced risk of injury for both algorithms.

Table 6-1: Descriptive and univariable analysis for the cholestatic liver injury matched case control study, for the low- and high- specificity algorithms

		Low-specificity algorithm			High-specificity algorithm		
		Controls (N = 77 476) n (%)	**Cases (N = 19 891)** n (%)	**Univariable OR (95% CI)**	**Controls (N = 22 176)** n (%)	**Cases (N = 5 681)** n (%)	**Univariable OR (95% CI)**
Age at index date[1]	18 – 29	4209 (5)	1093 (5)	-	433 (2)	113 (2)	-
	30 – 39	5380 (7)	1386 (7)	-	807 (4)	209 (4)	-
	40 – 49	7966 (10)	2012 (10)	-	1634 (7)	414 (7)	-
	50 – 59	11152 (14)	2816 (14)	-	2819 (13)	711 (13)	-
	60 – 69	15458 (20)	3921 (20)	-	4719 (21)	1198 (21)	-
	70 – 79	17855 (23)	4553 (23)	-	5983 (27)	1521 (27)	-
	80+	15456 (20)	4110 (21)	-	5781 (26)	1515 (27)	-
	median (25 - 75%):	*66 (51 – 77)*	66 (51 – 78)	-	*71 (59 – 80)*	*71 (59 – 80)*	
Gender[1]	Male	41054 (53)	10503 (53)	-	11444 (52)	2929 (52)	-
	Female	36422 (47)	9388 (47)	-	10732 (48)	2752 (48)	-
Date of cholestatic liver injury[1]	1992 – 1993	3524 (5)	917 (5)	-	64 (0)	17 (0)	-
	1994 – 1996	5987 (8)	1554 (8)	-	250 (1)	66 (1)	-
	1997 – 1999	7955 (10)	2050 (10)	-	833 (4)	218 (4)	-
	2000 – 2002	11678 (15)	2993 (15)	-	2318 (10)	597 (11)	-
	2003 – 2005	13934 (18)	3576 (18)	-	4513 (20)	1158 (20)	-
	2006 – 2008	13713 (18)	3507 (18)	-	5287 (24)	1350 (24)	-
	2009 – 2011	12258 (16)	3137 (16)	-	5215 (24)	1331 (23)	-
	2012 – 2014	8427 (11)	2157 (11)	-	3696 (17)	944 (17)	-
Prescriptions for causes of cholestatic injury[2]	None	47383 (61)	8526 (43)	1	20022 (90)	2150 (38)	1
	Less common cause	28974 (37)	9834 (49)	2.08 (2.00 - 2.15)	2087 (9)	3007 (53)	16.66 (15.21 - 18.26)
	More common cause	1119 (1)	1531 (8)	8.28 (7.62 - 9.00)	87 (0)	524 (9)	68.41 (52.52 - 89.11)

		Low-specificity algorithm			High-specificity algorithm		
		Controls (N = 77 476) n (%)	**Cases (N = 19 891)** n (%)	**Univariable OR (95% CI)**	**Controls (N = 22 176)** n (%)	**Cases (N = 5 681)** n (%)	**Univariable OR (95% CI)**
Smoking status	Non-smoker	33408 (43)	8182 (41)	1	9595 (43)	2227 (39)	1
	Ex-smoker	25726 (33)	6272 (32)	0.99 (0.96 - 1.03)	8521 (38)	2174 (38)	1.11 (1.03 - 1.19)
	Current smoker	14169 (18)	4285 (22)	1.27 (1.21 - 1.32)	3434 (15)	1138 (20)	1.48 (1.36 - 1.61)
	Unknown	4162 (5)	1152 (6)	1.12 (1.04 - 1.21)	621 (3)	142 (2)	0.98 (0.80 - 1.19)
BMI	<20	3993 (5)	1295 (7)	1	1132 (5)	378 (7)	1
	20 - 25	23042 (30)	5790 (29)	0.77 (0.72 - 0.83)	6576 (30)	1743 (31)	0.78 (0.69 - 0.89)
	25+	38930 (50)	9639 (48)	0.76 (0.71 - 0.82)	11981 (54)	2848 (50)	0.70 (0.62 - 0.80)
	Unknown	11511 (15)	3167 (16)	0.85 (0.78 - 0.91)	2487 (11)	712 (13)	0.85 (0.74 - 0.99)
Alcohol intake	Non-drinker	9098 (12)	2394 (12)	1	2364 (11)	586 (10)	1
	Ex-drinker	2884 (4)	993 (5)	1.34 (1.23 - 1.46)	1177 (5)	358 (6)	1.24 (1.06 - 1.44)
	Current NOS	1821 (2)	516 (3)	1.08 (0.97 - 1.21)	577 (3)	157 (3)	1.11 (0.91 - 1.36)
	2 or less u/d	13621 (18)	3274 (16)	0.92 (0.86 - 0.97)	4240 (19)	1032 (18)	0.99 (0.88 - 1.11)
	3/6 u/d	33959 (44)	7750 (39)	0.88 (0.84 - 0.93)	10007 (45)	2318 (41)	0.96 (0.86 - 1.06)
	>6 u/d	5590 (7)	2269 (11)	1.61 (1.50 - 1.73)	1488 (7)	617 (11)	1.76 (1.53 - 2.02)
	Unknown	10503 (14)	2695 (14)	0.97 (0.91 - 1.03)	2323 (10)	613 (11)	1.07 (0.94 - 1.22)
Socioeconomic status	1 (Highest SES)	13882 (18)	3396 (17)	1	4463 (20)	1075 (19)	1
	2	13957 (18)	3403 (17)	1.03 (0.96 – 1.10)	4261 (19)	1056 (19)	1.07 (0.96 - 1.21)
	3	13359 (17)	3458 (17)	1.14 (1.06 – 1.22)	3984 (18)	1033 (18)	1.17 (1.03 - 1.32)
	4	11193 (14)	2972 (15)	1.21 (1.12 – 1.31)	3258 (15)	871 (15)	1.25 (1.09 - 1.44)
	5 (Lowest SES)	10199 (13)	2827 (14)	1.36 (1.25 – 1.49)	2594 (12)	713 (13)	1.36 (1.16 - 1.60)
	Unknown	14886 (19)	3835 (19)	-[3]	3636 (16)	933 (16)	-[3]
Ethnicity	White	39005 (50)	8544 (43)	1	12607 (57)	2878 (51)	1
	South Asian	734 (1)	125 (1)	0.75 (0.61 - 0.92)	168 (1)	25 (0)	0.61 (0.39 - 0.94)
	Black	396 (1)	59 (0)	0.66 (0.50 - 0.88)	99 (0)	11 (0)	0.46 (0.24 - 0.87)

		Low-specificity algorithm			High-specificity algorithm		
		Controls (N = 77 476) n (%)	**Cases (N = 19 891)** n (%)	**Univariable OR (95% CI)**	**Controls (N = 22 176)** n (%)	**Cases (N = 5 681)** n (%)	**Univariable OR (95% CI)**
	Other	337 (0)	75 (0)	1.02 (0.79 - 1.32)	83 (0)	12 (0)	0.62 (0.34 - 1.15)
	Mixed	88 (0)	11 (0)	0.57 (0.30 - 1.07)	32 (0)	4 (0)	0.53 (0.18 - 1.50)
	Not Stated	5851 (8)	1799 (9)	1.47 (1.39 - 1.57)	1758 (8)	544 (10)	1.41 (1.26 - 1.58)
	Unknown	31065 (40)	9278 (47)	1.79 (1.71 - 1.87)	7449 (34)	2207 (39)	1.64 (1.50 - 1.79)

Note 1: Matched variables

Note 2: Prescriptions were counted if they occurred anytime from 45 days prior to index date. List of drugs did not include those under study (see Chapter 3 section 3.4.1.5 for full list). Less common categories were created using flucloxacillin as a standard (so less than or more common than that caused by flucloxacillin), as reported in the literature (see Chapter 3 section 3.4.1.5). Therefore, less common are drugs reported to cause cholestatic liver injury at a frequency of less than approximately 1 per 1000 users, more common at a frequency of over 1 per 1000.

Note 3: Omitted because of no within-group variance.

6.4 Relative effect estimates: associations between each drug and cholestatic liver injury

The results of the crude and multivariable analysis of the association between each drug exposure and cholestatic liver injury as detected by the low and high specificity algorithms are shown in Table 6-2, and a forest plot of the multivariable results is provided in Figure 6.2.

Risperidone: Of the four putative causes of cholestatic liver injury, risperidone was the most strongly associated with the outcome. Current users of risperidone experienced 2.03 times the rate of liver injury as detected by the low specificity algorithm with a 95% confidence interval (CI) of 1.53 to 2.70, after multivariable adjustments. When the same analysis was performed with the high specificity algorithm, the rate ratio (RR) for current users of risperidone increased but the estimate was less precise (multivariable RR 2.59, 95% CI 1.41 – 4.75). Being a recent user of risperidone did not seem to increase the rate of liver injury as detected by either algorithm, while there was a suggestion that past users had a slightly increased rate (low specificity algorithm multivariable RR 1.24 95% CI 0.96 – 1.60, high specificity algorithm multivariable RR 1.59 95% CI 0.85 – 2.95).

Celecoxib: Recent users of celecoxib were also shown to have an increased rate of cholestatic liver injury, with a multivariable rate ratio of 1.89 (95% CI 1.11 – 3.22) as detected by the low specificity algorithm. When applying the high specificity algorithm, the estimated rate ratio was consistent with an increased rate for recent users, although the association lacked power (RR 1.93 95% CI 0.51 – 7.27). No association was observed for current or past users as detected by either algorithm.

Ramipril: There was a slightly increased rate of cholestatic liver injury for current users of ramipril, although the high specificity estimate lacked power (low specificity RR 1.13 95% CI 1.04 – 1.22, high specificity RR 1.12 95% CI 0.96 – 1.30). When applying the low specificity algorithm, recent users of ramipril had a further increased risk of liver injury (RR 1.73 95% CI 1.20 – 2.50), but this association was not observed when applying the high specificity algorithm.

Duloxetine: There was a suggestion that current users of duloxetine experience an increased rate of liver injury, with similar point estimates across both algorithms but a loss of precision with the high specificity algorithm (low specificity multivariable RR 1.56 95% CI 0.93 – 2.61, high specificity multivariable RR 1.5 to 95% CI 0.55 – 4.25).

Carbamazepine: Carbamazepine use did not seem to be associated with liver injury, while the known cause of cholestatic liver injury flucloxacillin showed strong

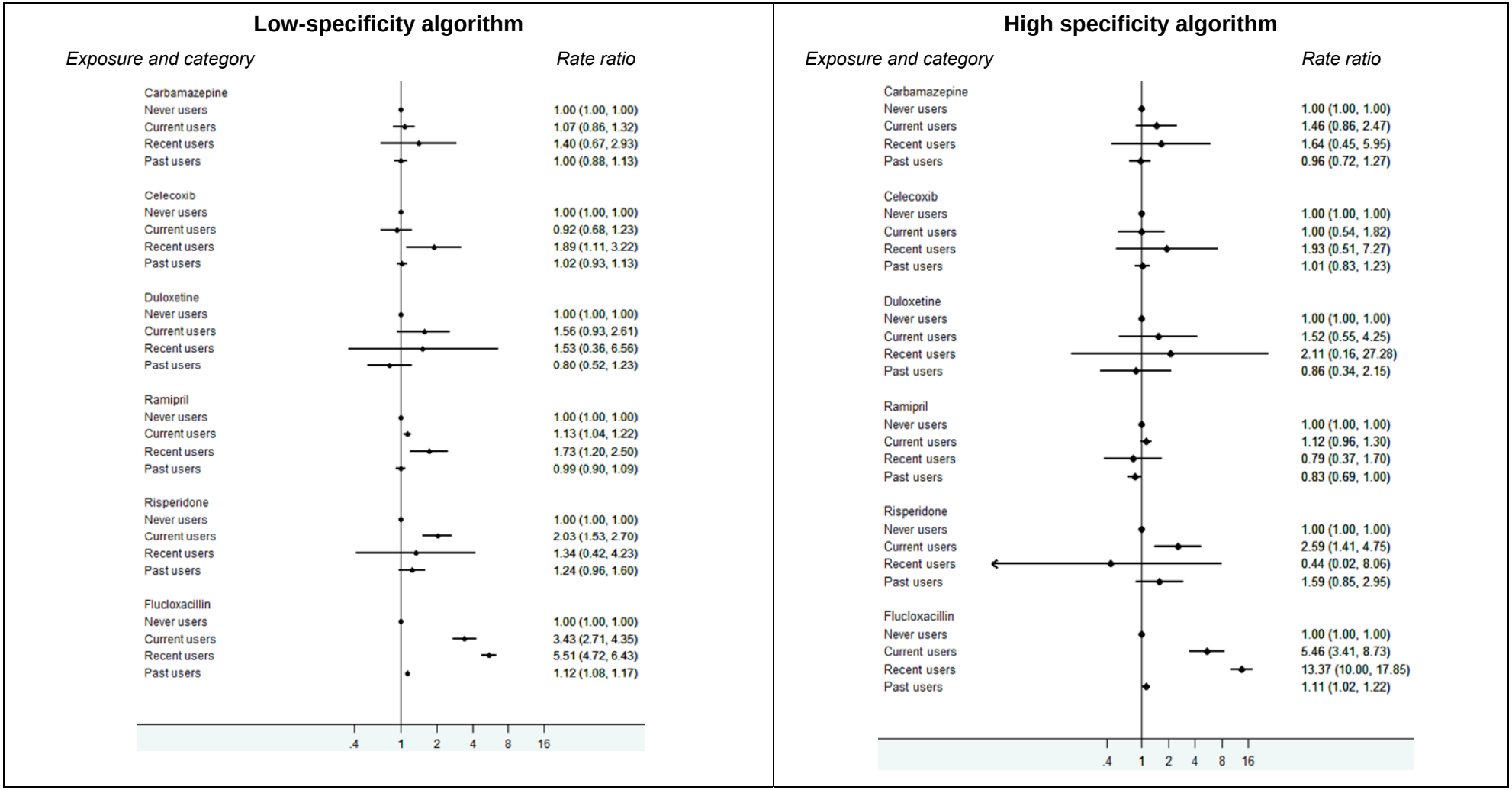
associations with the outcome for both current and recent users, with the high specificity algorithm measuring associations approximately twice the strength of those detected by the low specificity (but with wider confidence intervals).

Table 6-2: Crude and multivariable association between each drug and cholestatic liver injury as defined by the low and high specificity algorithms in the matched case control study (matched on age, gender and date of cholestatic liver injury)

		Low-specificity algorithm				High-specificity algorithm			
		Controls (N = 77 476) n (%)	Cases (N = 19 891) n (%)	Crude RR (95% CI)	Multivariable[1] RR	Controls (N = 22 176) n (%)	Cases (N = 5 681) n (%)	Crude RR (95% CI)	Multivariable[1] RR
Carbamazepine	Never users	75814 (98)	19382 (97)	1	1[1]	21650 (98)	5509 (97)	1	1[1]
	Current users[1]	414 (1)	133 (1)	1.27 (1.05 - 1.55)	1.07 (0.87 – 1.32)	103 (0)	36 (1)	1.38 (0.94 - 2.01)	1.46 (0.86 – 2.47)
	Recent users	30 (0)	11 (0)	1.44 (0.72 - 2.88)	1.40 (0.67 – 2.93)	10 (0)	7 (0)	2.73 (1.04 - 7.17)	1.65 (0.45 – 5.95)
	Past users	1218 (2)	365 (2)	1.18 (1.04 - 1.32)	1.00 (0.88 – 1.13)	413 (2)	129 (2)	1.23 (1.00 - 1.50)	0.96 (0.72 – 1.27)
Celecoxib	Never users	74977 (97)	19197 (97)	1	1[2]	21087 (95)	5393 (95)	1	1[2]
	Current users	270 (0)	58 (0)	0.84 (0.63 – 1.13)	0.92 (0.68 – 1.23)	114 (1)	20 (0)	0.69 (0.43 - 1.10)	1.00 (0.54 – 1.82)
	Recent users	45 (0)	23 (0)	2.04 (1.23 – 3.39)	1.89 (1.11 – 3.22)	16 (0)	8 (0)	1.95 (0.83 - 4.56)	1.93 (0.51 – 7.27)
	Past users	2184 (3)	613 (3)	1.11 (1.01 – 1.21)	1.02 (0.93 – 1.13)	959 (4)	260 (5)	1.06 (0.92 - 1.23)	1.01 (0.83 – 1.23)
Duloxetine	Never users	77300 (100)	19837 (100)	1	1[3]	22089 (100)	5655 (100)	1	1[3]
	Current users	57 (0)	22 (0)	1.52 (0.93 - 2.49)	1.56 (0.93 – 2.61)	33 (0)	10 (0)	1.20 (0.59 - 2.44)	1.52 (0.55 – 4.25)
	Recent users	6 (0)	3 (0)	2.00 (0.50 - 8.00)	1.53 (0.36 – 6.56)	1 (0)	3 (0)	11.21 (1.16 - 108.11)	2.11 (0.16 – 27.28)
	Past users	113 (0)	29 (0)	1.00 (0.66 - 1.50)	0.80 (0.52 – 1.23)	53 (0)	13 (0)	0.96 (0.52 - 1.75)	0.86 (0.34 – 2.15)
Ramipril	Never users	71231 (92)	18134 (91)	1	1[4]	19348 (87)	4931 (87)	1	1[4]
	Current users	3728 (5)	1021 (5)	1.09 (1.01 - 1.18)	1.13 (1.04 – 1.22)	1680 (8)	432 (8)	1.01 (0.90 - 1.14)	1.12 (0.96 – 1.30)
	Recent users	102 (0)	45 (0)	1.78 (1.25 - 2.54)	1.73 (1.20 – 2.50)	54 (0)	17 (0)	1.23 (0.71 - 2.14)	0.79 (0.37 – 1.70)
	Past users	2415 (3)	691 (3)	1.14 (1.05 - 1.25)	0.99 (0.90 – 1.09)	1094 (5)	301 (5)	1.08 (0.94 - 1.24)	0.83 (0.69 – 1.00)
Risperidone	Never users	77071 (99)	19700 (99)	1	1[5]	22035 (99)	5615 (99)	1	1[5]
	Current users	157 (0)	86 (0)	2.13 (1.64 - 2.78)	2.03 (1.53 – 2.70)	53 (0)	33 (1)	2.42 (1.55 - 3.76)	2.59 (1.41 – 4.75)
	Recent users	10 (0)	5 (0)	2.09 (0.71 - 6.11)	1.34 (0.42 – 4.23)	4 (0)	1 (0)	1.00 (0.11 - 8.95)	0.44 (0.03 – 8.06)
	Past users	238 (0)	100 (1)	1.66 (1.31 - 2.10)	1.24 (0.96 – 1.60)	84 (0)	32 (1)	1.50 (1.00 - 2.27)	1.59 (0.85 – 2.95)
Flucloxacillin	Never users	57746 (75)	13965 (70)	1	1	15536 (70)	3687 (65)	1	1
	Current users	215 (0)	118 (1)	2.30 (1.83 - 2.88)	3.43 (2.71 – 4.35)	70 (0)	36 (1)	2.18 (1.45 - 3.27)	5.46 (3.41 – 8.73)
	Recent users	392 (1)	333 (2)	3.57 (3.08 - 4.14)	5.51 (4.72 – 6.44)	138 (1)	144 (3)	4.37 (3.45 - 5.54)	13.37 (10.01 – 17.85)
	Past users	19123 (25)	5475 (28)	1.21 (1.16 - 1.25)	1.12 (1.08 – 1.17)	6432 (29)	1814 (32)	1.21 (1.13 - 1.29)	1.11 (1.02 – 1.22)

Note 1: Adjusted for all variables in Table 6-1 except calendar period (low-specificity algorithm) and age and calendar period (high-specificity algorithm) as additional stratification on these matched variables was not possible

Figure 6.2: Forest plot of the multivariable association between each drug and cholestatic liver injury as defined by the low and high specificity algorithms in the matched case control study (matched on age, gender and date of cholestatic liver injury and adjusted as detailed in Table 6-2)



6.4.1 Interactions/Effect modification

As discussed in Chapter 3, all co-variates included in the DAG analysis were investigated as potential effect modifiers. Evidence of effect modification was strongest for the low-specificity algorithm across all drugs (where there was more power to detect interactions), and all results discussed in this section relate to those obtained for the low-specificity algorithm. Likelihood ratio tests (LRT) for effect modification showed evidence that prescription with other hepatotoxic drugs, age, and ethnicity were possible effect modifiers of the association between drug exposure and cholestatic liver injury, particularly for risperidone (LRT p-values: other prescriptions – 0.001, age – 0.017, ethnicity - 0.041). Table 6-3 shows the association between risperidone and cholestatic liver injury (low-specificity algorithm) stratified by each of these variables.

Risperidone users who were not being prescribed another cause of liver injury had an increased rate of liver injury when compared to those being prescribed another hepatotoxic drug. For example, RR in current risperidone users (compared to never users) who were not prescribed another hepatotoxic drug: 3.22 (95% CI 2.20 – 4.73) compared with 1.38 (95% CI 0.86 – 2.17) for current risperidone users who were prescribed a hepatotoxic drug. This difference between categories of prescriptions for another hepatotoxic drug was apparent across all categories of risperidone exposure (e.g. within past users of risperidone, those not prescribed another cause of liver injury had a RR of 2.24, 95% CI 1.39 – 3.59 while those prescribed a more common cause had an RR of 0.67, 95% CI 0.36 – 1.22). A similar pattern of effect modification by prescriptions for other hepatotoxic drugs was also observed for celecoxib (Chapter 6 Appendix Table 1a).

When considering the effect of age, there was a trend for the effect of risperidone on cholestatic liver to be stronger in younger age groups (e.g. RR for current users of risperidone in the 18-49 yr old age group 4.79, 95% CI 2.35 – 9.76 compared to 1.38, 95% CI 0.94 – 2.03 in those aged over 70). A similar clear trend was apparent for Celecoxib (Chapter 6 Appendix Table 1b).

Finally, although there was insufficient power to obtain results for all categories of ethnicity, stratification by this variable suggested that the effect of risperidone on cholestatic liver injury was particularly marked in South Asian users (RR for current users compared to never users 26.06, 95% CI 2.78 – 244.21).

Table 6-3: Association between risperidone and cholestatic liver injury (low-specificity algorithm) by prescription with other hepatotoxic drugs, age at date of liver injury and ethnicity

		Multivariable RR[1] (95% CI[2]) by risperidone exposure category			
		Never users	Current users	Recent users	Past users
Prescription with other hepatotoxic drugs[3]	Not prescribed another cause	1	3.22 (2.20 – 4.73)	3.57 (0.49 – 25.98)	2.24 (1.39 – 3.59)
	Prescribed a less common cause	1	1.38 (0.86 – 2.17)	0.55 (0.06 – 4.61)	1.17 (0.85 – 1.61)
	Prescribed a more common cause	1	1.14 (0.54 – 2.41)	1.54 (0.13 – 17.81)	0.67 (0.36 – 1.22)
Age at date of liver Injury[4]	18 - 49	1	4.79 (2.35 – 9.76)	-	1.49 (0.87 – 2.54)
	50 - 69	1	2.91 (1.63 – 5.20)	1.91 (0.17 – 21.57)	1.30 (0.76 – 2.22)
	70+	1	1.38 (0.94 – 2.03)	0.83 (0.20 – 3.52)	1.13 (0.80 – 1.59)
Ethnicity[5]	White	1	1.93 (1.31 – 2.84)	-	1.20 (0.84 – 1.71)
	South Asian	1	26.06 (2.78 – 244.21)	-	7.08 (1.02 – 49.02)
	Black	1	7.60 (0.44 – 130.67)	-	1.50 (0.14 – 16.39)
	Other	1	-	-	-
	Mixed	1	-	-	-
	Not stated	1	4.11 (1.72 – 9.81)	0.64 (0.05 – 8.52)	0.88 (0.36 – 2.08)
	Unknown	1	1.40 (0.83 – 2.36)	3.01 (0.55 – 16.30)	1.31 (0.87 – 1.98)

[1]Multivariable rate ratio, with adjustments as described in Table 6-2
[2]95% confidence interval
[3]p-value for Likelihood Ratio Test of interaction with prescription with other hepatotoxic drugs: 0.001
[4]p-value for Likelihood Ratio Test of interaction with age at date of liver injury: 0.017
[5]p-value for Likelihood Ratio Test of interaction with ethnicity: 0.041

6.5 Absolute effect estimates: frequency (risk) of cholestatic liver injury

Following detailed and blinded review of the electronic record 1 year prior to case assignment of the 91 current and recent users of risperidone who were identified as cases in the case-control analysis, 39 were found to have no other documented cause of liver injury (19 of whom were identified as cases by the high specificity algorithm as well as the low specificity algorithm). Searching the entire CPRD database for the total number of people exposed to risperidone over the age of 18 during the study period resulted in a total number of 40 161 people, giving a risk of 97.11 per 100 000 users (95% CI 69.06 – 132.73) as estimated by the low specificity algorithm, and 47.31 per 100 000 users (28.49 – 73.87) as measured by the high specificity algorithm (Table 6-4).

Applying a similar review for celecoxib resulted in the identification of 47 people who were likely to have experienced their injury as a result of exposure to celecoxib, and 150 854 total exposed people in the database during the study period. The risk of liver injury per 100 000 users was therefore estimated to be 21.88 (15.06 – 30.72) when using the low specificity algorithm, and 7.95 (4.11 – 13.89) according to the high specificity.

The absolute effect estimate obtained for flucloxacillin for the high-specificity algorithm (included to allow a comparison between this method for estimating absolute effects and the cohort study performed in Chapter 5) of 8.52 per 100 00 users (95% CI 7.10 – 10.15) was slightly higher but consistent with the estimate obtained in Chapter 5 (6.15 per 100 000 users, 95% CI 4.61 – 8.04).

Table 6-4: Absolute effect estimates

Drug exposure	Total exposed people[1]	High specificity algorithm		Low specificity algorithm	
		Cases[2]	Risk[3] (CI[4]) (per 100 000 people prescribed the drug)	Cases	Risk (CI) (per 100 000 people prescribed the drug)
Risperidone	40 161	19	47.31 (28.49 – 73.87)	39	97.11 (69.06 – 132.73)
Celecoxib	150 854	12	7.95 (4.11 – 13.89)	33	21.88 (15.06 – 30.72)
Flucloxacillin[5]	1 478 303	126	8.52 (7.10 – 10.15)	344	23.27 (20.88 – 25.86)

Note 1: All those over the age of 18 with at least one prescription in CPRD for the drug in question during the study period
Note 2: Cases identified who were current or recent users of the drug in question and had no other cause of their liver injury in the previous year (assessed by 2 reviewers, including a clinician blinded to exposure status)
Note 3: Risk amongst those people who were either (1) current users or (2) recent users of the drug (i.e. the prescription ended within 30 days)
Note 4: 95% confidence interval
Note 5: Flucloxacillin results included in order to compare how closely this approach for estimating the absolute effect compared to the cohort study performed in Chapter 5. Number of cases in this table was obtained by multiplying the number cases identified in Table 6-2 by the proportion of cases found to be drug-induced following blinded clinician review in Chapter 5 Table 5-2 (i.e. 0.72 for the high specificity, 0.68 for the low specificity).

6.6 Discussion

In the case control study of the association between 5 drug exposures and cholestatic liver injury as detected by a low and a high specificity case definition algorithm, a strong association with liver injury was observed for risperidone (high-specificity algorithm: current users 2.59 times the risk of never-users, 95% CI 1.41 – 4.75). A weaker association was observed for users of celecoxib, but this was only significant for recent users (not current users) and was only detected using the low specificity algorithm (RR 1.89, 95% CI 1.11 – 3.22). Finally, ramipril use was associated with cholestatic liver injury, although this was only detected by the low specificity algorithm and was most pronounced in recent users with (RR 1.73, 95% CI 1.20 – 2.50). Stratified analysis of variables identified as effect modifiers suggested that for risperidone the effect differed by age, ethnicity and concomitant prescriptions. Similar (although less pronounced) effect modification by age and concomitant prescriptions was observed for celecoxib.

6.6.1 Comparison of relative effect estimates obtained by each algorithm

For those drugs that were shown to be associated with cholestatic liver injury, the low-specificity algorithm generally obtained a more conservative rate ratio (RR) than the high specificity algorithm, but with narrower confidence intervals. For example, the low specificity celecoxib recent users result was RR 1.89 (95% CI 1.11 – 3.22) compared to RR 1.93 (95% CI 0.51 – 7.27) for the high specificity algorithm. Similarly, for risperidone, the low specificity algorithm estimate for current users was RR 2.03 (95% CI 1.53 – 2.70) compared to a high specificity algorithm estimate of RR 2.59 (95% CI 1.41 – 4.75). This observation also applied to the results for flucloxacillin obtained here and in the study performed in Chapter 5.

This effect-dilution could be attributed to the non-differential measurement error that is being introduced by using a relatively non-specific case definition, with increased false positives biasing the estimate towards the null (so underestimating the true effect). One might expect though that there would be a degree of non-differential measurement error caused by the highly specific algorithm identifying more false negatives. While this will also be true, it seems here that identification of people who are not true cases as cases (in the unexposed group) causes the relative effect to be reduced when applying the more sensitive algorithm. The increase in precision is a result of the larger sample sizes due to the larger number of cases that are classified as cases by a low-specificity definition. As discussed previously (Chapter 5 Section 5.9), the fact that a relatively non-specific case definition for liver injury obtains estimates that are lower

than (although still similar to) and more precise than a more specific case definition is encouraging for settings where power may be an issue. It supports the idea that relatively accurate results can be obtained with good precision in settings where there are insufficient numbers of people with complete case records available to apply a highly specific case definition.

The results for ramipril contrasted to this pattern, as the effect estimated by the low specificity algorithm for recent users (RR 1.73, 95% CI 1.20 – 2.50) was in a different direction to that estimated by the high specificity algorithm (RR 0.79, 0.37 – 1.70). Furthermore, the 95% CI for the high specificity algorithm estimate excluded an effect as large as the point estimate obtained by the low specificity algorithm. Underlying biases that may have contributed to this result are discussed in the following section on interpretation of results.

A final point to note when considering the performance of each of the algorithms within this case control study relates to effect modification. Use of a low-specificity algorithm allowed a number of effect modifiers to be investigated, as there was sufficient power to detect the interaction and subsequently present and interpret the stratified results. Use of only a high specificity algorithm would have restricted this analysis.

6.6.2 Interpretation of relative and absolute effect estimates

6.6.2.1 Risperidone

The results suggest that current users of risperidone have over twice the risk of cholestatic liver injury, whichever algorithm is applied. Subsequent calculation of the risk revealed a frequency of cholestatic liver injury of 4.73 per 10 000 recent or current users (95% CI 2.84 – 7.39) or 9.71, 95% CI 6.91 – 13.27 if measured using the high specificity algorithm. Due to the population exposed to risperidone, however, it is necessary to consider these results carefully.

One possibility is that the increased rate of liver injury in the exposed group represents confounding by behaviours that may be associated with the potentially chaotic lifestyle of people who have (e.g.) schizophrenia. For example, individuals with schizophrenia may be users of illegal drugs, something that may not be recorded in electronic medical records but could increase the likelihood of liver injury. Furthermore, they may exhibit paranoia or be at least suspicious of GPs or medical centres, which could mean they consult less, missing vaccinations or other appointments which could mean other underlying unrecorded liver pathologies could be causing the liver injury detected in this study (such as infection with hepatitis). In an ad-hoc analysis performed to try and look at this, characteristics of people exposed to risperidone compared to people not

exposed to risperidone in this study were tabulated (Chapter 6 Appendix Table 2). Generally, this analysis did not really suggest that people exposed to risperidone had a greater proportion of missing data, however.

Interestingly, although there was a suggestion (in an underpowered analysis) that recent users of risperidone had a small increased risk of cholestatic liver injury as measured by the low-specificity algorithm, the high specificity algorithm result did not suggest an increased risk. The result was underpowered, but it is also possible that some people who have recently stopped risperidone prescriptions in primary care are being admitted to hospital due to worsening of their condition, which would means that although there could still be cholestatic liver test results these are not recorded in primary care.

The effect of risperidone on cholestatic liver injury was shown to be modified by age at date of liver injury and use of other hepatotoxic drugs, with the association being strongest in the lowest categories (youngest age group and not prescribed other drugs). One possible explanation for this pattern is that the liver event associated with risperidone is more common towards the beginning of a course of therapy, and those starting risperidone therapy are more likely to be younger and prescribed less other drugs than those who are someway into their period of therapy. A similar interpretation has been applied previously in a study of drug-induced liver injury relating to non-steroidal anti-inflammatory drugs [64]. An alternative possibility (relating to other hepatotoxic drug prescriptions) is that people who are able to tolerate other hepatotoxic drugs are also able to tolerate risperidone. Those who are not being prescribed other hepatotoxic drugs could therefore represent a subgroup who have had liver-related adverse events when prescribed other potentially hepatotoxic drugs, and are therefore also more likely to suffer liver injury when prescribed risperidone. A common genetic basis for liver injury in response to drug administration may explain this (for example, polymorphisms in genes encoding the cytochrome P450 (CYP) enzymes, which are known to be involved in the metabolism of a number of drugs including antipsychotics [121]).

Although the ethnicity data derived from CPRD and HES data suffers from the fact that many people do not have ethnicity recorded at all, the results hint at a possible interaction between South Asian ethnicity and the association between risperidone and cholestatic liver injury. Despite the confidence intervals for current users of South Asian ethnicity obtained being very wide, the lower bound of the 95% confidence interval (RR 2.78) excludes the point estimate obtained for White current users (RR 1.93). As there were only 4 people in the South Asian current user category, this result should be considered with caution. If it were true, it would be of interest as it may be

that people of South Asian ethnicity are the least likely to be living a chaotic lifestyle (for example, drug dependency in England in 2013 was lowest within this ethnic group [122]). A strong effect in this group could indicate that the detected liver injury may indeed be due to risperidone, and that there could be genetic traits linked to South Asian ethnicity that increase susceptibility. The CYP2D6 gene is known to effect the metabolism of risperidone [121] and interethnic variation in its expression is well established [123]. CYP2D6 poor metabolisers are known to occur more frequently amongst the white population than South Asians, however, so an alternative gene may be involved in this case. Alternatively, the effect seen could be because the baseline incidence of liver injury is lower within South Asians than it is in the White population.

There are a lack of published epidemiological studies on liver injury associated with either risperidone or with other atypical antipsychotics. Typical antipsychotics are known causes of liver injury: chlorpromazine, for example, has had frequent case reports of liver-injury published since its launch in 1951 [124-126] and newer atypical antipsychotics such as risperidone are generally assumed to have better side-effect profiles [107, 127]. Chlorpromazine has been studied in a number of large epidemiological studies, two of which were performed using primary care data stored in the VAMP database (a predecessor of the UK CPRD) and obtained estimates of cholestatic liver injury incidence of 28.6 per 100 000 users (95% CI 5.9 – 83.5) [66] and 135.4 per 100 000 users (49.7 – 294.7) [74]. The estimates obtained in this study for risperidone (47.31 per 100 000 users, 95% CI 28.49 – 73.87 for the high specificity algorithm, 97.11 per 100 000 users, 95% CI 69.06 – 132.73 for the lower specificity) suggests that the frequency of cholestatic liver injury may in fact be comparable to that of chlorpromazine.

As mentioned earlier in this section, there are many other factors that could be influencing the rate of liver injury observed in this study, but it should be noted that the studies on chlorpromazine performed previously will have also been susceptible to the same unmeasured confounding. The perception of risperidone (and possibly other antipsychotics) as being safer drugs than chlorpromazine could well be due to the fact that prescription habits had changed by the time that risperidone was launched (1990) such that it was (and is) not prescribed as freely as chlorpromazine was after its launch, and therefore a comparable number of cases of jaundice were not observed, despite a similar risk.

Celecoxib

The results suggest that recent users of celecoxib have around twice the risk of cholestatic liver injury than never users. The low-specificity algorithm was sufficiently powered to detect the association (RR 1.89, 95% CI 1.11 – 3.22), while the high

specificity algorithm result was consistent with a similar increase, although underpowered (RR 1.93, 95% CI 0.51 – 7.27). Subsequent calculation of the risk revealed a frequency of cholestatic liver injury of 7.95 per 100 000 current or recent users (95% CI 4.11 – 13.89) or 21.88 per 100 000 (95% CI 15.06 – 30.72) if applying the low specificity algorithm.

Of note is that while there was an association detected in recent users, this was not apparent in current users (for both the high and low specificity algorithms). One possibility is that recent celecoxib users represent a group of users who have generally been on celecoxib therapy for a longer period of time than those who are still current users, and could have reached some kind of threshold of exposure that was needed for liver injury to occur. An ad-hoc analysis was performed in order to investigate this possibility, but in fact found the opposite: the group of current users of celecoxib had a median of 9 prescriptions (spanning a median period of 416 days), while recent users had a median exposure of 3 prescriptions (spanning 181 days).

An alternative possibility, therefore, is that the effect seen in this study is due to the characteristics of the people who have had a shorter course of therapy (i.e. recent users). For example they could be finishing their prescriptions early due to other health issues which could be also lead to jaundice and a cholestatic liver test result (such as congestive heart failure). If this were the case, then one would expect that when the cases identified by the algorithm were reviewed for the purpose of calculating the absolute effect presented in Table 6-4, then a greater proportion of recent users would have been identified as non-cases and removed before calculating the risk. An ad-hoc analysis looking at this proportion indicated that it stayed constant, however (28% of cases were recent users before and after the review required for the absolute effect calculation). Another possibility is that recent users could include current users who have taken their prescription irregularly throughout their current use period and continue to do so during their recent use period.

If the effect is real, then it suggests that celecoxib is a rare cause of liver injury, and symptoms associated with the reaction may be prolonged before jaundice or a cholestatic liver test result. Existing case studies of celecoxib-induced liver injury have reported relatively short latencies between drug exposure and jaundice of less than one month, which is in contrast to this finding [128, 129]. Single case reports are more likely to be generated for occasions where the reaction has occurred very shortly after the drug administration (and is relatively straightforward to associated with the drug in question), however, which could explain this disparity.

Effect modification patterns were comparable to risperidone, and as discussed there (and in a previous study on NSAIDs [68]) could be due to the lower background level of

injury in those who are younger or do not have prescriptions for any other hepatotoxic drugs. It could also be due to a shared genetic susceptibility for liver injury in response to exposure to a number of drugs [9]. The apparent susceptibility of South Asian users to risperidone but not celecoxib could indicate the involvement of distinct genetic loci, however.

Although a number of epidemiological studies have been published looking at the hepatotoxicity of conventional NSAIDs (such as ibuprofen, diclofenac and mefenamic acid), there is a lack of data on COX-2 inhibitors such as celecoxib. One published result estimates a rate ratio for any liver injury associated with celecoxib of 1.0 (95 % CI 0.1–7.3) and a risk of 15.1 per 100 000 person years, but has very wide confidence intervals (95% CI 0.4 – 84.2) [130, 131]. The result obtained here suggests that the risk of cholestatic liver injury (confirmed by biochemical test i.e. the high specificity algorithm) is lower than this (assuming that one person is receiving celecoxib therapy for approximately 1 year) at 7.95 per 100 000 current or recent users (95% CI 4.11 – 13.89). If considering those without lab test results available (but still with a diagnosis of jaundice in order to qualify as low-specificity cases), the risk is higher, however (21.88 per 100 000 current or recent users, 95% CI 15.06 – 30.72). Of note is that both the (high and low specificity) estimates obtained in this study suggest that celecoxib may be a more common cause of liver injury than the older NSAIDs [68, 74], although still a rare cause.

6.6.2.2 Ramipril

For ramipril, there was a small increase in the rate of cholestatic liver injury observed in current users as detected by the low-specificity algorithm (RR 1.13, 95% CI 1.04 – 1.22), which was also detected by the high-specificity algorithm, but the analysis lacked power (RR 1.12, 95% CI 0.96 – 1.30). As discussed previously, for recent users the low specificity algorithm estimated a relatively strong association (RR 1.73, 95% CI 1.20 – 2.50) that was in a different direction and did not seem to be compatible with the effect estimated by the high specificity algorithm (RR 0.79, 0.37 – 1.70).

Such a discordant result suggests that there is likely to be an underlying bias, because if a drug increases the rate of jaundice (required to be a low specificity algorithm case), one would normally expect that the rate of biochemically confirmed cholestatic liver injury (required to be a high specificity case) would also increase. For example, this is the pattern observed for flucloxacillin (a known cause of cholestatic liver injury) in this analysis, and for each of the other drugs analysed.

Therapy with ramipril is likely to be long term, as once a person is diagnosed with (e.g.) hypertension, this requires ongoing management. People who have recently stopped

using the drug are either likely to have reacted badly to it, or the disease requiring ramipril treatment may have worsened. One indication that ramipril can be used for is the treatment of portal hypertension associated with chronic liver disease [132]. People who have recently stopped ramipril therapy could represent a group whose condition has deteriorated sufficiently that their current prescriptions are stopped and/or they are admitted to secondary care (where they may still receive drugs but these prescriptions would not be recorded in CPRD). If amongst these people there are individuals with (previously well managed) chronic liver disease, a worsening of condition could result in jaundice, either prior to admission to hospital (and therefore recorded in CPRD) or after being admitted to hospital (and entered retrospectively in CPRD).

This could explain the increased rate observed in recent users of ramipril detected by the low-specificity algorithm. A similar increase is not observed when applying the high-specificity algorithm because this relies on the presence of cholestatic liver test results, and if these tests were performed in hospital, the results would not be available in primary care. Therefore, the observed association detected in recent users by the low-specificity algorithm likely represents confounding by a condition that is an underlying cause of the indication being treated.

A post-hoc investigation was performed to try and assess whether this could be an explanation for the observed results, in which the CPRD records of twenty randomly selected ramipril recent user cases were reviewed again. All twenty were low-specificity algorithm cases; five were also high-specificity cases. Although it was not possible to determine the presence of portal hypertension and chronic liver disease was not documented in any of the cases, more than half of the cases (11/20, 55%) were hospitalised around the time of their final ramipril prescription or liver injury date. 4/11 (37%) of those hospitalised went on to be diagnosed with conditions that were more likely to have caused the observed symptoms than a drug exposure. These included conditions such as malignant neoplasm of the pancreas and congestive heart failure, which are more common in those leading a lifestyle that is likely to also require a prescription for ramipril (such as heavy smokers, drinkers and those who are overweight). Confounding by indication is therefore a likely explanation for the results obtained here.

6.6.2.3 Carbamazepine, duloxetine and flucloxacillin

Although the point estimates for carbamazepine and duloxetine were consistent with an increased rate of cholestatic liver injury, the analyses were underpowered in this setting. Performing a similar analysis in a database where drug utilisation was higher (for example, a U.S. database for Duloxetine) could be advisable. Alternatively,

combining datasets from multiple databases might be one way of increasing power sufficiently for a valid analysis to be performed.

The results obtained for flucloxacillin provide encouragement that the methods applied for the calculation of relative and absolute effects in this case-control study were valid. Flucloxacillin is a known cause of cholestatic liver injury, so one would expect that (as seen here) decreasing the specificity of a cholestatic liver injury algorithm would result in a reduction of the measured rate ratio (as the non-differential measurement error dilutes the effect estimate). Furthermore, the absolute effect estimate for the high specificity algorithm (8.52, 95% CI 7.10 – 10.15) is very similar to that obtained in Chapter 5 (6.15 per 100 000 users, 95% CI 4.61 – 8.04) and in previous studies performed in the same setting [53, 55].

6.6.3 Limitations

6.6.3.1 Chance

The same considerations as those detailed in Chapter 5 applied to this study.

6.6.3.2 Method for absolute effect estimation

For the calculation of the absolute effect in this analysis, the risk of cholestatic liver injury was calculated by dividing (1) the total number of cases identified for the drug by (2) the total number of people in CPRD with at least one prescription during the study period and the risk of cholestatic liver injury per 100 000 current and recent users was presented. This is only provides a crude estimate of the risk, as it does not take into account follow-up time or time that each person is on the drug in the analysis. Analysis that could allow further characterisation of the absolute effect could involve a cohort-design that factors in the time that each individual was exposed to the drug prior to their injury (allowing estimation of the rate).

6.6.3.3 Bias

Misclassification of main exposures

As discussed in Chapter 5, CPRD records indicating a prescription for a drug do not provide any information about how much of the prescription was actually taken by the individual. If the compliance to the drug was linked to case or control status, then this could bias the result in unexpected ways. Being a case could be related to drug compliance because early symptoms of liver injury may lead to a person stopping taking the drug during the time that the prescription covers. In this study this is unlikely to have led an exposed case to be incorrectly assigned as an unexposed case however, because a person was considered exposed from the beginning of their

prescription for the entire period that the prescription covered (plus an additional grace period). The most likely misclassification is between recent and current users. The grace period added on to the end of a prescription period was included to help cope with this (providing a period at the end of a prescription during which a person may still be taking drugs provided as part of that prescription). Furthermore, if a small number of people were still taking the drug but ended up being assigned to the wrong exposure group, the impact on the conclusions drawn would be minimal (the person would still be considered as exposed to the drug, whether or not they were recent or current users).

Misclassification of outcomes

As the case-control analysis performed here included the use of algorithms of varying specificity and sensitivity, there was very likely to be misclassification of outcome status, as case status was assigned based upon relatively sparse information (for example, a CPRD diagnostic code of jaundice only, with no other information). Therefore, a degree of non-differential measurement error here is inevitable, which would likely increase absolute measures of effect and reduce relative measure of effect. This was observed and commented on throughout this study (for example the increasing risk measurements with decreasing sensitivity and the reduction in rate ratios for the risk factor analysis when comparing the less specific algorithms with the more specific ones). A further point that should be noted is that for the absolute risk estimate (requiring close review of the electronic record in order to verify whether the injury was drug-induced), after review there were often cases identified as “probably” due to the drug exposure. These were considered as non-cases and not included in the final risk calculations (i.e. a conservative approach was adopted so that if there was any doubt, the individual was considered a non-case). This was done so that the resulting absolute effect estimates were very unlikely to be overestimated.

Observer/ascertainment bias and selection bias

Ascertainment bias has the potential to impact this study in 2 ways. Firstly, at the level of the general practitioners who are responsible for diagnosing patients in the source population. All CPRD health records used in this study were routinely collected during primary care consultations before the design of this study, meaning that prescribing/diagnosing clinicians would not be aware which particular exposure group of this study patients had been assigned to. Clinicians would also have been unlikely to have suspected the drugs under study to be causes of cholestatic liver injury, as none were established causes of liver injury. Then at the stage of review of electronic record for this study, blinding to exposure status would have greatly minimised the likelihood of observer bias contributing to the results.

Selection bias was minimised in this study in terms of selection of controls by ensuring that the source population that the controls were drawn from was the same as that which the cases were drawn. In the past (and as discussed in Chapter 2), case-control studies of cholestatic liver injury performed in electronic health records have been careless in this respect, and have included a close review and exclusion process on potential cases but not potential controls. This effectively makes the source population of controls different to that of the cases, and risks selection bias that could alter the effect estimate unpredictably. The omission of this type of potential case review increases non-differential measurement error in the case control study (as more people will be included who may not actually be cases), but this is known to underestimate the effect rather than bias it in an unknown direction. Close review of case information was then subsequently performed in the study, but only when required to obtain accurate risk estimates.

6.6.3.4 Co-variates, confounding and missing data

Careful consideration and planning around potential confounders was performed in relation to this study, including the use of a causal diagram (or DAG). As many of the co-variates identified in the DAG as possible were included in the analysis, but there were some that could not be included in the analysis due to the routine nature of the data. Where this occurred, the impact on the results was discussed and considered (for example, unmeasured confounders such as illicit drug use that may have affected the results for risperidone).

The primary method for handling missing data was by including “missing” or “unknown” categories, allowing the extent of missing data to be observed for each variable. Smoking status, BMI and alcohol intake had missing values (all under 17% of records in all cases), as did socioeconomic status (20% missing) and ethnicity (>40% missing). This may not be an ideal approach, as the true distribution of the covariates categories within the missing group is unknown and may mean that the confounder has not been appropriately adjusted for [133]. Given that that nearly half of the cohort had ethnicity data missing, a sensitivity analysis was performed by repeating the analysis with a model that included all co-variates apart from ethnicity. The results obtained without adjusting for ethnicity were very similar to those obtained with the ethnicity co-variate included (the biggest difference across all drugs and algorithms being for ramipril using the high specificity algorithm: with ethnicity recent users vs. never users RR 2.11, 95% CI 0.16 – 27.28, without ethnicity RR 1.81, 0.16 – 19.91).

A complete records analysis was also performed for the low specificity algorithm, meaning that only records that had values recorded for all variables analysed were

included. For risperidone, the results obtained for the complete records analysis were consistent with those obtained for the main analysis. Of note was that the magnitude of the association between current users of risperidone and cholestatic liver injury was increased from an RR of 2.13 (95% CI 1.64 – 2.78) to an RR of 3.10 (2.05 – 4.70). This does suggest that the involvement of unmeasured confounders associated with a chaotic lifestyle may not be substantial, because people with complete records are likely to be those with the least chaotic lifestyles yet the rate ratio increases if they are the only people included in the analysis.

For celecoxib, restricting to those with complete records only altered the point estimates by a maximum of 0.08, but the reduction in sample size meant that there was a loss of precision such that the 95% CIs crossed the null value for recent users. This was predominantly due to the fact that so many people had ethnicity data missing. Although ethnicity was included as a covariate in the DAG, whether or not it was included as a covariate in the final model of the main analysis had minimal impact on the results obtained. If the complete records analysis was performed in those with complete records apart from ethnicity, then the results obtained were very similar to those in the main analysis.

Considering missing data more closely, for a complete case analysis to provide sensible results, the probability of being a complete case needs to be independent of the outcome (conditional on the covariates) [134] [135]. One way that this condition could be fulfilled is if the data were missing completely at random (MCAR), where the probability of data being missing for a variable is unrelated to the value of the variable itself and also unrelated to the value of (an)other fully observed variable(s) [136]. This is unlikely to apply to studies using routinely collected data (unless, for example, a file system storing the records becomes corrupt and a random sample of those records are lost). Although MCAR is unlikely to apply, it could be argued that after adjusting for all co-variates in this study, the probability of being a complete case is independent of the outcome (suffering acute liver injury), because the outcome is relatively rapid onset, severe and of (again, relatively) short duration. In contrast to a chronic outcome that could well influence attendance at health clinics over a long period of time and therefore the availability of information on co-variates, it is unlikely that the idiosyncratic liver injury studied here would influence the availability of information on co-variates. Therefore, a complete case analysis could well be valid.

If one were not convinced that this condition held, one could consider that the mechanism for missingness might be missing at random (MAR), where the chance of values for a particular variable being missing could depend on the values of a fully

observed variable. If this was the case, multiple imputation is one method that could be used to allow a valid analysis, by imputing the missing data. In fact, within this study a missing not at random (MNAR) mechanism is possible, meaning that whether an individual has missing data for a variable could depend on the underlying value of the variable itself. For example, a reason that somebody has missing smoking information could be because they are heavy smokers who attend primary care infrequently as they don't want to be told to stop smoking. Additional work could therefore be performed where multiple imputation assuming MAR is performed in order to impute missing data, with sensitivity analyses included to investigate the plausibility of this model.

6.6.4 Generalisability

The CPRD is a good representation of the geographic distribution, age and sex structure of the UK population [119] and has a very large coverage (around 8% of the population [120]). In this study we used a total sample of approximately 100 000 people from this database, so our results are likely to be as generalisable to the UK population as the CPRD database itself. UK population groups not covered might include those not registered with a primary care practice which for example may include people who have immigrated to the UK and do not trust the health system or the homeless/prisoners. Given the rarity of the outcome under study, it is unlikely that omission of such groups would occur at a high enough level to impact the results of this study. Overall, it is likely that our results could be generalised to the UK as a whole and other countries with a similar demography to the UK.

6.7 Conclusions and recommendations

Use of the cholestatic liver injury algorithm in a case-control study confirmed that it is possible for a low specificity algorithm to obtain results that will be smaller in magnitude than high specificity algorithm results but with narrower confidence intervals. This finding is consistent with a previous study that applied algorithms for identifying acute liver injury with varying specificity to the UK CPRD and the Spanish BIFAP database, suggesting that it is likely to be generalisable to databases other than just the UK CPRD [112]. If results are obtained that do not fit this pattern, this can be an aid to identifying possible biases with the underlying data. As described in Chapter 5, both low specificity and high specificity algorithms for identifying cholestatic liver injury can be of use depending on setting. Low specificity analysis may also allow interactions to be studied that might not have otherwise been detected.

The antipsychotic risperidone and the non-steroidal anti-inflammatory celecoxib were both shown to be associated with cholestatic liver injury in this study. The risk

associated with celecoxib was low (less than 1 per 10 000 users) than might have been expected based on the literature and prescribing information for risperidone (almost 1 per 1000 users and comparable with the older generation of antipsychotics). Independent of whether the increased rate of liver injury in this study is wholly caused by risperidone, the finding that those prescribed the drug have a relatively high rate of serious liver injury is of concern, as this group are likely to be particularly vulnerable.

Based on the results obtained in this study additional work is recommended. A cohort analysis looking at the risk of cholestatic liver injury in risperidone compared to the use of another antipsychotic (such as chlorpromazine) would be advisable. This could help deduce how much of the effect is due to risperidone and how much is related to characteristics of population who receive risperidone. Within the study, risk estimates by age and other characteristics could be obtained, and further characterisation of the nature of the liver injury could be obtained (for example, time between first or last prescription and liver injury, occurrence of other symptoms). Similarly, a cohort study comparing celecoxib with the older non-steroidal anti-inflammatory drugs could be performed. Finally, given the widespread use of ramipril and the public health impact of even a small increase risk associated with its use, a further study is recommended to help understand the risk that current users of the drug may experience, given that a small increased risk was detected by the low specificity algorithm (and was suggested by the high specificity). A carefully designed cohort or self-controlled case series design could help minimise confounding.

6.8 Chapter 6 Summary

- In this chapter, the results were presented for the case-control study of the association between cholestatic liver injury and carbamazepine, celecoxib, duloxetine, ramipril and risperidone

- When performing a case control study of cholestatic liver injury, a low specificity algorithm generally obtains comparable (although smaller) relative effect estimates than a high specificity algorithm and has narrower confidence intervals (e.g. risperidone current vs. never users: high specificity multivariable RR 2.59, 95% CI 1.41 – 4.75, low specificity 2.03, 1.53 – 2.70; celecoxib recent vs. never users: high specificity multivariable RR 1.93, 0.51 – 7.27, low specificity 1.89, 1.11 – 3.22).

- Inconsistencies between high and low specificity algorithm results can be used to help identify bias – for example, with Ramipril different relative effect measures (high specificity recent vs. never users multivariable rate ratio 1.73, 95% CI 1.20 – 2.50, low specificity 0.79, 95% CI 0.37 – 1.70) were likely to have been explained by confounding by a condition that was an underlying cause of the treatment indication (hepatic portal hypertension), leading to hospitalisation and an associated decreased recording of liver test results in primary care

- The anti-inflammatory drug celecoxib and the atypical antipsychotic risperidone were associated with cholestatic liver injury (celecoxib low-specificity algorithm multivariable RR recent vs. current users 1.89, 95% CI 1.11 – 3.22, high specificity risk per 100 000 people prescribed the drug 7.95, 4.11 – 13.89; risperidone high specificity multivariable RR 2.59, 95% CI 1.41 – 4.75, high specificity risk 47.31, 28.49 – 73.87)

- Absolute effect estimates for risperidone suggest it may be as common a cause of liver injury as the older antipsychotics

7 Summary and Conclusions

7.1 Introduction

The aim of this final chapter is to provide an overarching summary of findings, key discussion points, and future directions. Detailed discussion sections are provided at the end of each previous chapter of this thesis.

7.2 Summary of research and main findings

In this thesis, methods for identifying serious adverse drug reactions for epidemiological studies in electronic health records were investigated and developed, with a focus on drug-induced cholestatic liver injury. The concept of rare but serious drug reactions was introduced, and a likely genetic predisposition to such reactions was discussed. Challenges in detection of these reactions during drug discovery and limitations of current methods for post-marketing signal detection were identified. An increasing trend for using databases for pharmacoepidemiology studies was highlighted, including potential novel uses such as predictive genetic test development and adaptive licensing. The specific serious adverse reaction of cholestatic liver injury was then described (the cholestatic type of drug induced liver injury), with cholestatic liver injury caused by flucloxacillin cited as an important example.

A systematic literature review of studies with an outcome of cholestatic liver injury performed within routinely collected electronic health records then found 16 existing studies that had studied this outcome. 14 criteria were assessed, with criteria selected based upon existing standards for the reporting of observational studies with a particular focus on case-identification methods and their reproducibility. While 10 of the studies met the majority of assessed criteria, the following issues were identified as problematic: (1) exclusion criteria were often not defined clearly or applied appropriately for the study design (2), the availability and specific use of data was generally not described in detail (3) there was a potential overreliance on information requested from health centres (i.e. requests for data that was not included in the database) (4) time intervals that were applied between diagnoses and liver test results were not specified (5) measurement of validity was not performed and (6) multiple case status levels were not included . Suggested areas for further work included: linking existing databases (such as primary and secondary care databases), assessing the benefit of requesting notes from clinicians, allowing “real-time” identification and reducing reliance upon binary case definitions if at all possible.

Multiple electronic health records sources (UK CPRD and UK HES and ONS databases) were then utilised to construct a multisource algorithm for detection of

cholestatic liver injury. The multisource algorithm case status was used to develop and validate a CPRD cholestatic liver injury algorithm, using data only from a standard CPRD record. Testing of low, medium and high specificity algorithms in a study of the antibiotics oxytetracycline and flucloxacillin demonstrated that while absolute effects differed by specificity, relative effects were similar between the highest and lowest algorithms. Incidence estimates for the most specific algorithm were very similar to those obtained by previous studies in the literature that did rely on external information.

The algorithms were then implemented within a real-life pharmacoepidemiological database research setting by performing a case-control study of cholestatic liver injury for a number of putative drug causes. This study showed that a low specificity algorithm obtained comparable (although smaller) relative effect estimates to that estimated by a high specificity algorithm, with narrower confidence intervals. Inconsistencies between high and low specificity algorithm results for the same drug were used to help identify likely underlying biases. The anti-inflammatory drug celecoxib and the atypical antipsychotic risperidone were associated with cholestatic liver injury. Absolute effect estimates for risperidone suggest that it may be as common a cause of liver injury as some older antipsychotics.

7.3 Comparison with existing research

7.3.1 Comparison of algorithm and study methodology

The most well-known and well established algorithm for detection of drug-induced liver injury (including cholestatic liver injury) was developed in 1990 by CIOMS and is known as the RUCAM method [45]. This algorithm provides a system for assigning scores for specific characteristics of a potential drug-induced liver injury event, and the total score assigned can then be used to indicate the probability of whether a drug suspected of causing the liver injury was responsible (i.e. excluded, unlikely, possible, probable and definite). With the increasing incidence of non-alcoholic fatty liver disease, the threshold for defining DILI based upon biochemical parameters were increased (so, for example, DILI based upon raised ALT required ALT to be more than five times the upper limit of normal compared to two times the upper limit as was originally suggested in the RUCAM method). These up to date parameters were discussed in a paper written by a group of international experts in 2011 [46], and these updated parameters were used in the CPRD algorithm developed in this thesis.

Other characteristics that the CPRD algorithm (and subsequent clinician review and study analysis steps) have in common with the RUCAM-type method include the careful exclusion of non-drug causes, consideration of concomitant therapies when assessing drug causes and consideration of time periods between the drug prescription

and the event (e.g. 90 days for cholestatic reactions). There were a number of ways in which the approaches differed, as detailed in Table 7-1.

Table 7-1: Contrasting characteristics of the RUCAM-type algorithm approach and the CPRD algorithm

#	RUCAM	CPRD algorithm*
1	For use in a range of settings, although likely to be difficult to apply in a database setting. Not specifically for epidemiological studies.	Designed specifically for use in electronic health records (CPRD, although could be amended relatively easily for other databases), with a particular focus on epidemiology.
2	Main focus on causality (i.e. how to assess whether a drug caused a particular injury)	Focuses on how to identify an event qualifying as (cholestatic) liver injury in electronic health records, which then allows further investigation into drug causes
3	Includes criteria obtained from (1) monitoring patient after injury event and (2) drug rechallenge	Does not include criteria on monitoring after injury event or drug rechallenge (difficult generally, but particularly in databases)
4	Considers the presence of risk factors as adding to the evidence for likelihood of DILI	Does not consider risk factors as part of causality, but allows the effect of these to be investigated as part of subsequent analysis
5	Blinding to drug exposure status not specified (in fact, prior knowledge of drug hepatotoxicity is a criteria)	Blinding to drug exposure status required during any review step

***CPRD algorithm:** includes the electronic algorithm and the subsequent case review and study analysis performed in this thesis

One reason for these differences relates to the complexity of identifying any outcome within (routinely collected) electronic health records. A second reason is that the CPRD algorithm is designed for epidemiological studies and not for use in clinical settings, where there will certainly be different approaches required. For example, when comparing two drugs in an epidemiological study, blinding to exposure status is very important in order to prevent observer bias. In contrast, blinding to exposure status is unlikely to help a clinician who is trying to establish whether a single patient is suffering from drug induced liver injury caused by a drug they have prescribed in a clinic.

Chapter 2 of this thesis provides an overview of studies that have developed algorithms specifically for use in electronic health record database settings. These were a useful basis for development of the CPRD algorithm, as they provided lists of diagnostic codes used, descriptions of high-level approaches and external standards for biochemical criteria. Areas where it was identified that these algorithms could be improved or enhanced that were subsequently addressed within the CPRD algorithm are as follows:

1. Reduction in reliance on information other than that found in the routinely collected electronic health records for case identification, and assessment of the effect of doing this
2. The use of multiple levels of case status

3. Within case control analysis, ensuring equivalent review procedures of electronic health record data for potential cases and for potential controls
4. The use of information from multiple linked electronic health record databases
5. Specification of a maximum allowable time window between a liver test result and an associated diagnostic code
6. Inclusion of a maximum allowable period between separate liver enzyme tests used when calculating the 'R' value (used for determining type of liver injury)
7. Detailing of codelists of exclusion terms (that could then be provided to journals when submitting for publication)

7.3.2 Comparison of research findings (by drug)

7.3.2.1 Flucloxacillin

The CPRD algorithm estimated an overall risk of cholestatic liver injury associated with flucloxacillin that was comparable to results in the literature. This was despite the fact that (in contrast to all previous studies that had estimated the incidence of flucloxacillin-induced cholestatic liver injury) information external to the database was not used as part of the CPRD algorithm. Slight differences could be explained by chance and/or a lack of blinding to exposure status (whether or not this was done was not specified in previous studies). The median time between first prescription and injury was slightly longer than previously reported.

In previous studies, age (over 55) and duration of use (greater than 14 days) were shown to be important risk factors for flucloxacillin-induced liver injury. The results obtained here confirmed a substantial increased risk for those over the age of 50, and in those receiving more than 2 prescriptions, but also suggested a particular susceptibility in the over 70 year aged old group. Absolute effect estimates for flucloxacillin-induced liver injury stratified by these important characteristics were also presented for the first time.

7.3.2.2 Risperidone

This was the first large epidemiological study to assess whether risperidone is associated with liver injury. Typical antipsychotics such as chlorpromazine are established causes of liver injury, and the study performed here suggests that risperidone (one of the newer atypical antipsychotics generally assumed to have an improved side-effect profile) may be associated with the injury at a similar frequency.

7.3.2.3 Celecoxib

Although a number of epidemiological studies have been published looking at the hepatotoxicity of conventional NSAIDs (such as ibuprofen, diclofenac and mefenamic acid), there is a lack of data on COX-2 inhibitors such as celecoxib. The absolute risk estimated in this study was low (and was also lower than in another published study of the drug), but higher than studies of the older NSAIDs.

7.3.2.4 Ramipril

Recent case studies had suggested Ramipril might be associated with liver injury. The analyses performed here did not detect that it was associated with liver injury, but highlighted difficulties relating to confounding by indication that should be considered in any future studies.

7.3.2.5 Carbamazepine and duloxetine

This study did not detect that carbamazepine or duloxetine were associated with cholestatic liver injury, which contrasts with case reports in the literature. It should be noted that the analyses were underpowered, however, and a large association was not ruled out by the upper 95% confidence interval boundary.

7.4 Strengths

The strengths of the approaches used in this thesis were as follows:

1. The preparation of a systematic literature review in order to inform the design of the case-identification algorithm
2. The use of clearly defined existing biochemical standards for (cholestatic) liver injury as part of the case definition algorithms
3. The novel use of multiple linked data sources for development of a case identification algorithm
4. The clear description of all diagnostic codes, exclusions, and time windows applied in the development of the algorithm
5. Development of an algorithm that could be implemented into a program to allow “real-time” detection of cholestatic liver injury within a single database
6. Use of multiple versions of the algorithm (varying specificity and sensitivity) to allow the effect of clinical uncertainty and/or the impact of data availability to be presented
7. The use of this algorithm in conjunction with detailed medical review of electronic records where appropriate for risk estimation (but not for case control

analysis to minimise the introduction of bias due to different handling of potential case and potential control populations)

8. Testing of the algorithm on a known cause of cholestatic liver injury

7.5 Limitations and suggested further work

7.5.1 Challenges in using routinely-collected electronic health data

There are likely to be fundamental limitations of using routinely collected electronic data alone for the identification of drug-induced liver injury. For example, there may always be some degree of misclassification with this approach compared to the "ideal" of having all available information, copies of letters/free text, and the ability to query treating clinicians. Although this is an acknowledged limitation of this approach, the fact that sensible and comparable results have been obtained throughout this thesis without the use of such information suggests that this misclassification is unlikely to have as much impact as people may often intuitively think that it will.

7.5.2 Detection of hepatocellular injury

In this thesis, the primary outcome of interest was a cholestatic liver injury, which represents only a subtype of all DILI. A driver for this was the development of an algorithm that could be used to further progress genetic work already performed on flucloxacillin-induced liver injury (which is primarily cholestatic in nature) [58]. Although cholestatic liver injury may represent a significant proportion of all DILI, hepatocellular liver injury can be at least if not more serious for the patient – for example, if a patient presents with hepatocellular injury and jaundice, case fatality may be between 10 and 50% [46, 137].

Updating the algorithm to cover all DILI subtypes would be relatively straightforward. For example, the liver test result parameters could be updated to include the criteria for hepatocellular injury (i.e. $R^* \geq 5$, see Chapter 1 Table 1-2). Minor changes to the diagnostic lists used based upon review of the literature could be made, and the algorithm could then be utilised for pharmacoepidemiological studies of DILI as an outcome. It would remain straightforward to analyse results for each subtype separately, however. This could be particularly important for any genetic studies, where it is possible that the hepatocellular and cholestatic injury subtypes have a distinct genetic basis. Having said this, the fact that the biochemical pattern of a single episode of a drug-induced liver injury can change from one subtype to another over time may suggest a common genetic aetiology [46], and genetic work to date has focused more on DILI caused by specific drugs as an outcome, rather than on the injury subtype [57, 138]). It would be a worthwhile exercise to repeat the analyses performed in this thesis

with the algorithm updated in this way, as it is possible that some of the drugs studied may be associated with hepatocellular injury (as well as or even instead of cholestatic injury).

7.5.3 Further characterisation of injury

While working on the flucloxacillin analysis, there were many people found in the database who had liver function tests that met the criteria for drug-induced liver injury, but did not have symptoms such as jaundice recorded in the database. These people did not meet any of the case definitions used in the analyses, but amending the algorithm to include them could help improve understanding of the genetic and cellular processes underlying DILI. Furthermore, this amendment could allow updated absolute effects to be estimated that include those with less severe liver injury (i.e. possibly including a group not presenting with jaundice, although the fact that people may have had jaundice without it being recorded in the database would also need to be considered). An additional (related) change would be to amend the algorithm so that it was able to categorise cases into different severity groups, for example based upon how far their liver test values deviated from normal and/or the presence or absence of specific symptoms. This could also help identify any specific groups of cases with different underlying genetic, clinical or environmental characteristics.

Finally, the algorithm could be amended to make it possible to detect if the cases of liver injury identified displayed any clinical or biochemical precursors to their injury. For example, did the majority of people have serious liver injury without any signs of illness prior to the injury, or were there a common set of clinical and biochemical features present in the database that preceded the case date (such as a rash, or perhaps raised liver test values prior to the value that met the DILI standard threshold).

7.5.4 Use of a probabilistic method for case identification

A potential weakness of the case definition approach used is the requirement to select a cut-off for each case definition above which a person is considered a case and below which they are considered a non-case. Although the analysis in this thesis did allow the use of a cut-off “score” system for defining injury, and multiple score cut-offs were used to try and allow the impact of different decisions around case definition to be assessed, using such cut-offs is still applying a binary case definition (i.e. a person is either a case or a non-case). With such an approach, it could be said that there is some “wasted” information, i.e. the presence or absence of other characteristics that could be contributing to the overall probability of a person being a case is not considered. For example, with a highly specific definition that requires a diagnosis of jaundice and a cholestatic liver test result, an individual may or may not have other (perhaps less

critical although still contributory) symptoms whose presence could contribute to the overall probability that the person has cholestatic liver injury (such as a rash, or general malaise). With a binary classification, these characteristics are basically ignored. One option could be to include them as part of the case definition, although this may well result in far too specific a definition if only a proportion of all cases present with these additional symptoms.

In order to try and handle this issue, it might be possible to adopt a probabilistic system of case identification for analysis, where instead of either being a case or not, all cohort members have a *probability* of being a case. Analyses of association could then be carried out by performing a regression model directly on that probability. One key challenge with this method would be how to obtain the probabilities of being a true case (when there is no gold standard to compare against). Latent class analysis is one method which could enable probabilities to be obtained based upon observed indicator variables [139, 140]. In order for this approach to work, indicator variables for membership of each latent class would need to be defined. The indicators identified as predictors for multisource algorithm case status from chapter 4 could be used as predictors of latent class membership, which would allow conditional properties of being in each latent class to be assigned. Application of such an approach would be difficult to apply to a real-time case identification model, but it could be of interest to apply to a pharmacoepidemiology study and compare with the methods developed within this thesis.

7.5.5 Facilitation of collaboration with other groups

When a researcher develops an algorithm for case identification within databases of electronic health records as part of a study for publication, the reproducibility of that algorithm is facilitated by clearly defining the methodology used for case identification (as has been highlighted throughout this thesis). Including the codelists themselves in published articles is particularly helpful, as is inclusion of any search terms used to generate the codelists. Making these codelists and/or search terms available for download from dedicated repositories means that sharing with other research teams is even simpler. ClinicalCode.org is an example of one such repository [141]. Although codelists were not uploaded during the preparation of this thesis, all codelists will be uploaded to this site as part of the preparation of any scientific publications. To aid in the understanding of how the codelists were generated, the search terms used to generate the terms and codes (where appropriate) will also be uploaded.

7.6 Context and future applications

7.6.1 Real-time detection of liver injury for newly licensed drugs

As discussed in Chapter 1, prior to launch of a new medicine, the relatively low numbers of people that a new medicine is tested on and the strict eligibility criteria of Phase III clinical trials means that it is difficult to detect (1) rare events (2) events that might occur within specific sub groups or (3) events that might occur when a drug is used differently than it was by trial participants (for a longer duration, for example). Spontaneous reporting systems for pharmacovigilance were also introduced in Chapter 1. Such systems (e.g. the UK's yellow card system) are very prone to inaccuracies and bias.

Large stored databases (such as the CPRD) provide an alternative setting for active surveillance of serious adverse drug reactions associated with newly marketed drugs. In contrast to a spontaneous reporting system, such databases provide information on the number of people exposed to the drug, and not just those who have been exposed and might have experienced a reaction (i.e. valid denominator information is provided). Furthermore, data that is entered into these databases is standardised, and careful development of algorithms such as the ones developed in this thesis allows standardised approaches for case definition to be applied. Finally, susceptibility to reporting biases are likely to be somewhat reduced, as longitudinal data captured routinely during (e.g.) primary and secondary care for an individual can allow a much more reliable analysis of whether the drug actually caused the injury to be applied (particularly if including clinician review of records).

Active surveillance of databases such as the UK CPRD could play an important role in a move away from all or nothing safety decisions and towards a phased regulatory approval approach (as per an adaptive drug licensing system [38]). Here, an initial restricted licence could be based upon results from a trial powered for efficacy, but then broadening of the licence could be based upon subsequent studies performed in real-world settings (for example, pragmatic trials using databases of electronic health records). Continued updating of the licence based upon surveillance of large databases would then take place throughout the product's lifecycle. In order for this approach to work, it is likely to require collaboration between pharmaceutical company, regulatory agency and possibly an external group who manage the detection algorithm and analysis of results. A suggested process is detailed in Table 7-2.

Table 7-2: High level outline of process and responsible parties for safety surveillance within an adaptive licensing approach

#	Step	Responsible
1	Identify potential safety issues pre-marketing (based on results of animal studies, human safety studies, biomarker studies or knowledge of similar drugs)	Pharmaceutical company, regulatory agency
2	Develop algorithm for identifying the likely or possible event in association with the newly launched drug	Pharmaceutical company working with external partner
3	Launch drug	Pharmaceutical company
4	Run algorithm on monthly basis	External partner
5	Analyse results	Pharmaceutical company with external partner
6	Update label (restrict or expand as appropriate)	Pharmaceutical company, regulatory agency

7.6.2 Application to genetic and genomic studies

As discussed in Chapter 1, a genetic susceptibility to Type B reactions is becoming well established. For DILI, for example, there are a number of studies of genetic associations, including identifying involvement of the HLA-B*5701 genotype in liver injury associated with flucloxacillin [58] . Despite this strong association, translation to clinical practice would not yet be feasible, as >13 000 individuals would need to be screened to prevent a single case [59]. The algorithms developed here could be used to recruit cases on an ongoing basis from the CPRD, in order to increase sample sizes, which could help obtain cohorts big enough for more in-depth genetic analysis. An alternative (and complimentary) approach would be to utilise newly developed methods for investigating cellular processes (functional genomics), which could likely provide insights into the mechanism and underlying processes of DILI. Cell lines could be developed from gene samples obtained from cases of DILI, and a detailed analysis of the underlying cellular mechanism performed [142, 143]. The algorithm developed within this thesis would allow for recruitment of both case and control patients for this research, and these patients would have detailed historical medical records that could be linked to their genetic and genomic profiles.

Bringing together large databases of electronic health records and genetic/genomic research in this way does not necessarily have to be limited to DILI, or adverse drug reactions, or even rare outcomes. Setting up pilot studies and testing the methodology for more common outcomes, and/or for outcomes that are considered to be hard to identify could represent very important areas of future work. Success in these pilots

could allow the potential of using EHR databases in this way to be realised and in doing so facilitate real progress in the understanding of disease treatment, prevention and aetiology.

7.6.3 Conclusion

Although large databases of stored electronic health records have been used as a setting for epidemiological and pharmacoepidemiological studies for over 20 years, there remain challenges for methods of case identification within such studies. These challenges intensify if we start trying to maximise the potential of such databases for drug safety and genetic epidemiology.

Using the cholestatic type of serious drug-induced liver injury as an example, the work in this thesis first of all focused on understanding case-identification approaches that had been applied in existing epidemiological studies of this reaction. This knowledge was then used to help develop an algorithm that could be applied to the UK CPRD database, validated against an algorithm that used CPRD data but also data from UK HES and ONS data. Testing of the algorithm on a known cause of the injury (flucloxacillin) demonstrated that it was able to obtain similar absolute and relative effect estimates to previous studies. When subsequently used to examine a number of possible drug-causes of the injury, associations with risperidone and with celecoxib were identified. Key features of the new algorithm included the possibility of applying different levels of specificity, and the ability to detect cases without the need for information external to the database. Both these characteristics could facilitate innovative uses of large databases including real-time detection of injury events for pharmacovigilance (and adaptive licensing) and rolling recruitment to genetic association studies.

7.7 Chapter 7 Summary

- This chapter provided an overarching summary of findings, key discussion points, and future directions

- Following a detailed review of the literature, a new algorithm for identifying cholestatic liver injury in the UK CPRD was developed, validated using data from the UK HES and ONS databases

- The algorithm detected similar absolute and relative effects for flucloxacillin (a known cause of cholestatic liver injury) as previous studies, and also detected associations with risperidone and celecoxib (suspected causes of the injury for which there was a lack of epidemiological data)

- Key characteristics of the new algorithm that could facilitate the use of electronic health records for (1) real-time detection for pharmacovigilance and (2) rolling recruitment to genetic association studies include the ability to (a) apply varying levels of specificity and (b) detect cases without using information external to the electronic health record

- Further development of the algorithm could be to allow detection of hepatocellular (as well as cholestatic) liver injury, and the development of an approach allowing analysis by probability of case status (removing the need for binary case assignment)

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Appendices

Chapter 2 Appendix

Chapter 2 Appendix Table A1: alphabetical list of all database diagnostic codes used to select potential cases in the retrieved studies of cholestatic liver injury performed in databases of stored electronic health records

Database code	Diagnosis
OXMIS	
9669XX	Abnormal drug reactions/effects
L3264AB	Abnormal hepatic function
L3263AB	Abnormal liver enzymes
L3260AB	Abnormal liver function test
5710HA	Alcoholic hepatitis
L4720N	Alkaline phosphatase level
L109H	ALT raised
L1002CR	Aspartate aminotransferase level raised
L110H	AST raised
L1151NA	Bilirubin serum level abnormal
L1151	Bilirubin serum level normal
L3262AB	Biochemical liver dysfunction
574AL	Cholelithiasis
7852JC	Cholestatic jaundice
5719CH	Chronic hepatitis
9779PN	Drug-induced jaundice
7851XX	Enlarged liver
7516JA	Familial intrahepatic cholestasis
070F	Fulminant hepatitis
574XX	Gallbladder disorders
570XX	Hepatitis/liver necrosis
5730D	Hepatocellular damage
57300	Hepatocellular damage
K5091	Hepatostomy
070	Infectious hepatitis
7852XX	Jaundice
7852	Jaundice
K501	Liver biopsy
K501XX	Liver biopsy
L3263H	Liver enzymes raised
L3260	Liver function test
070N	Non-A non-B hepatitis
576A	Obstructive jaundice
575XX	Other gallbladder disorders (cholangitis/cholecystisis)
573XX	Other liver disorders
785CP	Pale stools
ICD-9	
782.4	Jaundice (ICD-9)
570	Necrosis of the liver (ICD-9)
573.3	Unspecified hepatitis (ICD-9)
READ	
J633.00	Hepatitis unspecified (READ)
1675.11	Jaundice - symptom (READ)
R024.00	Jaundice (not of newborn) (READ)
R024111	Jaundice (READ)
J66y600	Obstructive jaundice nos (READ)

*XX = category including a range of diagnoses

Chapter 2 Appendix Table A2: alphabetical list of all exclusions applied during identification of patients with cholestatic liver injury in the sixteen studies performed in databases of stored electronic health records that have cholestatic liver injury as the outcome

Exclusion (as described in article)
Alcoholism
ALT or AST elevations above normal levels during 1 year prior
Cancer of the gallbladder
Cancer of the liver
Cancer of the pancreas
Cholecystitis
Cholelithiasis
Concomitant use of medications associated with hepatotoxicity
Chronic liver disease
Crohn's disease
Cirrhosis
Congestive heart failure
Crohn's disease
Gallbladder or pancreatic disease
Hepatitis after blood transfusion
HIV infection
Inflammatory bowel disease
Liver disease
Liver-related diagnosis
Malignant neoplasm
Normal liver function test results
Not referred to a specialist/not admitted to hospital
Other co-morbidity associated with liver chemistry elevations
Other liver disorders
Other well-defined pathology or disease
Pancreatic disease
Rheumatoid arthritis
Sarcoidosis
Systemic lupus
Ulcerative colitis
Viral hepatitis (based on serology)
Viral infection (serologically confirmed)
Well-defined systemic condition affecting the liver

Chapter 3 Appendix

Chapter 3 Appendix Table 1: List of CPRD clinical diagnosis terms and codes used to identify patients with potential cholestatic liver injury, with strength of evidence for cholestatic liver injury groupings indicated

Term	*READ code*	*Strength of evidence marker*[1]
toxic liver disease with cholestasis	j635000	Group 1
[d]jaundice (not of newborn)	r024.00	Group 2
hepatitis unspecified	j633.00	Group 2
[d]jaundice	r024111	Group 2
obstructive jaundice nos	j66y600	Group 2
hepatitis unspecified nos	j633z00	Group 2
o/e – jaundiced	2274.11	Group 2
jaundice – symptom	1675.11	Group 2
[d]icterus nos	r024100	Group 2
yellow/jaundiced colour	1675.00	Group 2
o/e - jaundiced colour	2274.00	Group 2
[d]jaundice (not of newborn) nos	r024z00	Group 2
infective hepatitis	a701.11	Group 3
other liver disorders	j63..00	Group 3
chronic hepatitis	j614.00	Group 3
chronic aggressive hepatitis	j614200	Group 3
acute alcoholic hepatitis	j611.00	Group 3
other specified liver disorder nos	j63yz00	Group 3
[d]cholaemia nos	r024000	Group 3
acute hepatic failure	j600000	Group 3
o/e - liver grossly enlarged	25g4.00	Group 3
alcoholic hepatitis	j617.00	Group 3
open wedge biopsy of lesion of liver	7804200	Group 3
Cholangitis	j661.00	Group 3
biopsy of liver nec	780b000	Group 3
liver disorder nos	j63z.00	Group 3
primary sclerosing cholangitis	j661700	Group 3
chronic hepatitis nos	j614z00	Group 3
toxic hepatitis	j633000	Group 3
recurrent cholangitis	j661200	Group 3
acute hepatitis – noninfective	j600100	Group 3
o/e -liver moderately enlarged	25g3.00	Group 3
hepatic failure	j62y.13	Group 3
needle biopsy of liver nec	780a112	Group 3
toxic liver disease with chronic persistent hepatitis	j635300	Group 3
alcoholic hepatic failure	j613000	Group 3
hepatitis non a non b	a705400	Group 3
fh: hepatitis	12e3.11	Group 3
subacute hepatitis – noninfective	j601100	Group 3
encephalopathy – hepatic	j622.11	Group 3
hepatic coma	j622.00	Group 3
chronic persistent hepatitis	j614000	Group 3
calculus of bile duct with cholangitis	j646.00	Group 3
[x] hepatic failure	j625.00	Group 3
liver abscess due to cholangitis	j620100	Group 3
other specified liver disorder	j63y.00	Group 3
subacute hepatic failure	j601000	Group 3

Term	*READ code*	*Strength of evidence marker*[1]
nonspecific reactive hepatitis	j63y100	Group 3
other cholangitis	j661y00	Group 3
other non-alcoholic chronic liver disease nos	j61yz00	Group 3
ascending cholangitis	j661400	Group 3
percutaneous transvascular biopsy of lesion of liver	780a000	Group 3
toxic liver disease with hepatic necrosis	j635100	Group 3
hepatic failure as a complication of care	sp14200	Group 3
toxic liver disease	j635.00	Group 3
toxic liver disease with chronic active hepatitis	j635500	Group 3
toxic liver disease with acute hepatitis	j635200	Group 3
acute hepatic failure due to drugs	j635700	Group 3
acute necrosis of liver	j600.00	Group 3
toxic liver disease, unspecified	j635x00	Group 3
other non-alcoholic chronic liver disease	j61y.00	Group 3
toxic liver disease with fibrosis and cirrhosis of liver	j635600	Group 3
sclerosing cholangitis unspecified	j661900	Group 3
other sequelae of chronic liver disease	j62y.00	Group 3
acute and subacute liver necrosis	j60..00	Group 3
chronic cholangitis	j661100	Group 3
recurrent hepatitis	j614300	Group 3
acute yellow atrophy	j600200	Group 3
chronic hepatitis unspecified	j614y00	Group 3
acute necrosis of liver nos	j600z00	Group 3
hepatic failure nos	j62y.11	Group 3
subacute necrosis of liver	j601.00	Group 3
cholangitis nos	j661z00	Group 3
central haemorrhagic necrosis of liver	j636.00	Group 3
toxic liver disease with chronic lobular hepatitis	j635400	Group 3
menghini needle biopsy of liver	780a111	Group 3
acute and subacute liver necrosis nos	j60z.00	Group 3
toxoplasma hepatitis	ad05.00	Group 3
cholangitis lenta	j661500	Group 3
chronic lobular hepatitis	j614400	Group 3
subacute yellow atrophy	j601200	Group 3
subacute necrosis of liver nos	j601z00	Group 3
endoscopic ultrasound examination liver biopsy lesion liver	780f000	Group 3
obliterative cholangitis	j661600	Group 3
sheeba needle biopsy of liver	780a113	Group 3

Note 1: Group 1=strongest evidence for cholestatic liver injury, Group 2=weaker evidence for cholestatic liver injury, Group 3=weakest evidence for cholestatic liver injury

Chapter 3 Appendix Table 2: List of HES clinical diagnosis terms and codes used to identify individuals with potential cholestatic liver injury, with strength of evidence for cholestatic liver injury groupings indicated

Term	*ICD code*	*Strength of evidence marker*[1]
toxic liver disease with cholestasis	K71.0	Group 1
unspecified jaundice	R17	Group 2

Note 1: Group 1=strongest evidence for cholestatic liver injury, Group 2=weaker evidence for cholestatic liver injury

Chapter 3 Appendix Table 3: List of HES liver related procedure terms and codes used to identify hospital procedures likely to elucidate the type of liver injury

Term	*OPCS code*
laparoscopic ultrasound examination of liver nec	J093
other specified diagnostic endoscopic examination of liver using laparoscope	J098
unspecified diagnostic endoscopic examination of liver using laparoscope	J099
biopsy of liver nec	J141
other specified endoscopic ultrasound examination of liver	J178
unspecified endoscopic ultrasound examination of liver	J179
computed tomography of abdomen nec	U081
ultrasound of abdomen	U082
magnetic resonance imaging of abdomen	U085
other specified diagnostic imaging of abdomen	U088
unspecified diagnostic imaging of abdomen	U089

Chapter 3 Appendix Table 4: List of CPRD potential explanatory variables created for the CPRD cholestatic liver injury algorithm

CPRD characteristic type	Potential explanatory variables (all binary)
Liver test	Had cholestatic CPRD liver test result
Specialist referral	Had any referrals <30 days from index date
	Had liver-related referral <30 days from index
	Top liver referral code (strength of evidence for cholestatic liver injury of referral code: Group 1 or Group 2=1, Group 3 or no referral=0))
	Referred for liver scan <30 days from index
Index diagnosis	Toxic liver disease with cholestasis (single code)
	Obstructive jaundice (single code)
	Jaundice or similar terms
	Hepatitis or similar terms
	Chronic hepatitis or similar terms
	Hepatic failure related
	Liver-enlargement related
	Cholangitis related
	Liver biopsy related
	Alcohol-related
	Liver-necrosis related
	Toxic liver disease (other than cholestasis)
	Cholaemia
	Other or non-specific code
Other liver-related diagnoses	Had additional liver-related diagnoses on index date
	Had additional liver-related diagnoses <30 days from index
	Evidence grouping for additional liver-related diagnosis <30 days from index (strength of evidence for cholestatic liver injury of additional liver-related diagnosis: Group 1 or Group 2=1, Group 3 or no additional diagnosis=0)

Chapter 3 Appendix Table 5: List of exclusion terms applied in epidemiological studies

Term	*READ code*
[v]contact with and exposure to viral hepatitis	zv01b00
[v]personal history of alcoholism	zv11300
[v]personal history of malignant neoplasm of liver	zv10015
[v]screening for alcoholism	zv79100
[v]screening for rheumatoid arthritis	zv7y100
[v]viral hepatitis carrier	zv02600
[x]acute alcoholic drunkenness	eu10011
[x]alcoholic dementia nos	eu10711
[x]alcoholic hallucinosis	eu10511
[x]alcoholic jealousy	eu10512
[x]alcoholic paranoia	eu10513
[x]alcoholic psychosis nos	eu10514
[x]chronic alcoholic brain syndrome	eu10712
[x]chronic alcoholism	eu10212
[x]dementia in human immunodef virus [hiv] disease	eu02400
[x]hiv disease result/haematological+immunologic abnorms,nec	ayucb00
[x]hiv disease resulting in multiple infections	ayuc300
[x]hiv disease resulting in other non-hodgkin's lymphoma	ayuc600
[x]hiv disease resulting in other specified conditions	ayucc00
[x]hiv disease resulting/other infectious+parasitic diseases	ayuc400
[x]other and unspecified cirrhosis of liver	jyu7100
[x]other cholecystitis	jyu8100
[x]other cholelithiasis	jyu8000
[x]other crohn's disease	jyu4000
[x]other forms of systemic lupus erythematosus	nyu4300
[x]other seropositive rheumatoid arthritis	nyu1100
[x]other specified acute viral hepatitis	ayub000
[x]other specified rheumatoid arthritis	nyu1200
[x]other ulcerative colitis	jyu4100
[x]rheumatoid arthritis+involvement/other organs or systems	nyu1000
[x]sarcoidosis of other and combined sites	cyu0600
[x]sequelae of viral hepatitis	ayuj900
[x]seropositive rheumatoid arthritis, unspecified	nyu1g00
[x]unspecified human immunodeficiency virus [hiv] disease	ayucd00
[x]viral hepatitis	ayub.00
accidental poisoning by alcoholic beverages	t900.00
acute alcoholic hepatitis	j611.00
acute alcoholic intoxication in alcoholism	e230.00
acute alcoholic intoxication in alcoholism nos	e230z00
acute alcoholic intoxication in remission, in alcoholism	e230300
acute alcoholic intoxication, unspecified, in alcoholism	e230000
acute angiocholecystitis	j650100
acute cholecystitis	j650.00
acute cholecystitis nos	j650z00
acute cholecystitis unspecified	j650000
acute congestive heart failure	g580000

Term	*READ code*
acute emphysematous cholecystitis	j650200
acute gangrenous cholecystitis	j650400
acute polyarticular juvenile rheumatoid arthritis	n043100
acute suppurative cholecystitis	j650300
acute viral hepatitis nos	a70z100
alcohol dependence with acute alcoholic intoxication	e230.11
alcoholic cardiomyopathy	g555.00
alcoholic cirrhosis of liver	j612.00
alcoholic dementia nos	e012.11
alcoholic encephalopathy	f11x011
alcoholic fatty liver	j610.00
alcoholic fibrosis and sclerosis of liver	j612000
alcoholic gastritis	j153.00
alcoholic hepatic failure	j613000
alcoholic hepatitis	j617.00
alcoholic liver damage unspecified	j613.00
alcoholic myopathy	f394100
alcoholic paranoia	e015.00
alcoholic polyneuropathy	f375.00
alcoholic psychoses	e01..00
alcoholic psychosis nos	e01z.00
alcoholics anonymous	13y8.00
Alcoholism	e23..11
alcoholism counselling	z4b1.00
arthropathy in crohn's disease	n031100
arthropathy in ulcerative colitis	n031000
aversion therapy - alcoholism	8g32.00
bacterial portal cirrhosis	j615d00
benign neoplasm of gallbladder	b715200
benign neoplasm of liver	b715000
benign neoplasm of liver and biliary ducts	b715.00
bile duct calculus + acute cholecystitis - obstruct nos	j643z00
bile duct calculus + acute cholecystitis and no obstruction	j643000
bile duct calculus + acute cholecystitis and obstruction	j643100
bile duct calculus + other cholecystitis - obstruction nos	j644z00
bile duct calculus + other cholecystitis and obstruction	j644100
bile duct calculus with acute cholecystitis	j643.00
bile duct calculus with other cholecystitis	j644.00
bile duct calculus without cholecystitis nos	j645z00
bile duct calculus without cholecystitis with obstruction	j645100
bile duct calculus without cholecystitis, no obstruction	j645000
bile duct calculus without mention of cholecystitis	j645.00
biliary cirrhosis	j616.00
biliary cirrhosis nos	j616z00
biliary cirrhosis of children	j616200
bmast - brief michigan alcoholism screening test	zra1111
brief michigan alcoholism screening test	zra1100
capsular portal cirrhosis	j615600
carcinoma gallbladder	b160.11

Term	*READ code*
carcinoma in situ of liver	b808000
carcinoma in situ of liver and biliary system	b808.00
carcinoma in situ of liver or biliary system nos	b808z00
carcinoma in situ of pancreas	b80z000
cardiac portal cirrhosis	j615700
cdai - crohn's disease activity index	zr3s.11
cerebellar ataxia due to alcoholism	f144000
cerebral degeneration due to alcoholism	f11x000
cholecystitis nos	j651z00
cholelithiasis	j64..00
cholelithiasis nos	j64z.00
cholelithiasis nos	j64zz00
cholelithiasis with obstruction nos	j64z100
cholelithiasis without obstruction nos	j64z000
chronic alcoholic brain syndrome	e012000
chronic alcoholic hepatitis	j617000
chronic alcoholism	e231.00
chronic alcoholism in remission	e231300
chronic alcoholism nos	e231z00
chronic cholecystitis	j651000
chronic congestive heart failure	g580100
chronic liver disease nos	j61z.00
chronic viral hepatitis	a707.00
chronic viral hepatitis b with delta-agent	a707000
chronic viral hepatitis b without delta-agent	a707100
chronic viral hepatitis c	a707200
chronic viral hepatitis, unspecified	a707x00
cirrhosis - non alcoholic	j615.00
cirrhosis and chronic liver disease	j61..00
cirrhosis of liver nos	j615z13
congenital viral hepatitis	q409.00
congestive heart failure	g580.00
congestive heart failure due to valvular disease	g580400
congestive heart failure monitoring	662t.00
continuous acute alcoholic intoxication in alcoholism	e230100
continuous chronic alcoholism	e231100
crohn's disease	j40..11
crohn's disease activity index	zr3s.00
crohn's disease nos	j40z.11
crohn's disease of the ileum nos	j400400
crohn's disease of the ileum unspecified	j400300
crohn's disease of the large bowel nos	j401z00
crohn's disease of the small bowel nos	j400z00
crohn's disease of the terminal ileum	j400200
cryptogenic cirrhosis of liver	j615z12
cystic fibrosis related cirrhosis	c370800
cytomegaloviral hepatitis	a785200
delivery of rehabilitation for rheumatoid arthritis	7p20300
diffuse nodular cirrhosis	j615300
disease activity score 28 joint in rheumatoid arthritis	38dz000
disease activity score in rheumatoid arthritis	38dz.00
drug-induced systemic lupus erythematosus	n000200
episodic acute alcoholic intoxication in alcoholism	e230200
episodic chronic alcoholism	e231200
exacerbation of crohn's disease of large	j401200

Term	*READ code*
intestine	
exacerbation of crohn's disease of small intestine	j400500
exacerbation of ulcerative colitis	j410400
except rheumatoid arthritis qual indicator: informed dissent	9hr1.00
except rheumatoid arthritis quality indicator: pt unsuitable	9hr0.00
exception reporting: rheumatoid arthritis quality indicators	9hr..00
fatty portal cirrhosis	j615400
fh: alcoholism	1282.00
fh: crohn's disease	12e5.00
fh: gallbladder disease	12e4.11
fh: rheumatoid arthritis	12i1.00
fh: ulcerative colitis	12e2.11
fibrosing alveolitis associated with rheumatoid arthritis	n04y012
flare of rheumatoid arthritis	n040t00
florid cirrhosis	j612.11
gallbladder calculus with acute cholecystitis	j640.00
gallbladder calculus with acute cholecystitis - obst nos	j640z00
gallbladder calculus with acute cholecystitis + obstruction	j640100
gallbladder calculus with acute cholecystitis +no obstruct	j640000
gallbladder calculus with other cholecystitis	j641.00
gallbladder calculus with other cholecystitis - obstruct nos	j641z00
gallbladder calculus with other cholecystitis + obstruct	j641100
gallbladder calculus with other cholecystitis +no obstruct	j641000
gallbladder calculus without cholecystitis and obstruct nos	j642z00
gallbladder calculus without mention cholecystitis + obstruc	j642100
gallbladder calculus without mention cholecystitis +no obstr	j642000
gallbladder calculus without mention of cholecystitis	j642.11
gallbladder calculus without mention of cholecystitis	j642.00
gallstones	j64..15
glycogenosis with hepatic cirrhosis	c310400
h/o: alcoholism	1462.00
h/o: gallbladder disease	14c7.11
h/o: rheumatoid arthritis	14g1.00
h/o: ulcerative colitis	14c4.11
hepatic granulomas in sarcoidosis	j63a.00
hepatoblastoma of liver	b150100
history of viral hepatitis	141f.00
hiv disease complicating pregnancy childbirth puerperium	l179.00
hiv disease result/haematological+immunologic abnorms,nec	a788u00
hiv disease resulting in burkitt's lymphoma	a789600

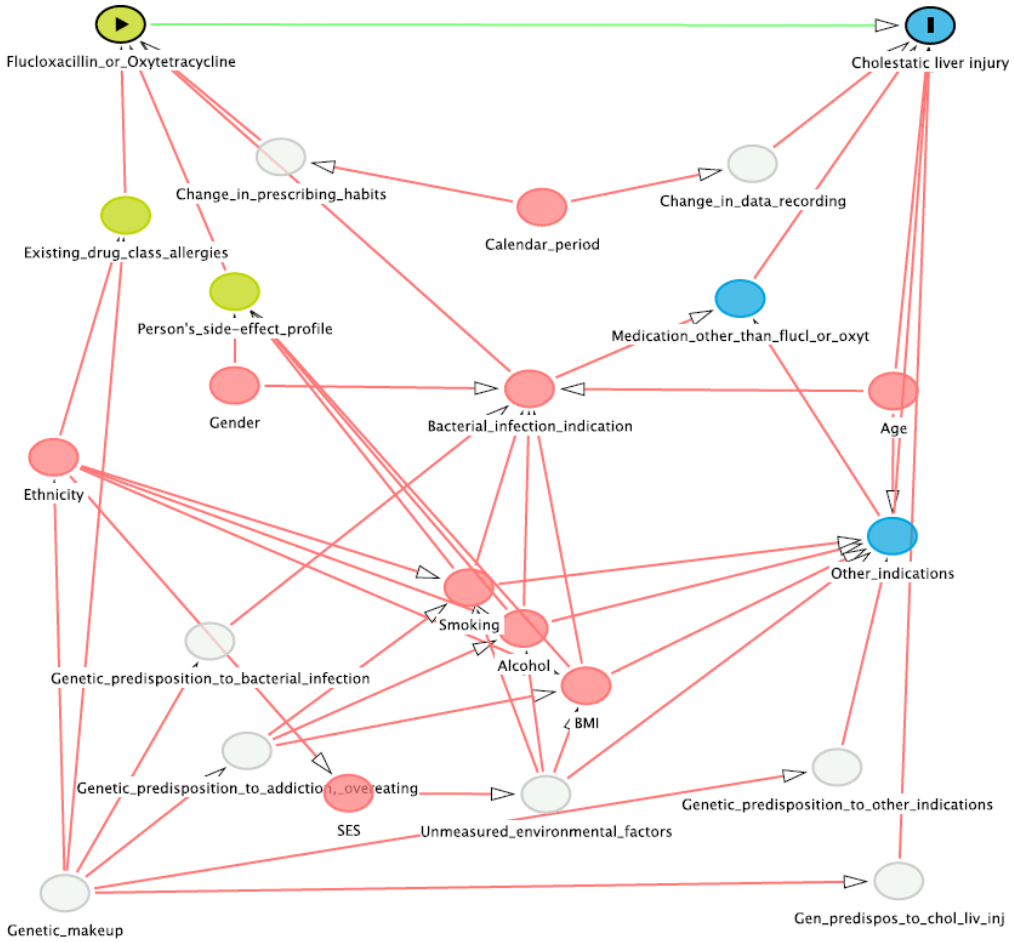
Term	*READ code*
hiv disease resulting in candidiasis	a789200
hiv disease resulting in cytomegaloviral disease	a789100
hiv disease resulting in kaposi's sarcoma	a789500
hiv disease resulting in lymphoid interstitial pneumonitis	a789900
hiv disease resulting in multiple infections	a789400
hiv disease resulting in multiple malignant neoplasms	a789800
hiv disease resulting in mycobacterial infection	a789000
hiv disease resulting in pneumocystis carinii pneumonia	a789300
hiv disease resulting in pneumocystis jirovecii pneumonia	a789311
hiv disease resulting in unspecified malignant neoplasm	a788w00
hiv disease resulting in wasting syndrome	a789a00
hiv disease resulting/unspcf infectious+parasitic disease	a788x00
hiv infection with persistent generalised lymphadenopathy	a788200
hiv positive	43c3.11
husband alcoholic	13l3.13
hypertrophic portal cirrhosis	j615500
indian childhood cirrhosis	j615812
infectious cirrhosis nos	j615h00
inflammatory bowel disease	j4...12
juvenile arthritis in crohn's disease	n045300
juvenile arthritis in ulcerative colitis	n045400
juvenile portal cirrhosis	j615800
juvenile rheumatoid arthritis	n045500
juvenile rheumatoid arthritis - still's disease	n043.00
juvenile rheumatoid arthritis nos	n043z00
korsakoff's non-alcoholic psychosis	e040.11
korsakov's alcoholic psychosis	e011000
korsakov's alcoholic psychosis with peripheral neuritis	e011100
laennec's cirrhosis	j612.12
liver abscess and sequelae of chronic liver disease	j62..00
liver metastases	b577.11
lung disease with systemic lupus erythematosus	h57y400
macronodular cirrhosis of liver	j615z11
malignant neoplasm gallbladder and extrahepatic bile ducts	b16..00
malignant neoplasm gallbladder/extrahepatic bile ducts nos	b16z.00
malignant neoplasm of body of pancreas	b171.00
malignant neoplasm of gallbladder	b160.00
malignant neoplasm of head of pancreas	b170.00
malignant neoplasm of liver and intrahepatic bile ducts	b15..00
malignant neoplasm of liver and intrahepatic bile ducts nos	b15z.00
malignant neoplasm of liver unspecified	b152.00
malignant neoplasm of other specified sites of pancreas	b17y.00
malignant neoplasm of pancreas	b17..00
malignant neoplasm of pancreas nos	b17z.00

Term	*READ code*
malignant neoplasm of specified site of pancreas nos	b17yz00
malignant neoplasm of tail of pancreas	b172.00
malignant neoplasm other gallbladder/extrahepatic bile duct	b16y.00
mast - michigan alcoholism screening test	zra1.11
meningitis due to sarcoidosis	f013.00
michigan alcoholism screening test	zra1.00
monarticular juvenile rheumatoid arthritis	n043300
multilobular portal cirrhosis	j615100
multiple cranial nerve palsies in sarcoidosis	f326300
munich alcoholism test	zrau.00
myopathy due to rheumatoid arthritis	f396400
myopathy due to sarcoidosis	f396500
myositis in sarcoidosis	n233200
neoplasm of uncertain behaviour of liver	b903000
neoplasm of uncertain behaviour of liver and biliary passage	b903.00
nephrotic syndrome in systemic lupus erythematosus	k01x400
non-alcoholic cirrhosis nos	j615z00
non-alcoholic fatty liver	j61y100
o/e - alcoholic breath	2577.11
oesophageal varices in alcoholic cirrhosis of the liver	g852300
oesophageal varices in cirrhosis of the liver	g852200
orofacial crohn's disease	j08z900
other alcoholic dementia	e012.00
other alcoholic psychosis	e01y.00
other alcoholic psychosis nos	e01yz00
other cholecystitis	j651.00
other cholecystitis os	j651y00
other non-alcoholic chronic liver disease	j61y.00
other non-alcoholic chronic liver disease nos	j61yz00
other rheumatoid arthritis of spine	n040100
other sequelae of chronic liver disease	j62y.00
other specified viral hepatitis with coma	a704.00
other specified viral hepatitis with hepatic coma nos	a704z00
other specified viral hepatitis without coma	a705.00
other specified viral hepatitis without mention of coma nos	a705z00
pauciarticular juvenile rheumatoid arthritis	n043200
pigmentary cirrhosis of liver	c350012
polyneuropathy in rheumatoid arthritis	f371200
polyneuropathy in sarcoidosis	f374900
portal cirrhosis	j615.11
portal cirrhosis unspecified	j615y00
portal fibrosis without cirrhosis	j61y300
primary biliary cirrhosis	j616000
primary carcinoma of liver	b150000
primary malignant neoplasm of liver	b150.00
primary malignant neoplasm of liver nos	b150z00
pulmonary sarcoidosis	h57y200
regional enteritis - crohn's disease	j40..00
rheumatoid arthritis	n040.00
rheumatoid arthritis - multiple joint	n040s00
rheumatoid arthritis and other inflammatory	n04..00

Term	*READ code*
polyarthropathy	
rheumatoid arthritis annual review	66hb000
rheumatoid arthritis of 1st mtp joint	n040k00
rheumatoid arthritis of acromioclavicular joint	n040400
rheumatoid arthritis of ankle	n040f00
rheumatoid arthritis of cervical spine	n040000
rheumatoid arthritis of dip joint of finger	n040a00
rheumatoid arthritis of distal radio-ulnar joint	n040600
rheumatoid arthritis of elbow	n040500
rheumatoid arthritis of hip	n040b00
rheumatoid arthritis of knee	n040d00
rheumatoid arthritis of lesser mtp joint	n040l00
rheumatoid arthritis of mcp joint	n040800
rheumatoid arthritis of other tarsal joint	n040j00
rheumatoid arthritis of pip joint of finger	n040900
rheumatoid arthritis of sacro-iliac joint	n040c00
rheumatoid arthritis of shoulder	n040200
rheumatoid arthritis of subtalar joint	n040g00
rheumatoid arthritis of talonavicular joint	n040h00
rheumatoid arthritis of wrist	n040700
rheumatoid arthritis particle agglutination test	43b9.00
rheumatoid arthritis screen	68f1.00
rheumatoid arthritis screening test	43c6.00
sarcoidosis	ad5..00
sarcoidosis of inferior turbinates	ad54.00
sarcoidosis of lung	ad50.00
sarcoidosis of lung with sarcoidosis of lymph nodes	ad52.00
sarcoidosis of lymph nodes	ad51.00
sarcoidosis of skin	ad53.00
secondary biliary cirrhosis	j616100
secondary malignant neoplasm of liver	b577.00
secondary malignant neoplasm of liver	b153.00
sequelae of viral hepatitis	ae23.00
seronegative rheumatoid arthritis	n040p00
seropositive errosive rheumatoid arthritis	n047.00
seropositive rheumatoid arthritis, unspecified	n04x.00
slam - systemic lupus activity measure	zrq8.11
suspected gallstones	1j5..00
systemic lupus activity measure	zrq8.00
systemic lupus erythematosus	n000.00
systemic lupus erythematosus disease activity index	zrq9.00
systemic lupus erythematosus nos	n000z00
systemic lupus erythematosus with organ or sys involv	n000300
systemic lupus erythematosus with pericarditis	n000400
toxic liver disease with fibrosis and cirrhosis of liver	j635600
ulcerative colitis	j410100
ulcerative colitis and/or proctitis	j41..12
unspecified chronic alcoholism	e231000
unspecified viral hepatitis	a70z.00
viral (serum) hepatitis b	a703.00
viral hepatitis	a70..00
viral hepatitis a with coma	a700.00
viral hepatitis b with coma	a702.00
viral hepatitis c with coma	a704000

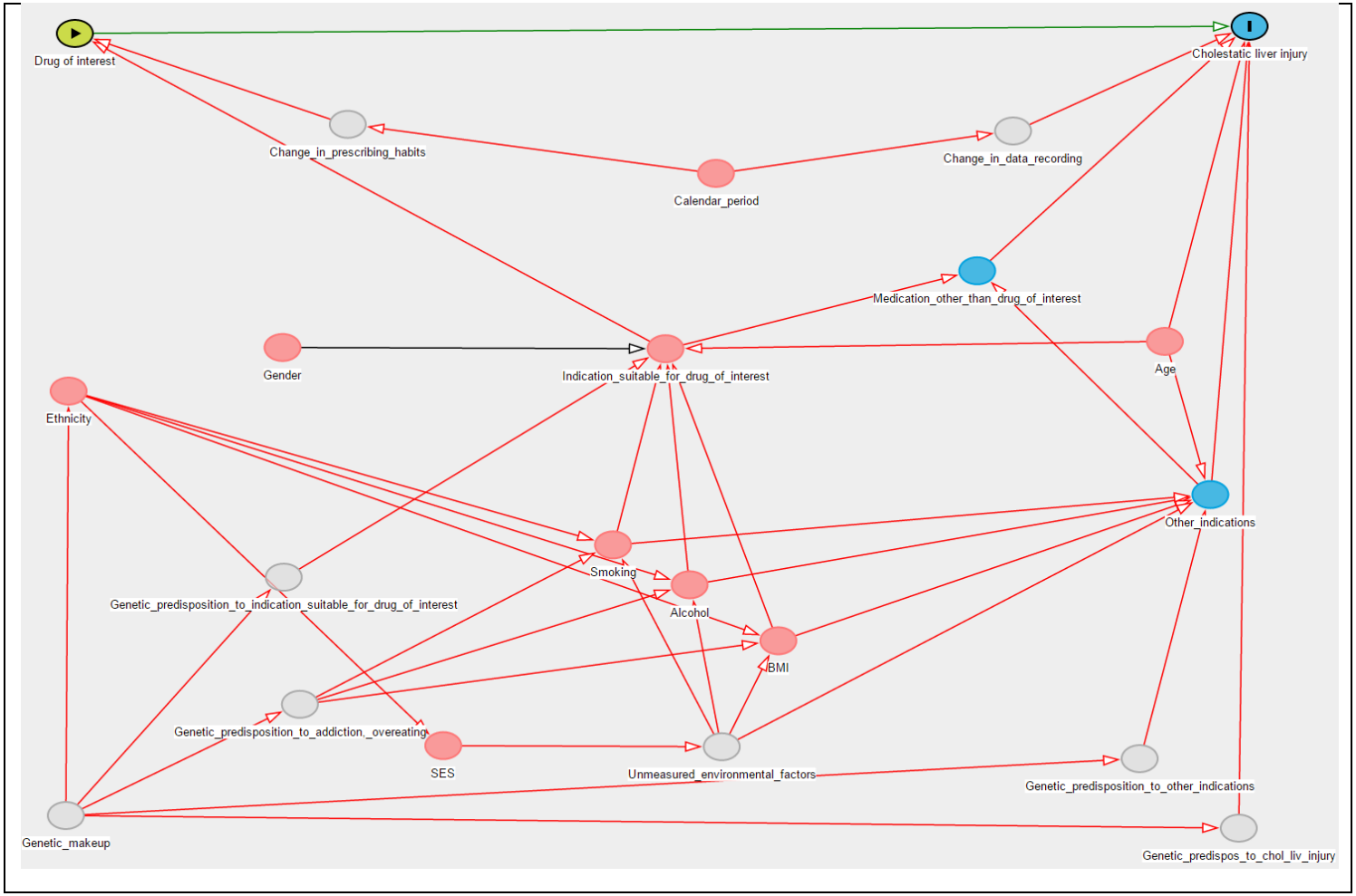
Term	*READ code*
viral hepatitis c without mention of hepatic coma	a705000
viral hepatitis carrier	65q7.00
viral hepatitis comp pregnancy, childbirth & the puerperium	l176500
viral hepatitis screening test	4jrf.00
viral hepatitis without hepatic coma	a709.00
xanthomatous portal cirrhosis	j615c00

Chapter 3 Appendix Table 6a: Causal diagram used to assist in the identification of co-variates likely to confound (or be effect modifiers of) the association between flucloxacillin and cholestatic liver injury



Note re: Ethnicity: Both social aspects of health related to ethnicity and genetic variation which can influence health are considered relevant [144]

Chapter 3 Appendix Table 6b: Causal diagram used to assist in the identification of co-variates likely to confound (or be effect modifiers of) the association between the drug exposures carbamazepine, celecoxib, duloxetine, ramipril or risperidone and cholestatic liver injury



Chapter 3 Appendix Table 7: List of drug substances considered as other potential causes of cholestatic liver injury, with assignment to co-variate category marked (=less frequent cause of cholestastic liver injury vs. more frequent cause of cholestatic liver injury)

Drug substance	*Frequency category* *(category=less frequent unless marked)*
acebutolol hydrochloride/hydrochlorothiazide	
allopurinol	
aluminium hydroxide/bismuth subnitrate/magnesium carbonate/sodium bicarbonate/deglycyrrhizinised liquorice	
amiloride hydrochloride/hydrochlorothiazide	
amiodarone hydrochloride	
amitriptyline hydrochloride	
amitriptyline hydrochloride/perphenazine	
amlodipine	
amlodipine besilate	
amlodipine besilate/hydrochlorothiazide/olmesartan medoxomil	
amlodipine besilate/olmesartan medoxomil	
amlodipine besilate/valsartan	
amoxicillin sodium	
amoxicillin sodium/potassium clavulanate	More frequent
amoxicillin trihydrate	
amoxicillin trihydrate/potassium clavulanate	More frequent
ampicillin	
ampicillin sodium	
ampicillin trihydrate	
aspirin	
aspirin/aloxiprin/caffeine citrate	
aspirin/caffeine	
aspirin/caffeine/citric acid/sodium bicarbonate/paracetamol	
aspirin/codeine phosphate	
aspirin/ethoheptazine citrate/meprobamate	
aspirin/isosorbide mononitrate	
aspirin/papaveretum	
aspirin/paracetamol	
aspirin/paracetamol/caffeine	
atenolol/amiloride hydrochloride/hydrochlorothiazide	
atorvastatin calcium	
atorvastatin calcium trihydrate	
auranofin	
azathioprine	More frequent
bacampicillin	
benzoyl peroxide/clindamycin phosphate	
benzoyl peroxide/erythromycin	
betahistine dihydrochloride	
bezafibrate	
bisoprolol fumarate/hydrochlorothiazide	
bupropion hydrochloride	
captopril	
carbamazepine	More frequent
carmustine	
cefalexin	
cefuroxime	
cefuroxime axetil	

Drug substance	*Frequency category* *(category=less frequent unless marked)*
cefuroxime sodium	
celecoxib	
cetirizine hydrochloride	
chlorambucil	
chlorothiazide	
chlorpromazine embonate	More frequent
chlorpromazine hydrochloride	More frequent
chlortetracycline hydrochloride	
chlortetracycline hydrochloride/tetracycline hydrochloride/demeclocycline hydrochloride	
chlortetracycline hydrochloride/triamcinolone acetonide	
cimetidine	
cinnarizine	
ciprofloxacin	
ciprofloxacin hydrochloride	
ciprofloxacin lactate	
clarithromycin	
clindamycin hydrochloride	
clindamycin phosphate	
clobetasone butyrate/oxytetracycline calcium/nystatin	
clomethiazole	
clomethiazole edisilate	
clomipramine hydrochloride	
clopidogrel	
clopidogrel hydrogen sulphate	
cloxacillin	
clozapine	
codeine phosphate/aspirin	
cyclophosphamide	
cyclophosphamide monohydrate	
cyproheptadine hydrochloride	
danazol	
desogestrel/ethinylestradiol	
dexibuprofen	
dexketoprofen trometamol	
dextromethorphan hydrobromide	
dextromethorphan hydrobromide/ephedrine hydrochloride	
dextromethorphan hydrobromide/menthol	
dextromethorphan hydrobromide/pseudoephedrine hydrochloride	
dextromethorphan hydrobromide/terpin hydrate/menthol/pumilio pine oil/eucalyptus oil	
dextromethorphan hydrobromide/triprolidine hydrochloride	
dextropropoxyphene hydrochloride	
dextropropoxyphene hydrochloride/paracetamol	
dextropropoxyphene napsylate	
diazepam	
diclofenac diethylammonium	
diclofenac epolamine	
diclofenac potassium	
diclofenac sodium	
diclofenac sodium/misoprostol	
diflunisal	

Drug substance	*Frequency category* *(category=less frequent unless marked)*
digoxin	
dimenhydrinate/cinnarizine	
diphenhydramine hydrochloride/dextromethorphan hydrobromide	
diphenhydramine hydrochloride/menthol/dextromethorphan hydrobromide	
dipyridamole/aspirin	
disulfiram	
dosulepin hydrochloride	
doxazosin mesilate	
doxycycline hyclate	
doxycycline monohydrate	
drospirenone/estradiol hemihydrate	
drospirenone/ethinylestradiol	
duloxetine hydrochloride	
dydrogesterone/estradiol	
enalapril maleate	
enalapril maleate/hydrochlorothiazide	
erythromycin	
erythromycin ethyl succinate	
erythromycin lactobionate	
erythromycin stearate	
erythromycin/isotretinoin	
erythromycin/tretinoin	
erythromycin/zinc acetate	
escitalopram oxalate	
esomeprazole magnesium	
esomeprazole magnesium dihydrate	
esomeprazole magnesium trihydrate	
esomeprazole sodium	
estradiol	
estradiol acetate	
estradiol hemihydrate	
estradiol valerate	
estradiol valerate/norethisterone	
estradiol/levonorgestrel	
estradiol/norethisterone acetate	
estrone/estriol/estradiol	
ethinylestradiol	
ethinylestradiol/cyproterone acetate	
ethinylestradiol/etonogestrel	
etodolac	
etoricoxib	
ezetimibe/simvastatin	
fenofibrate	
fenofibrate micronised	
fenoprofen calcium	
flucloxacillin magnesium	
flucloxacillin magnesium/ampicillin trihydrate	
flucloxacillin sodium	
flucloxacillin sodium/ampicillin trihydrate	
fluconazole	
fluphenazine decanoate	
fluphenazine enantate	
fluphenazine hydrochloride	

Drug substance	*Frequency category* *(category=less frequent unless marked)*
fluphenazine hydrochloride/nortriptyline hydrochloride	
flutamide	
fluvastatin sodium	
fosinopril sodium	
fosphenytoin sodium	
gabapentin	
gemcitabine hydrochloride	
gestodene/ethinylestradiol	
glibenclamide	
glimepiride	
griseofulvin	
haloperidol	
haloperidol decanoate	
halothane	
hydrochlorothiazide	
hydrochlorothiazide/amlodipine besilate/olmesartan medoxomil	
hydrochlorothiazide/amlodipine/olmesartan medoxomil	
hydrochlorothiazide/captopril	
hydrochlorothiazide/irbesartan	
hydrochlorothiazide/losartan potassium	
hydrochlorothiazide/metoprolol tartrate	
hydrochlorothiazide/olmesartan medoxomil	
hydrochlorothiazide/quinapril hydrochloride	
hydrochlorothiazide/telmisartan	
hydrochlorothiazide/valsartan	
hydrocortisone/nystatin/oxytetracycline calcium	
ibuprofen	
ibuprofen lysine	
ibuprofen sodium dihydrate	
ibuprofen/codeine phosphate	
ibuprofen/levomenthol	
ibuprofen/paracetamol	
ibuprofen/phenylephrine hydrochloride	
ibuprofen/pseudoephedrine hydrochloride	
imipramine hydrochloride	
infliximab	
interferon beta-1a	
iproniazide	
irbesartan	
irbesartan/hydrochlorothiazide	
isoflurane	
isoniazid	More frequent
itraconazole	
ketoprofen	
ketoprofen/omeprazole	
ketorolac trometamol	
lamotrigine	
leflunomide	
levocetirizine dihydrochloride	
levofloxacin	
levofloxacin hemihydrate	
levonorgestrel	
levonorgestrel/ethinylestradiol	

Drug substance	*Frequency category* *(category=less frequent unless marked)*
linagliptin/metformin hydrochloride	
lisinopril	
lisinopril/hydrochlorothiazide	
lithium carbonate	
lithium citrate	
losartan potassium/hydrochlorothiazide	
loxapine succinate	
lysine acetylsalicylate/metoclopramide hydrochloride	More frequent
medroxyprogesterone acetate/estradiol valerate	
mefenamic acid	
meloxicam	
mepivacaine hydrochloride/nicotinamide/polyestradiol phosphate	
mercaptopurine	
metformin	
metformin hydrochloride	
metformin hydrochloride/rosiglitazone maleate	
metformin hydrochloride/saxagliptin hydrochloride	
metformin hydrochloride/sitagliptin phosphate	
metformin hydrochloride/vildagliptin	
methyldopa anhydrous	
methyldopate hydrochloride	
methyltestosterone	
methyltestosterone/pemoline/yohimbine hydrochloride	
metoclopramide hydrochloride	More frequent
metoclopramide hydrochloride/paracetamol	More frequent
metoprolol tartrate/hydrochlorothiazide	
mianserin hydrochloride	
minocycline hydrochloride	
nabumetone	
nandrolone decanoate	
naproxen	
naproxen sodium	
naproxen/esomeprazole	
nefazodone hydrochloride	
nevirapine	
nevirapine anhydrate	
nevirapine hemihydrate	
nitrofurantoin	
nomegestrol/estradiol hemihydrate	
norelgestromin/ethinylestradiol	
norethisterone acetate/estradiol	
norethisterone acetate/ethinylestradiol	
norethisterone/ethinylestradiol	
norfloxacin	
norgestimate/ethinylestradiol	
olanzapine	
olanzapine embonate monohydrate	
olmesartan medoxomil/amlodipine besilate	
olmesartan medoxomil/hydrochlorothiazide	
omeprazole	
omeprazole magnesium	
omeprazole sodium	

Drug substance	*Frequency category* *(category=less frequent unless marked)*
orlistat	
oxandrolone	
oxymetholone	
oxytetracycline dihydrate	
oxytetracycline hydrochloride	
oxytetracycline hydrochloride/hydrocortisone	
paracetamol/caffeine/aspirin	
paracetamol/dextropropoxyphene hydrochloride	
paracetamol/ibuprofen	
paracetamol/metoclopramide hydrochloride	More frequent
paracetamol/promethazine hydrochloride/dextromethorphan hydrobromide	
paracetamol/pseudoephedrine hydrochloride/doxylamine succinate/dextromethorphan hydrobromide	
paroxetine hydrochloride	
perphenazine	
phenoxymethylpenicillin potassium	
phenylbutazone	
phenytoin	
phenytoin sodium	
pimozide	
pioglitazone hydrochloride	
pioglitazone hydrochloride/metformin hydrochloride	
piroxicam	
piroxicam betadex	
pivampicillin	
polymyxin b sulphate/trimethoprim	
prochlorperazine maleate	
prochlorperazine mesilate	
propafenone hydrochloride	
pseudoephedrine hydrochloride/dextromethorphan hydrobromide	
pseudoephedrine hydrochloride/ibuprofen	
pseudoephedrine hydrochloride/levomenthol/diphenhydramine hydrochloride/dextromethorphan hydrobromide	
pseudoephedrine hydrochloride/triprolidine hydrochloride/dextromethorphan hydrobromide	
pyrazinamide/rifampicin/isoniazid	More frequent
quetiapine fumarate	
racemic camphor/aspirin/methyl salicylate/menthol	
ranitidine bismuth citrate	
ranitidine hydrochloride	
repaglinide	
rifampicin	
rifampicin/isoniazid	More frequent
risperidone	
rofecoxib	
rosiglitazone maleate	
rosiglitazone maleate/metformin hydrochloride	
sertraline hydrochloride	
simvastatin	
simvastatin/ezetimibe	
sodium aurothiomalate	

Drug substance	*Frequency category* *(category=less frequent unless marked)*
sodium fusidate	
sodium fusidate/hydrocortisone acetate	
sodium valproate	More frequent
spironolactone/chlorothiazide	
stanozolol	
sulfamethoxazole/trimethoprim	
sulindac	
sulpiride	More frequent
talampicillin hydrochloride	
tamoxifen citrate	
telmisartan/hydrochlorothiazide	
tenoxicam	
terbinafine	
terbinafine hydrochloride	
terfenadine	
testosterone	
testosterone enantate	
testosterone phenylpropionate/testosterone propionate/testosterone decanoate/testosterone isocaproate	
testosterone propionate	
testosterone propionate/testosterone phenylpropionate/testosterone isocaproate	
testosterone undecanoate	
tetracycline hydrochloride	
thioridazine	
thioridazine hydrochloride	
ticlopidine hydrochloride	
timolol maleate/hydrochlorothiazide/amiloride hydrochloride	
tolbutamide	
tolmetin sodium	
triamterene/hydrochlorothiazide	
trifluoperazine hydrochloride	
trimethoprim	
trimethoprim/sulfamethoxazole	
trimipramine maleate	
valproate semisodium	More frequent
valsartan/amlodipine besilate	
valsartan/hydrochlorothiazide	
vildagliptin/metformin hydrochloride	
zinc sulphate/lithium succinate	

Chapter 3 Appendix Table 8: Study protocols and approvals

ISAC PROTOCOL

Development and testing of new methods for the identification of cases of cholestatic hepatitis in GPRD

Aims and objectives

Aim

To develop optimal methods for the accurate identification of cholestatic hepatitis within the GPRD.

Specific objectives

Develop an algorithm (the chol-hep multisource algorithm) using data sources within and external to GPRD that enables cholestatic hepatitis to be accurately identified as an outcome in patients.

Use the chol-hep multisource algorithm to develop a second algorithm (the chol-hep GPRD algorithm) that allows patients with cholestatic hepatitis to be accurately identified using data only held within GPRD.

Test the chol-hep GPRD algorithm by utilising it in a comparison of the rate of cholestatic hepatitis in people prescribed flucloxacillin with the rate in people prescribed oxytetracycline (an antibiotic which has a similar range of indications but is not associated with cholestatic hepatitis).

Study type

The primary aim of this study is to design new methodologies for the definition of cholestatic hepatitis within the General Practice Research Database (described in part 1 of the following sections).

To assist with analysis of the effectiveness of the new methods, a hypothesis testing study will be performed, testing the null hypothesis that flucloxacillin is not associated with an increased rate of cholestatic hepatitis (described in part 2 of the following sections).

Study population

Study population will be selected from patients registered in the GPRD aged 18+ registered with a GPRD practice during the study period of 1st January 2000 to April 2012.

Part 1 – Design of new methodologies

We will be developing two algorithms for case definition: a chol-hep multisource algorithm and a chol-hep GPRD algorithm.

The chol-hep multisource algorithm

Data sources

Our chol-hep multisource algorithm will utilise the following data sources:

GPRD READ codes

HES ICD10 codes

Other GPRD/HES information (e.g. laboratory test results)

Hospital discharge letters from GPs

GPRD freetext

ONS mortality data

Development of case definition

Based upon standard clinical definitions for cholestatic liver injury [45, 46, 145], we have developed an algorithm that will allow patients to be classified as definite, probable, possible (and non-) cases. To start with, we will use a combination of coded information and laboratory test results from GPRD and HES, as defined in the table below. In this table, the groups of GPRD/HES codes represent groups according to strength of evidence for cholestatic hepatitis (group 1: highest, group 3: lowest) - full details of the codes contained in each group are included in the appendices.

Case status	GPRD (READ) codes *See Appendix 1*	HES (ICD10) codes *See Appendix 2*	Laboratory test results[1] (in GPRD)
Definite	Group 1	Group 1	Cholestatic[2]
Definite	Group 1	Group 2 or 3	Cholestatic
Definite	Group 2 or 3	Group 1	Cholestatic
Definite	Group 1	No HES record	Cholestatic
Probable	Group 1	Group 1	Abnormal liver function, but not cholestatic
Probable	Group 1	Group 2 or 3	Abnormal liver function, but not cholestatic
Probable	Group 2 or 3	Group 1	Abnormal liver function, but not cholestatic
Probable	Group 2 or 3	Group 2 or 3	Cholestatic
Probable	Group 2 or 3	No HES record	Cholestatic
Probable	Group 1	Group 1	None available
Possible	Group 1	Group 2 or 3	Abnormal liver function, but not cholestatic

Possible	Group 1	No HES record	Abnormal liver function, but not cholestatic
Possible	Group 2 or 3	Group 1	Abnormal liver function, but not cholestatic
Possible	Group 1	No HES record	None available

[1]*Laboratory test results: need to be recorded in the patient's medical record within two months of being diagnosed with a code from one of the groups.*

[2]*Cholestatic: Either (1) an increase of over twice the upper limit of the normal range in alkaline phosphatase (AP) alone or (2) when the ratio (R) of serum activity of alanine aminotransferase to serum activity of AP is <=2.*

We will then use other information held within the GPRD or HES records (such as abdominal ultrasound test results, biopsy results), questionnaires sent to GPs (questionnaire to be provided at a later date), GPRD freetext and ONS mortality data to assist with further classification of the probable/possible cases. Finally, a sample of all possible/probable/definite cases will be reviewed by a physician in the project team. We will then use the probable and definite cases to assist in preparation of the chol-hep GPRD algorithm.

The chol-hep GPRD algorithm

Data sources

Our major goal is to develop an algorithm for cholestatic hepatitis case definition in GPRD that uses only data available within a standard GPRD record (i.e. without free-text, hospital discharge letters from GPs or linkages to HES/ONS data). This ensures that the method would be compatible with "real time" identification of (e.g. flucloxacillin-induced) cholestatic hepatitis cases for studies that aim to recruit patients with flucloxacillin-induced cholestatic hepatitis for genetic analysis. These would use the recently developed Randomised Controlled Trial IT infrastructure at GPRD, which would allow patients to be flagged as potential cases based on exposure to flucloxacillin (assessed by daily downloads of GP data into GPRD) and subsequently designated as probable/definite cases based upon application of the chol-hep GPRD algorithm.

Development of case definition

We will use a data-driven approach to develop a chol-hep GPRD algorithm, using the case definitions (probable and definite) from the chol-hep multisource algorithm as the outcome, and the following information within GPRD as the exposures:

READ codes

Other descriptions of the outcome in the GPRD standard record (e.g. laboratory test results, information on referrals)

We will then use cross tabulations and logistic regression to assess the combination of these exposures that best predict the probable and definite case definitions obtained from the chol-hep multisource algorithm. The log Odds Ratios (OR) for exposures in the final model will be

used to obtain a cholestatic-hepatitis diagnosis score for (1) each patient classified (by the chol-hep multisource algorithm) as having cholestatic hepatitis and (2) a 5:1 random sample of those without cholestatic hepatitis by assigning the value of the log OR for each of the exposures present in their GPRD record and then summing these values to produce an overall score for the individual [79]. We will then determine the cut-off score that best separates individuals without cholestatic hepatitis from those with cholestatic hepatitis. STATA version 12 will be used for all statistical analysis.

This cut-off score will be used as the case definition (outcome) under test in part 2.

Part 2 – Testing the methods using a cohort analysis of the association between flucloxacillin and cholestatic hepatitis

This part describes the study we will perform that will allow us to test the performance of the chol-hep GPRD algorithm for case definition versus (1) case definitions used in published data (2) cases identified by obtaining hospital discharge notes from GPs (3) cases identified using HES/ONS data only (4) cases identified using the chol-hep multisource algorithm.

Study design

A cohort analysis of the association between flucloxacillin and cholestatic hepatitis.

Exposed group

Inclusion criteria

People with at least one prescription for flucloxacillin and at least 12 months of computerised prescription history in GPRD prior to their first prescription.

Exclusion criteria

Patients who have any of the following in their GPRD record anytime prior to their first flucloxacillin prescription and within a 12 month period after the first prescription will be excluded: any documented liver disease, alcoholism, malignant neoplasm, cholelithiasis, viral hepatitis, chronic liver disease, cirrhosis, congestive heart failure, hepatitis following blood transfusion, HIV, RA, sarcoidosis, systemic lupus or inflammatory bowel disease.

Comparison group

Inclusion criteria

People with at least one prescription for oxytetracycline and at least 12 months of computerised prescription history in GPRD prior to their first prescription.

Oxytetracycline users are likely to be more comparable to the exposed group with respect to exposures (in e.g. health-seeking behaviour, level of illness) than non-users but not the outcome (oxytetracycline has not been shown to be associated with cholestatic hepatitis).

We will also use the exposed group as their own comparison group for a part of the analysis, comparing the number of outcome events within 60 days with the following 60 days.

Exclusion criteria

Same criteria as for the exposed group, in relation to oxytetracycline.

Sample size/power calculations

A recent GPRD cohort study [53] demonstrated the risk of cholestatic liver disease to be 8.5 per 100 000 first time users for flucloxacillin (95% CI 5.4 – 12.6) and 0.8 per 100 000 first time users for oxytetracyline (95% CI 0.02 – 4.3), a risk ratio of 8.5/0.8 = 10.63. We performed a power calculation using STATA version 12 and in order to detect a difference of this size with 95% precision and 90% power, calculated that we would require a sample size of 189892 in each exposure group.

A feasibility count in GPRD was performed by obtaining the number of first time prescriptions for flucloxacillin and for oxytetracycline in a sample of 1 million people (extracted from the database October 2011) and multiplying this appropriately to reflect the total number of people in GPRD in October 2011 (11.6 million). There were 2241712 people with a first time prescription for flucloxacillin with records in the database in October 2011 and 618025 people with a first time prescription for oxytetracycline, meaning that our study will be adequately powered to detect the likely difference, even after applying exclusion criteria.

Exposures, outcomes and co-variates

Exposures

Exposures will be determined from prescription records within GPRD for these antibiotics. A person will be considered exposed up to 45 days after a prescription of flucloxacillin or oxytetracycline, as flucloxacillin-induced cholestatic hepatitis may occur up 6-8 weeks after administration of the drug [53].

Outcomes

The main study will be performed using an outcome definition determined by the chol-hep GPRD algorithm.

As the purpose of this cohort study is to assess the performance of the chol-hep GPRD algorithm, we will then repeat the analysis using cases defined by the methods listed below, and compare the results obtained with those obtained using the chol-hep GPRD algorithm:

The chol-hep multisource algorithm

Requests for hospital discharge notes from GPs

HES/ONS cases

Co-variates

We will consider adjusting for age, gender, smoking, SES, ethnicity, BMI and alcohol intake.

We will take account of the possible impact of the use of other drugs associated with liver toxicity during the exposure period (e.g. chlorpromazine, amoxicillin/clavulanic acid, macrolides, tetracyclines, metoclopramide, chlorpheniramine, betahistine, sulphasalazine, azathioprine, diclofenac, antiepileptics [74]) by performing sensitivity analyses that exclude patients on these drugs from the analysis.

If any patients are exposed to both oxytetracycline and flucloxacillin, we will perform a sensitivity analysis to assess if there is any impact of excluding these patients.

Data/statistical analysis and data management

Bias

If any assessment of outcome is required separate from using the algorithm prepared in part 1, any reviewers will be blinded to drug exposure status.

Statistical Analysis

We will use Cox regression to:

Compare the rate of cholestatic hepatitis during the 45 days after a first prescription of flucloxacillin to the rate during the 45 days after a first prescription of oxytetracycline

Compare the rate of cholestatic hepatitis during the 45 days after a first prescription of flucloxacillin to the rate between 46 and 90 days after a first prescription of flucloxacillin

We will compare the results obtained from applying Cox regression when using the chol-hep GPRD algorithm for outcome definition with the results obtained when using the other outcome definitions (as defined in the Outcomes section above). We will also compare the results with the incidence rates and rate ratios obtained in comparable studies in the literature.

Handling of missing values

For variables with missing values, a “missing” category will be included containing the number of individuals with the missing data. Sensitivity analysis will be performed to assess the impact of changing missing values to the most extreme values.

Patient or user group involvement

We do not believe that this research would benefit from patient involvement (or vice versa) at this stage.

Limitations of the study design, data sources, and analytical methods

As this is an observational study, confounding must be considered a limitation. We have included a list of confounders that we will assess, and will also carry out sensitivity analysis to assess the role of drugs other than those under study. Our comparison group has been selected to also receive an antibiotic, which should further reduce the likelihood that exposures other than those under study are causing observed effects.

Plans for disseminating and communicating study results

We plan to publish our findings in a peer-reviewed journal and to present them at relevant scientific conferences.

ISAC APPROVAL

ISAC EVALUATION OF PROTOCOLS FOR RESEARCH INVOLVING GPRD DATA

FEED-BACK TO APPLICANTS

CONFIDENTIAL	*by e-mail*
PROTOCOL NO:	12_049_Appendix1
PROTOCOL TITLE:	Development and testing of new methods for the identification of cases of cholestatic hepatitis in GPRD: APPENDIX TO THE PROTOCOL MOLECULAR GENETICS OF ADVERSE DRUG REACTIONS
APPLICANT:	Prof TP van Staa, GPRD,

APPROVED ☒	APPROVED SUBJECT TO MINOR AMENDMENT (resubmission not required) ☐	REVISION/ RESUBMISSIO REQUESTED ☐

INSTRUCTIONS:

Please include your response/s to the Reviewer's feedback below only if you are required to Revise/ Resubmit your protocol.

Protocols with an outcome of 'Approved' or 'Approved subject to minor amendments' do not require resubmission to the ISAC

REVIEWER COMMENTS:

The appendix to this protocol is approved.

APPLICANTS RESPONSE

DATE OF ISAC FEEDBACK:	1 May 2012
DATE OF APPLICANT FEEDBACK:	

Chapter 4 Appendix

Chapter 4 Appendix Table 1: CPRD denominator (total number of patients with up-to-research standard data in the database) from 2000 onwards

Year	Denominator[1]	% of current size (increase)
2000	5606488	45% (-)
2001	6263789	50% (5%)
2002	7249997	58% (8%)
2003	7886617	63% (5%)
2004	8548007	68% (5%)
2005	9048301	72% (4%)
2006	9526092	76% (4%)
2007	10097134	80% (5%)
2008	10575875	85% (4%)
2009	11066617	89% (4%)
2010	11539683	92% (4%)
2011	12075098	97% (4%)
2012	12501163	100% (3%)

[1]**Denominator:** Total number of patients registered in the database on 30th June of that year

Chapter 4 Appendix Table 2: Characteristics of people included in the CPRD algorithm training and validation cohorts (data from CPRD record unless otherwise stated)

		Training cohort (N = 8020) n (%)	Validation cohort (N = 8020) n (%)
Age at index diagnosis date[1]	18 – 29	474 (6)	474 (6)
	30 – 39	697 (9)	755 (9)
	40 – 49	1096 (14)	1068 (13)
	50 – 59	1350 (17)	1386 (17)
	60 - 69	1494 (19)	1443 (18)
	70 - 79	1555 (19)	1572 (20)
	80+	1354 (17)	1322 (16)
	median (25 - 75%):	*62 (47 – 75)*	*62 (47 – 75)*
Gender	Male	4201 (52)	4205 (52)
	Female	3819 (48)	3815 (48)
Date of index diagnosis	2000 – 2002	1643 (20)	1693 (21)
	2003 – 2005	1982 (25)	1885 (24)
	2006 – 2008	1971 (25)	1991 (25)
	2009 - 2012	2424 (30)	2451 (31)

Note 1: Date of diagnosis with one of the potential cholestatic liver injury codes listed in Chapter 4 Appendix, Table 1

Chapter 4 Appendix Table 3: Algorithm score and corresponding sensitivity and specificity when comparing the complete CPRD algorithm (stage 1 and stage 2 case assignment) against the multisource algorithm case status of definite to possible

CPRD algorithm score (stage 1 and stage 2 case assignment)	Sensitivity	Specificity
-1.60	100.0%	0.0%
-1.20	100.0%	3.8%
-1.20	100.0%	4.7%
-0.81	100.0%	4.9%
-0.80	100.0%	5.1%
-0.47	100.0%	5.1%
-0.40	100.0%	9.3%
-0.07	100.0%	9.3%
-0.07	100.0%	11.9%
0.00	100.0%	12.0%
0.33	99.9%	27.1%
0.34	99.9%	27.4%
0.39	99.9%	27.5%
0.40	99.9%	32.3%
0.64	99.8%	34.0%
0.68	99.8%	41.5%
0.74	99.8%	42.0%
0.79	99.8%	42.0%
0.81	99.8%	43.3%
1.03	99.8%	43.6%
1.04	99.8%	45.6%
1.07	99.8%	46.3%
1.08	99.8%	46.8%
1.21	99.8%	46.8%
1.43	99.8%	47.0%
1.44	99.8%	47.4%
1.47	99.8%	47.4%
1.49	99.8%	47.5%
1.63	99.8%	47.6%
1.84	94.1%	65.2%
1.89	94.1%	65.2%
2.02	91.6%	70.7%
2.03	88.5%	81.5%
2.29	86.8%	86.1%
2.29	85.3%	89.7%
2.42	84.7%	90.9%
2.43	82.7%	96.4%
2.69	82.2%	97.1%
2.70	81.4%	98.4%
2.83	81.3%	98.6%
3.02	81.3%	99.6%
3.10	81.0%	99.7%
3.42	81.0%	99.9%
3.42	81.0%	100.0%
5	81.0%	100.0%

Chapter 4 Appendix Table 4: Algorithm score and corresponding sensitivity and specificity when comparing algorithm cut-off score (stage 2 case assignment only) against the multisource algorithm case status of definite to possible

CPRD algorithm score (stage 2 case assignment only)	Sensitivity	Specificity
-1.60	100.0%	0.0%
-1.20	100.0%	3.8%
-1.20	100.0%	4.7%
-0.81	100.0%	4.9%
-0.80	100.0%	5.1%
-0.47	100.0%	5.1%
-0.40	100.0%	9.3%
-0.07	100.0%	9.3%
-0.07	100.0%	11.9%
0.00	100.0%	12.1%
0.33	99.6%	27.1%
0.34	99.6%	27.4%
0.39	99.6%	27.5%
0.40	99.4%	32.3%
0.64	99.2%	34.0%
0.68	99.2%	41.5%
0.74	99.2%	42.0%
0.79	99.2%	42.0%
0.81	99.0%	43.3%
1.03	99.0%	43.6%
1.04	99.0%	45.6%
1.07	99.0%	46.3%
1.08	99.0%	46.8%
1.21	99.0%	47.0%
1.43	99.0%	47.4%
1.44	99.0%	47.4%
1.47	99.0%	47.5%
1.49	99.0%	47.6%
1.63	99.0%	65.2%
1.84	69.3%	65.2%
1.89	69.3%	70.7%
2.02	56.4%	81.5%
2.03	40.2%	86.1%
2.29	31.2%	89.7%
2.29	23.4%	90.9%
2.42	20.3%	96.4%
2.43	9.6%	97.1%
2.69	7.4%	98.4%
2.70	3.1%	98.6%
2.83	2.7%	99.6%
3.02	0.8%	99.7%
3.10	0.6%	99.9%
3.42	0.2%	100.0%
3.42	0.0%	100.0%

Chapter 4 Appendix Table 5: Number of people with liver test results recorded in CPRD before and after 2007 (=approximate date of automation of transfer of liver test result data between pathology laboratory and primary care centres)

	Pre-2007 N=8582 n (%)	Post-2007 N=7458 n (%)
No liver test result recorded	4739 (55)	2615 (35)
Liver test result recorded	3843 (45)	4843 (65)

Chapter 5 Appendix

Chapter 5 Appendix Table 1: Characteristics of participants included in the cohort analysis of the association between flucloxacillin (compared with oxytetracycline) and cholestatic liver injury, by exposure status for the HES-linked cohort only

		Oxytetracycline (N = 103,673) n (%)	Flucloxacillin (N = 517,803) n (%)
Age at index date	18 – 29	17669 (17)	87830 (17)
	30 – 39	17465 (17)	96745 (19)
	40 – 49	16968 (16)	88110 (17)
	50 – 59	16764 (16)	77080 (15)
	60 – 69	15723 (15)	66182 (13)
	70 – 79	11846 (11)	54925 (11)
	80+	7238 (7)	46931 (9)
	median (25 - 75%):	*49 (35 – 65)*	*48 (34 – 65)*
Gender	Male	45576 (44)	235721 (46)
	Female	58097 (56)	282082 (54)
Date of index prescription	2000 – 2001	18810 (18)	68736 (13)
	2002 - 2003	19759 (19)	86628 (17)
	2004 - 2005	17567 (17)	91412 (18)
	2006 - 2007	16504 (16)	95090 (18)
	2008 - 2009	16721 (16)	93035 (18)
	2010 - 2011	14312 (14)	82902 (16)
Prescriptions for other causes of cholestatic injury[1]	None	18924 (18)	254142 (49)
	Less common cause	81018 (78)	237303 (46)
	More common cause	3731 (4)	26358 (5)
Smoking status	Non-smoker	47903 (46)	228867 (44)
	Ex-smoker	22274 (21)	129469 (25)
	Current smoker	31621 (31)	149286 (29)
	Unknown	1875 (2)	10181 (2)
BMI	<20	6379 (6)	29670 (6)
	20 - 25	32179 (31)	151247 (29)
	25+	52439 (51)	266587 (51)
	Unknown	12676 (12)	70299 (14)
Alcohol intake	Non-drinker	10473 (10)	54603 (11)
	Ex-drinker	3134 (3)	17249 (3)
	Current NOS	3385 (3)	17200 (3)
	2 or less u/d	15980 (15)	80368 (16)
	3/6 u/d	49201 (47)	236106 (46)
	>6 u/d	7720 (7)	41191 (8)
	Unknown	13780 (13)	71086 (14)
Socioeconomic status[2]	1 (Highest SES)	26098 (25)	118154 (23)
	2	25099 (24)	120327 (23)
	3	20083 (19)	103849 (20)
	4	18254 (18)	97450 (19)
	5 (Lowest SES)	14139 (14)	78023 (15)
Ethnicity[3]	White	70393 (68)	355416 (69)
	South Asian	2310 (2)	11330 (2)
	Black	1126 (1)	6710 (1)
	Other	996 (1)	4643 (1)
	Mixed	316 (0)	1778 (0)
	Not Stated	12155 (12)	61973 (12)
	Unknown	16377 (16)	75953 (15)

Note 1: Prescription counted if it occurred anytime from 1 month prior to index date or between index date and before end of follow-up (see Chapter 3 section 3.4.1.5). Less or more common in relation to flucloxacillin, as reported in the literature (see Chapter 3 section 3.4.1.5)
Note 2: Linked data, only available for practices in England, based on index of Multiple Deprivation (individual patient postcode) or otherwise practice level score based upon practice postcode (if no individual-level data) (see Chapter 3 section 3.4.1.5).
Note 3: Obtained from CPRD, unless none found, in which case from HES if patient from a linked practice (see Chapter 3 section 3.4.1.5).

Chapter 5 Appendix Table 2

Chapter 5 Appendix Table 2a: Rates and crude rate ratios of cholestatic liver injury for co-variates of the flucloxacillin and cholestatic liver injury cohort, using the highest specificity CPRD algorithm cut-off score (5), within those exposed to flucloxacillin or oxytetracycline during the 1 – 45 day period from first prescription

		# with outcome
		n=56
			Patients[4]
			N=1046696
				45-day risk[5] (CI[6])	PMAR[7]	Rate[8] (CI)	Crude Rate Ratio (CI)	p-value[9]

Age at index date[1]	18 – 49	1	545975	0.18 (0.00 - 1.02)	7.99	0.13 (0.02 - 0.89)	1	<0.001
	50 – 59	9	159448	5.64 (2.58 - 10.71)	2.34	3.85 (2.00 - 7.39)	31.51 (3.99 - 248.68)	
	60 – 69	5	139597	3.58 (1.16 - 8.36)	2.05	2.44 (1.02 - 5.87)	20.33 (2.37 - 174.00)	
	70 – 79	21	112655	18.64 (11.54 - 28.49)	1.65	13.35 (8.30 - 20.14)	99.17 (13.31 - 738.93)	
	80+	20	89021	22.47 (13.72 - 34.70)	1.29	16.72 (10.56 – 24.44)	108.04 (14.42 - 809.31)	
Gender	Male	22	475441	4.63 (2.90 - 7.01)	6.96	3.16 (2.08 - 4.80)	1	0.366
	Female	34	571255	5.95 (4.12 - 8.32)	8.35	4.07 (2.91 - 5.70)	1.29 (0.74 - 2.23)	
Date of index prescription	2000 – 2001	2	144627	1.38 (0.17 - 5.00)	2.13	0.94 (0.23 - 3.76)	1	0.255
	2002 – 2003	8	178582	4.48 (1.93 - 8.83)	2.63	3.04 (1.52 - 6.09)	3.12 (0.66 - 14.70)	
	2004 – 2005	13	189423	6.86 (3.65 - 11.74)	2.79	4.66 (2.71 - 8.03)	4.65 (1.05 - 20.61)	
	2006 – 2007	12	189394	6.34 (3.27 - 11.07)	2.78	4.31 (2.45 - 7.59)	3.88 (0.86 - 17.50)	
	2008 – 2009	9	182896	4.92 (2.25 - 9.34)	2.69	3.35 (1.74 - 6.43)	3.29 (0.71 - 15.23)	
	2010 – 2011	12	161774	7.42 (3.83 - 12.96)	2.30	5.22 (2.96 - 9.19)	4.26 (0.93 - 19.45)	
Prescriptions for other causes of cholestatic injury[2]	None	11	450216	2.44 (1.22 - 4.37)	6.58	1.67 (0.93 - 3.02)	1	<0.001
	Rare cause	41	543010	7.55 (5.42 - 10.24)	7.95	5.16 (3.80 - 7.00)	3.59 (1.83 - 7.02)	
	More common cause	4	53470	7.48 (2.04 - 19.15)	0.78	5.12 (1.92 - 13.63)	3.26 (1.04 - 10.22)	
Smoking status	Non-smoker	17	467184	3.64 (2.12 - 5.83)	6.84	2.49 (1.55 - 4.00)	1	<0.001
	Current smoker	8	260101	3.08 (1.33 - 6.06)	3.81	2.101 (1.05 - 4.20)	0.76 (0.31 - 1.85)	
	Ex-smoker	30	297657	10.08 (6.80 - 14.39)	4.36	6.88 (4.81 - 9.84)	2.86 (1.55 - 5.26)	
	Unknown	1	21754	4.60 (0.12 - 25.61)	0.31	3.18 (0.45 - 22.61)	1.33 (0.18 - 10.04)	
BMI	<20	1	59374	1.68 (0.04 - 9.38)	0.87	1.15 (0.16 - 8.20)	1	0.163
	20 – 25	23	303272	7.58 (4.81 - 11.38)	4.44	5.18 (3.44 - 7.80)	4.29 (0.58 - 31.82)	
	25+	26	542418	4.79 (3.13 - 7.02)	7.95	3.27 (2.23 - 4.80)	2.59 (0.35 - 19.12)	
	Unknown	6	141632	4.24 (1.55 - 9.22)	2.06	2.91 (1.31 - 6.47)	2.45 (0.30 - 20.36)	

		# with outcome n=56	Patients[4] N=1046696	45-day risk[5] (CI[6])	PMAR[7]	Rate[8] (CI)	Crude Rate Ratio (CI)	p-value[9]
Alcohol intake	Non-drinker	4	117896	3.39 (0.92 - 8.69)	1.72	2.32 (0.87 - 6.18)	1	0.16
	Ex-drinker	1	33858	2.95 (0.07 - 16.45)	0.49	2.03 (0.29 - 14.38)	1.15 (0.12 - 11.03)	
	Current NOS	4	33304	12.01 (3.27 - 30.75)	0.49	8.23 (3.09 - 21.93)	4.72 (1.06 - 21.10)	
	2 or less u/d	15	169724	8.84 (4.95 - 14.58)	2.48	6.04 (3.64 - 10.01)	3.48 (1.01 - 12.02)	
	3/6 u/d	22	465596	4.73 (2.96 - 7.15)	6.82	3.22 (2.12 - 4.90)	1.69 (0.50 - 5.69)	
	>6 u/d	4	79808	5.01 (1.37 - 12.83)	1.16	3.42 (1.28 - 9.12)	1.94 (0.43 - 8.67)	
	Unknown	6	146510	4.10 (1.50 - 8.91)	2.14	2.81 (1.26 - 6.25)	1.60 (0.40 - 6.39)	
Socioeconomic status	1 (Highest SES)	11	186791	5.89 (2.94 - 10.54)	2.74	4.02 (2.23 - 7.26)	1	0.443
	2	9	175505	5.13 (2.34 - 9.73)	2.57	3.50 (1.82 - 6.73)	0.77 (0.31 - 1.91)	
	3	13	167976	7.74 (4.12 - 13.23)	2.46	5.29 (3.07 - 9.11)	1.30 (0.58 - 2.89)	
	4	9	158966	5.66 (2.59 - 10.75)	2.34	3.87 (2.01 - 7.44)	0.96 (0.40 - 2.31)	
	5 (Lowest SES)	5	121845	4.10 (1.33 - 9.58)	1.78	2.81 (1.17 - 6.75)	0.68 (0.24 - 1.96)	
	Unknown	9	235613	3.82 (1.75 - 7.25)	3.45	2.61 (1.36 - 5.01)	0.52 (0.20 - 1.34)	
Ethnicity[3]	White	33	534140	6.18 (4.25 - 8.68)	7.81	4.22 (3.00 - 5.94)	1	0.08
	South Asian	0	17497	0.00 (0.00 - 21.08)	0.25	-	-	
	Black	1	10011	9.99 (0.25 - 55.64)	0.15	6.86 (0.97 - 48.73)	1.57 (0.21 - 11.46)	
	Other	0	7672	0.00 (0.00 - 48.07)	0.11	-	-	
	Mixed	0	2630	0.00 (0.00 - 140.16)	0.04	-	-	
	Not Stated	8	85336	9.37 (4.05 - 18.47)	1.25	6.41 (3.21 - 12.82)	1.51 (0.70 - 3.26)	
	Unknown	14	389410	3.60 (1.97 - 6.03)	5.70	2.45 (1.45 - 4.14)	0.46 (0.23 - 0.91)	

Note 1: age groups 18-29, 30-39, 40-49 combined due to lack of events in the 18-29 or 30-39 age groups
Note 2: prescription counted if it occurred anytime from 1 month prior to index date or between index date and before end of follow-up (see Chapter 3 section 4.4.1.5)
Note 3: Obtained from CPRD, unless none found, in which case from HES (see Chapter 4 section 3.4.1.5)
Note 4: number of patients prescribed the drug
Note 5: per 100000 patients prescribed the drug
Note 6: 95% confidence interval
Note 7: 100000 person-months at risk
Note 8: per 100000 person months
Note 9: p-value results for LRT of an association over all categories of the variable

Chapter 5 Appendix Table 2b: Rates and crude rate ratios of cholestatic liver injury for co-variates of the flucloxacillin and cholestatic liver injury cohort, using the CPRD algorithm cut-off score with medium specificity (2.29), within those exposed to flucloxacillin or oxytetracycline during the 1 – 45 day period from first prescription

		# with outcome	Patients[4]	45-day risk[5] (CI[6])	PMAR[7]	Rate[8] (CI)	Crude Rate Ratio (CI)	p-value[9]
		n=80	N=1046696					
Age at index date[1]	18 – 49	3	545975	0.55 (0.11 - 1.61)	7.99	0.38 (0.12 - 1.16)	1	<0.001
	50 – 59	12	159448	7.53 (3.89 - 13.15)	2.34	5.13 (2.91 - 9.03)	14.00 (3.95 - 49.62)	
	60 – 69	13	139597	9.31 (4.96 - 15.92)	2.05	6.35 (3.68 - 10.94)	17.62 (5.02 - 61.82)	
	70 – 79	25	112655	22.19 (14.36 - 32.76)	1.65	15.16 (10.24 - 22.43)	39.67 (11.94 - 131.73)	
	80+	27	89021	30.33 (19.99 - 44.13)	1.29	20.93 (14.35 - 30.52)	50.02 (15.10 - 165.67)	
Gender	Male	30	475441	6.31 (4.26 - 9.01)	6.96	4.31 (3.01 - 6.16)	1	0.15
	Female	50	571255	8.75 (6.50 - 11.54)	8.35	5.9862 4.5370 7.8982	1.40 (0.88 - 2.22)	
Date of index prescription	2000 – 2001	6	144627	4.15 (1.52 - 9.03)	2.13	2.82 (1.27 - 6.27)	1	0.732
	2002 – 2003	12	178582	6.72 (3.47 - 11.74)	2.63	4.57 (2.59 - 8.04)	1.56 (0.59 - 4.16)	
	2004 – 2005	17	189423	8.97 (5.23 - 14.37)	2.79	6.10 (3.79 - 9.81)	2.03 (0.80 - 5.14)	
	2006 – 2007	15	189394	7.92 (4.43 - 13.06)	2.78	5.39 (3.25 - 8.94)	1.65 (0.63 - 4.28)	
	2008 – 2009	15	182896	8.20 (4.59 - 13.53)	2.69	5.58 (3.36 - 9.26)	1.83 (0.71 - 4.71)	
	2010 – 2011	15	161774	9.27 (5.19 - 15.29)	2.30	6.52 (3.93 - 10.82)	1.85 (0.70 - 4.86)	
Prescriptions for other causes of cholestatic injury[2]	None	17	450216	3.78 (2.20 - 6.05)	6.58	2.58 (1.61 - 4.15)	1	<0.001
	Rare cause	56	543010	10.31 (7.79 - 13.39)	7.95	7.04 (5.42 - 9.15)	3.24 (1.87 - 5.59)	
	More common cause	7	53470	13.09 (5.26 - 26.97)	0.78	8.95 (4.27 - 18.78)	3.69 (1.53 - 8.89)	
Smoking status	Non-smoker	29	467184	6.21 (4.16 - 8.91)	6.84	4.24 (2.95 - 6.10)	1	<0.001
	Current smoker	10	260101	3.84 (1.84 - 7.07)	3.81	2.63 (1.41 - 4.88)	0.56 (0.26 - 1.19)	
	Ex-smoker	40	297657	13.44 (9.60 - 18.30)	4.36	9.18 (6.73 - 12.51)	2.20 (1.35 - 3.57)	
	Unknown	1	21754	4.60 (0.12 - 25.61)	0.31	3.18 (0.45 - 22.61)	0.76 (0.10 - 5.59)	
BMI	<20	2	59374	3.37 (0.41 - 12.17)	0.87	2.31 (0.58 - 9.23)	1	0.03
	20 – 25	34	303272	11.21 (7.76 - 15.67)	4.44	7.66 (5.47 - 10.72)	3.22 (0.77 - 13.41)	
	25+	38	542418	7.01 (4.96 - 9.62)	7.95	4.78 (3.48 - 6.57)	1.94 (0.47 - 8.06)	
	Unknown	6	141632	4.24 (1.55 - 9.22)	2.06	2.91 (1.31 - 6.47)	1.23 (0.25 - 6.07)	
Alcohol intake	Non-drinker	6	117896	5.09 (1.87 - 11.08)	1.72	3.48 (1.56 - 7.75)	1	0.1

		# with outcome n=80	Patients[4] N=1046696	45-day risk[5] (CI[6])	PMAR[7]	Rate[8] (CI)	Crude Rate Ratio (CI)	p-value[9]
	Ex-drinker	2	33858	5.91 (0.72 - 21.34)	0.49	4.05 (1.01 - 16.20)	1.38 (0.27 - 7.09)	
	Current NOS	5	33304	15.01 (4.87 - 35.03)	0.49	10.29 (4.28 - 24.72)	3.54 (1.03 - 12.24)	
	2 or less u/d	21	169724	12.37 (7.66 - 18.91)	2.48	8.45 (5.51 - 12.96)	2.92 (1.10 - 7.75)	
	3/6 u/d	31	465596	6.66 (4.52 - 9.45)	6.82	4.54 (3.19 - 6.46)	1.47 (0.57 - 3.80)	
	>6 u/d	7	79808	8.77 (3.53 - 18.07)	1.16	5.99 (2.86 - 12.56)	2.04 (0.65 - 6.42)	
	Unknown	8	146510	5.46 (2.36 - 10.76)	2.14	3.75 (1.87 - 7.49)	1.28 (0.42 - 3.91)	
Socioeconomic status	1 (Highest SES)	17	186791	9.10 (5.30 - 14.57)	2.74	6.22 (3.86 – 10.00)	1	0.185
	2	13	175505	7.41 (3.94 - 12.67)	2.57	5.06 (2.94 - 8.71)	0.74 (0.36 - 1.56)	
	3	19	167976	11.31 (6.81 - 17.66)	2.46	7.73 (4.93 - 12.12)	1.23 (0.64 - 2.36)	
	4	12	158966	7.55 (3.90 - 13.19)	2.34	5.16 (2.93 - 9.09)	0.83 (0.39 - 1.73)	
	5 (Lowest SES)	7	121845	5.75 (2.31 - 11.84)	1.78	3.93 (1.87 - 8.25)	0.62 (0.26 - 1.49)	
	Unknown	12	235613	5.09 (2.63 - 8.90)	3.45	3.48 (1.98 - 6.13)	0.48 (0.22 - 1.05)	
Ethnicity[3]	White	49	534140	9.17 (6.79 - 12.13)	7.81	6.27 (4.74 - 8.30)	1	0.016
	South Asian	0	17497	0.00 (0.00 - 21.08)	0.25	-	-	
	Black	1	10011	9.99 (0.25 - 55.64)	0.15	6.86 (0.97 - 48.73)	1.06 (0.15 - 7.64)	
	Other	0	7672	0.00 (0.00 - 48.07)	0.11	-	-	
	Mixed	0	2630	0.00 (0.00 - 140.16)	0.04	-	-	
	Not Stated	11	85336	12.89 (6.43 - 23.06)	1.25	8.82 (4.88 - 15.92)	1.40 (0.73 - 2.68)	
	Unknown	19	389410	4.88 (2.94 - 7.62)	5.70	3.33 (2.12 - 5.22)	0.45 (0.26 - 0.79)	

Note 1: age groups 18-29, 30-39, 40-49 combined due to lack of events in the 18-29 or 30-39 age groups
Note 2: prescription counted if it occurred anytime from 1 month prior to index date or between index date and before end of follow-up (see Chapter 3 section 3.4.1.5)
Note 3: Obtained from CPRD, unless none found, in which case from HES (see Chapter 3 section 3.4.1.5)
Note 4: number of patients prescribed the drug
Note 5: per 100000 patients prescribed the drug
Note 6: 95% confidence interval
Note 7: 100000 person-months at risk
Note 8: per 100000 person months
Note 9: p-value results for LRT of an association over all categories of the variable

Chapter 5 Appendix Table 2c: Rates and crude rate ratios of cholestatic liver injury for co-variates of the flucloxacillin and cholestatic liver injury cohort, using the most sensitive/least specific CPRD algorithm cut-off score (1.63), within those exposed to flucloxacillin or oxytetracycline during the 1 – 45 day period from first prescription

		# with outcome	Patients[4]	45-day risk[5] (CI[6])	PMAR[7]	Rate[8] (CI)	Crude Rate Ratio (CI)	p-value[9]
		n=129	N=1046696					
Age at index date[1]	18 – 49	14	545975	2.56 (1.40 - 4.30)	7.99	1.75 (1.04 - 2.96)	1	<0.001
	50 – 59	19	159448	11.92 (7.17 - 18.61)	2.34	8.12 (5.18 - 12.73)	5.12 (2.53 - 10.36)	
	60 – 69	16	139597	11.46 (6.55 - 18.61)	2.05	7.82 (4.79 - 12.76)	5.00 (2.41 - 10.40)	
	70 – 79	42	112655	37.28 (26.87 - 50.39)	1.65	25.47 (18.82 - 34.46)	15.26 (8.16 - 28.53)	
	80+	38	89021	42.69 (30.21 - 58.59)	1.29	29.46 (21.43 - 40.48)	15.70 (8.28 - 29.75)	
Gender	Male	47	475441	9.89 (7.26 - 13.15)	6.96	6.75 (5.07 - 8.98)	1	0.03
	Female	82	571255	14.35 (11.42 - 17.82)	8.35	9.82 (7.91 - 12.19)	1.50 (1.03 - 2.17)	
Date of index prescription	2000 – 2001	11	144627	7.61 (3.80 - 13.61)	2.13	5.17 (2.86 - 9.33)	1	0.671
	2002 – 2003	20	178582	11.20 (6.84 - 17.30)	2.63	7.61 (4.91 - 11.80)	1.35 (0.64 - 2.83)	
	2004 – 2005	26	189423	13.73 (8.97 - 20.11)	2.79	9.33 (6.35 - 13.70)	1.63 (0.80 - 3.31)	
	2006 – 2007	28	189394	14.78 (9.82 - 21.37)	2.78	10.06 (6.94 - 14.56)	1.73 (0.86 - 3.49)	
	2008 – 2009	21	182896	11.48 (7.11 - 17.55)	2.69	7.81 (5.09 - 11.98)	1.33 (0.64 - 2.77)	
	2010 – 2011	23	161774	14.22 (9.01 - 21.33)	2.30	10.00 (6.65 - 15.05)	1.55 (0.74 - 3.23)	
Prescriptions for other causes of cholestatic injury[2]	None	35	450216	7.77 (5.41 - 10.81)	6.58	5.32 (3.82 - 7.41)	1	<0.001
	Rare cause	84	543010	15.47 (12.34 - 19.15)	7.95	10.56 (8.53 - 13.08)	2.28 (1.53 - 3.41)	
	More common cause	10	53470	18.70 (8.97 - 34.39)	0.78	12.79 (6.88 - 23.77)	2.56 (1.27 - 5.17)	
Smoking status	Non-smoker	49	467184	10.49 (7.76 - 13.87)	6.84	7.17 (5.42 - 9.48)	1	0.001
	Current smoker	23	260101	8.84 (5.61 - 13.27)	3.81	6.04 (4.01 - 9.09)	0.80 (0.48 - 1.33)	
	Ex-smoker	56	297657	18.81 (14.21 - 24.43)	4.36	12.85 (9.89 - 16.70)	1.85 (1.25 - 2.74)	
	Unknown	1	21754	4.60 (0.12 - 25.61)	0.31	3.18 (0.45 - 22.61)	0.46 (0.06 - 3.36)	
BMI	<20	8	59374	13.47 (5.82 - 26.55)	0.87	9.24 (4.62 - 18.47)	1	0.002
	20 – 25	53	303272	17.48 (13.09 - 22.86)	4.44	11.94 (9.13 - 15.63)	1.24 (0.59 - 2.62)	
	25+	60	542418	11.06 (8.44 - 14.24)	7.95	7.55 (5.86 - 9.72)	0.75 (0.36 - 1.58)	
	Unknown	8	141632	5.65 (2.44 - 11.13)	2.06	3.88 (1.94 - 7.75)	0.36 (0.13 - 0.99)	

		# with outcome	Patients[4]	45-day risk[5] (CI[6])	PMAR[7]	Rate[8] (CI)	Crude Rate Ratio (CI)	p-value[9]
		n=129	N=1046696					
Alcohol intake	Non-drinker	11	117896	9.33 (4.66 - 16.69)	1.72	6.38 (3.53 - 11.53)	1	0.032
	Ex-drinker	3	33858	8.86 (1.83 - 25.89)	0.49	6.08 (1.96 - 18.84)	1.15 (0.31 - 4.24)	
	Current NOS	7	33304	21.02 (8.45 - 43.30)	0.49	14.40 (6.87 - 30.21)	2.76 (1.03 - 7.40)	
	2 or less u/d	35	169724	20.62 (14.36 - 28.68)	2.48	14.09 (10.12 - 19.62)	2.55 (1.22 - 5.33)	
	3/6 u/d	52	465596	11.17 (8.34 - 14.65)	6.82	7.62 (5.81 – 10.00)	1.38 (0.68 - 2.81)	
	>6 u/d	9	79808	11.28 (5.16 - 21.41)	1.16	7.70 (4.01 - 14.80)	1.45 (0.58 - 3.67)	
	Unknown	12	146510	8.19 (4.23 - 14.31)	2.14	5.62 (3.19 - 9.90)	1.07 (0.45 - 2.53)	
Socioeconomic status	1 (Highest SES)	23	186791	12.31 (7.81 - 18.48)	2.74	8.41 (5.59 - 12.65)	1	0.866
	2	21	175505	11.97 (7.41 - 18.29)	2.57	8.17 (5.33 - 12.54)	0.87 (0.47 - 1.60)	
	3	25	167976	14.88 (9.63 - 21.97)	2.46	10.17 (6.88 - 15.06)	1.19 (0.68 - 2.10)	
	4	18	158966	11.32 (6.71 - 17.89)	2.34	7.74 (4.88 - 12.29)	0.86 (0.46 - 1.62)	
	5 (Lowest SES)	15	121845	12.31 (6.89 - 20.30)	1.78	8.43 (5.08 - 13.98)	0.91 (0.47 - 1.77)	
	Unknown	27	235613	11.46 (7.55 - 16.67)	3.45	7.83 (5.37 - 11.41)	0.85 (0.48 - 1.51)	
Ethnicity[3]	White	79	534140	14.79 (11.71 - 18.43)	7.81	10.11 (8.11 - 12.60)	1	0.026
	South Asian	0	17497	0.00 (0.00 - 21.08)	0.25	-	-	
	Black	1	10011	9.99 (0.25 - 55.64)	0.15	6.86 (0.97 - 48.73)	0.68 (0.09 - 4.89)	
	Other	0	7672	0.00 (0.00 - 48.07)	0.11	-	-	
	Mixed	0	2630	0.00 (0.00 - 140.16)	0.04	-	-	
	Not Stated	13	85336	15.23 (8.11 - 26.05)	1.25	10.42 (6.05 - 17.95)	1.06 (0.59 - 1.92)	
	Unknown	36	389410	9.24 (6.47 - 12.80)	5.70	6.31 (4.55 - 8.75)	0.58 (0.38 - 0.88)	

Note 1: age groups 18-29, 30-39, 40-49 combined due to lack of events in the 18-29 or 30-39 age groups
Note 2: prescription counted if it occurred anytime from 1 month prior to index date or between index date and before end of follow-up (see Chapter 3 section 3.4.1.5)
Note 3: Obtained from CPRD, unless none found, in which case from HES (see Chapter 3 section 3.4.1.5)
Note 4: number of patients prescribed the drug
Note 5: per 100000 patients prescribed the drug
Note 6: 95% confidence interval
Note 7: 100000 person-months at risk
Note 8: per 100000 person months
Note 9: p-value results for LRT of an association over all categories of the variable

Chapter 5 Appendix Table 2d: Rates and crude rate ratios of cholestatic liver injury for co-variates of the flucloxacillin and cholestatic liver injury cohort, using the multisource algorithm (within those exposed to flucloxacillin or oxytetracycline during the 1 – 45 day period from first prescription)

		# with outcome n=46	Patients[4] N=621476	Risk[5] (CI[6])	PMAR[7]	Rate[8] (CI)	Crude Rate Ratio (CI)	p-value[9]
Age at index date[1]	18 – 49	2	324787	0.62 (0.07 - 2.22)	4.75	0.42 (0.11 - 1.68)	1	<0.001
	50 – 59	6	93844	6.39 (2.35 - 13.92)	1.38	4.35 (1.96 - 9.69)	10.35 (2.09 - 51.27)	
	60 – 69	4	81905	4.88 (1.33 - 12.50)	1.20	3.33 (1.25 - 8.88)	7.92 (1.45 - 43.22)	
	70 – 79	16	66771	23.96 (13.70 - 38.91)	0.98	16.36 (10.03 - 26.71)	38.88 (8.94 - 169.12)	
	80+	18	54169	33.23 (19.69 - 52.51)	0.78	22.93 (14.45 - 36.40)	54.49 (12.64 - 234.86)	
Gender	Male	21	281297	7.47 (4.62 - 11.41)	4.12	5.10 (3.32 - 7.82)	1	0.963
	Female	25	340179	7.35 (4.76 - 10.85)	4.97	5.03 (3.40 - 7.44)	0.99 (0.55 - 1.76)	
Date of index prescription	2000 – 2001	2	87546	2.28 (0.28 - 8.25)	1.28	1.55 (0.39 - 6.20)	1	0.353
	2002 – 2003	10	106387	9.40 (4.51 - 17.29)	1.56	6.39 (3.44 - 11.88)	4.11 (0.90 - 18.78)	
	2004 – 2005	9	108979	8.26 (3.78 - 15.68)	1.60	5.61 (2.92 - 10.79)	3.62 (0.78 - 16.74)	
	2006 – 2007	10	111594	8.96 (4.30 - 16.48)	1.64	6.09 (3.28 - 11.32)	3.93 (0.86 - 17.92)	
	2008 – 2009	7	109756	6.38 (2.56 - 13.14)	1.61	4.34 (2.07 - 9.10)	2.80 (0.58 - 13.46)	
	2010 – 2011	8	97214	8.23 (3.55 - 16.21)	1.38	5.79 (2.90 - 11.59)	3.73 (0.79 - 17.58)	
Prescriptions for other causes of cholestatic injury[2]	None	9	273066	3.30 (1.51 - 6.26)	3.99	2.25 (1.17 - 4.33)	1	0.002
	Rare cause	34	318321	10.68 (7.40 - 14.93)	4.66	7.29 (5.21 - 10.21)	3.23 (1.55 - 6.74)	
	More common cause	3	30089	9.97 (2.06 - 29.14)	0.43	6.82 (2.20 - 21.16)	3.03 (0.82 - 11.18)	
Smoking status	Non-smoker	13	276770	4.70 (2.50 - 8.03)	4.05	3.21 (1.86 - 5.53)	1	0.003
	Current smoker	8	151743	5.27 (2.28 - 10.39)	2.22	3.60 (1.80 - 7.20)	1.12 (0.46 - 2.71)	
	Ex-smoker	25	180907	13.82 (8.94 - 20.40)	2.65	9.44 (6.38 - 13.97)	2.94 (1.50 - 5.75)	
	Unknown	0	12056	0.00 (0.00 - 30.59)	0.17	-	-	
BMI	<20	3	36049	8.32 (1.72 - 24.32)	5.71	5.71 (1.84 - 17.69)	1	0.024
	20 – 25	23	183426	12.54 (7.95 - 18.81)	8.56	8.57 (5.69 - 12.89)	1.50 (0.45 - 5.00)	
	25+	17	319026	5.33 (3.10 - 8.53)	3.63	3.64 (2.26 - 5.85)	0.64 (0.19 - 2.17)	
	Unknown	3	82975	3.62 (0.75 - 10.57)	2.48	2.481 (0.80 - 7.69)	0.43 (0.09 - 2.15)	
Alcohol intake	Non-drinker	3	65076	4.61 (0.95 - 13.47)	0.95	3.16 (1.02 - 9.789)	1	0.107

		# with outcome	Patients[4]	Risk[5] (CI[6])	PMAR[7]	Rate[8] (CI)	Crude Rate Ratio (CI)	p-value[9]
		n=46	N=621476					
	Ex-drinker	0	20383	0.00 (0.00 - 18.10)	0.30	-	-	
	Current NOS	4	20585	19.43 (5.29 - 49.75)	0.30	13.32 (4.99 - 35.49)	4.22 (0.94 - 18.85)	
	2 or less u/d	12	96348	12.45 (6.44 - 21.76)	1.41	8.51 (4.83 - 14.99)	2.70 (0.76 - 9.55)	
	3/6 u/d	20	285307	7.01 (4.28 - 10.83)	4.18	4.78 (3.08 - 7.41)	1.51 (0.45 - 5.10)	
	>6 u/d	3	48911	6.13 (1.26 - 17.92)	0.72	4.19 (1.35 - 12.98)	1.33 (0.27 - 6.57)	
	Unknown	4	84866	4.71 (1.28 - 12.07)	1.23	3.23 (1.21 - 8.62)	1.02 (0.23 - 4.58)	
Socioeconomic status	1 (Highest SES)	12	144252	8.32 (4.30 - 14.53)	2.11	5.68 (3.23 - 10.00)	1	0.89
	2	10	145426	6.88 (3.30 - 12.65)	2.13	4.70 (2.53 - 8.73)	0.83 (0.36 - 1.91)	
	3	11	123932	8.88 (4.43 - 15.88)	1.81	6.07 (3.36 - 10.96)	1.07 (0.47 - 2.42)	
	4	8	115704	6.91 (2.99 - 13.62)	1.69	4.73 (2.36 - 9.45)	0.83 (0.34 - 2.04)	
	5 (Lowest SES)	5	92162	5.43 (1.76 - 12.66)	1.35	3.71 (1.54 - 8.92)	0.65 (0.23 - 1.86)	
Ethnicity[3]	White	35	425809	8.22 (5.73 - 11.43)	6.23	5.62 (4.03 – 7.82)	1	0.079
	South Asian	0	13640	0.00 (0.00 - 27.04)	0.20	-	-	
	Black	0	7836	0.00 (0.00 - 47.06)	0.11	-	-	
	Other	0	5639	0.00 (0.00 - 65.40)	0.08	-	-	
	Mixed	0	2094	0.00 (0.00 - 176.01)	0.03	-	-	
	Not Stated	9	74128	12.14 (5.55 - 23.05)	1.08	8.30 (4.32 – 15.96)	1.48 (0.71 - 3.08)	
	Unknown	2	92330	2.17 (0.26 - 7.82)	1.35	1.48 (0.37 – 5.90)	0.26 (0.06 - 1.09)	

Note 1: age groups 18-29, 30-39, 40-49 combined due to lack of events in the 18-29 or 30-39 age groups
Note 2: prescription counted if it occurred anytime from 1 month prior to index date or between index date and before end of follow-up (see Chapter 3 section 3.4.1.5)
Note 3: Obtained from CPRD, unless none found, in which case from HES (see Chapter 3 section 3.4.1.5)
Note 4: number of patients prescribed the drug
Note 5: per 100000 patients prescribed the drug
Note 6: 95% confidence interval
Note 7: 100000 person-months at risk
Note 8: per 100000 person months
Note 9: p-value results for LRT of an association over all categories of the variable

Chapter 5 Appendix Table 3

Results of classical (Mantel Haenszel) analysis of the association between flucloxacillin and cholestatic liver injury, using three different CPRD algorithm outcome definitions and the multisource algorithm

	CPRD algorithm (score 5)		*CPRD algorithm (score 2.29)*		*CPRD algorithm (score 1.63)*		*Multisource algorithm*	
	Rate Ratio (CI[1])	**p-value[2]**	**Rate Ratio (CI)**	**p-value**	**Rate Ratio (CI)**	**p-value**	**Rate Ratio (CI)**	**p-value**
Crude	3.79 (1.19 – 12.13)	-	5.51 (1.74 – 17.46)	-	3.74 (1.75 – 8.01)	-	9.02 (1.24 – 65.48)	-
Age at index date[3]	3.65 (1.16 – 11.51)	0.634	5.35 (1.72 – 16.68)	0.553	3.65 (1.72 – 7.73)	0.201	8.64 (1.20 – 62.42)	<0.001
18 – 49	-		-		2.65 (0.35 – 20.26)		0.19 (0.01 – 3.06)	
50 – 59	-		-		-		-	
60 – 69	-		-		-		-	
70 – 79	4.64 (0.62 – 34.59)		5.57 (0.75 – 41.17)		4.64 (1.12 – 19.21)		-	
80+	1.50 (0.35 – 6.47)		2.08 (0.49 – 8.80)		1.42 (0.50 – 3.99)		-	
Gender	3.81 (1.19 – 12.20)	0.872	5.54 (1.75 – 17.58)	0.924	3.76 (1.76 – 8.06)	0.651	8.98 (1.25 – 64.69)	0.256
Male	4.34 (0.58 – 32.24)		5.99 (0.82 – 43.96)		3.03 (0.94 – 9.75)		3.87 (0.52 – 28.84)	
Female	3.54 (0.85 – 14.79)		5.32 (1.29 – 21.87)		4.32 (1.58 – 11.80)			
Date of index prescription	3.57 (1.13 – 11.30)	0.339	5.26 (1.68 – 16.47)	0.221	3.62 (1.70 – 7.71)	0.489	8.94 (1.20 – 66.80)	0.690
2000 – 2001	-		-		-		-	
2002 – 2003	-		-		4.61 (0.62 – 34.42)		2.06 (0.26 – 16.22)	
2004 – 2005	-		-		5.21 (0.71 – 38.42)		-	
2006 – 2007	2.08 (0.27 – 16.12)		2.65 (0.35 – 20.14)		5.11 (0.69 – 37.59)		-	
2008 – 2009	-		-		3.81 (0.51 – 28.37)		-	
2010 – 2011	0.94 (0.20 – 4.27)		1.22 (0.27 – 5.39)		1.25 (0.37 – 4.20)		-	
Prescriptions for other causes of cholestatic injury[2]	5.16 (1.58 – 16.89)	0.844	7.34 (2.27 – 23.80)	0.819	4.72 (2.16 – 10.32)	0.565	12.62 (1.69 – 94.11)	0.952
None	-		-		-		-	
Rare cause	4.54 (1.40 – 14.72)		6.34 (1.98 – 20.28)		3.95 (1.82 – 8.55)		11.29 (1.54 – 82.54)	
More common cause	-		-		-		-	
Smoking status	3.87 (1.22 – 12.32)	0.689	6.22 (0.85 – 45.74)	0.631	3.80 (1.78 – 8.12)	0.713	8.98 (1.28 – 63.20)	0.056
Non-smoker	3.56 (0.47 – 26.81)		1.69 (0.21 – 13.31)		3.41 (1.06 – 10.96)		-	
Current smoker	1.31 (0.16 – 10.66)		8.92 (1.23 – 64.90)		1.97 (0.46 – 8.39)		1.21 (0.15 – 9.82)	
Ex-smoker	6.63 (0.90 – 48.67)		-		6.17 (1.51 – 25.32)		-	
Unknown	-				-		-	

	CPRD algorithm (score 5)		*CPRD algorithm (score 2.29)*		*CPRD algorithm (score 1.63)*		*Multisource algorithm*	
	Rate Ratio (CI[1])	**p-value[2]**	**Rate Ratio (CI)**	**p-value**	**Rate Ratio (CI)**	**p-value**	**Rate Ratio (CI)**	**p-value**
BMI	3.82 (1.19 – 12.23)	0.866	5.57 (1.76 – 17.67)	0.888	3.78 (1.77 – 8.09)	0.564	9.29 (1.27 – 67.84)	0.813
<20	-		-		-		-	
20 – 25	4.96 (0.67 – 36.78)		7.44 (1.02 – 54.38)		5.75 (1.40 – 23.61)		5.12 (0.69 – 37.83)	
25+	2.56 (0.60 – 10.82)		3.84 (0.92 – 15.93)		2.98 (1.08 – 8.23)		-	
Unknown	-		-		1.35 (0.17 – 10.99)		-	
Alcohol intake	3.81 (1.19 – 12.22)	0.508	5.53 (1.74 – 17.57)	0.544	3.76 (1.76 – 8.07)	0.519	9.11 (1.25 – 66.53)	0.939
Non-drinker	0.65 (0.07 – 6.20)		1.08 (0.13 – 9.21)		0.97 (0.21 – 4.48)		-	
Ex-drinker	-		-		-		-	
Current unknown	-		-		-		-	
2 or less u/d	-		-		3.61 (0.87 – 15.04)		-	
3-6 u/d	2.21 (0.52 – 9.43)		3.20 (0.76 – 13.40)		3.60 (1.12 – 11.56)		3.96 (0.53 – 29.60)	
Over 6u/d	-		-		-		-	
Unknown	-		-		-		-	
Socioeconomic status	3.80 (1.17 – 12.32)	0.245	5.52 (1.72 – 17.71)	0.216	3.74 (1.74 – 8.04)	0.461	9.12 (1.25 – 66.57)	0.471
1 (Least deprived)	-		-		-		-	
2	1.65 (0.21 – 13.17)		2.47 (0.32 – 19.00)		1.96 (0.46 – 8.40)		1.88 (0.24 – 14.84)	
3	-		-		-		-	
4	-		-		3.57 (0.48 – 26.86)		-	
5 (Most deprived)	-		-		2.61 (0.34 – 19.87)		-	
Unknown	0.88 (0.18 – 4.22)		1.25 (0.27 – 5.72)		2.00 (0.60 – 6.66)		-	
Ethnicity	3.78 (1.17 – 12.15)	0.028	5.49 (1.73 – 17.49)	0.023	3.73 (1.74 – 8.01)	0.361	8.94 (1.23 – 64.85)	0.851
White	-		-		5.38 (1.70 – 17.05)		6.75(0.92 – 49.28)	
South Asian	-		-		-		-	
Black	-		-		-		-	
Other	-		-		-		-	
Mixed	-		-		-		-	
Not Stated	-		-		-		-	
Unknown	0.81 (0.23 – 2.91)		1.18 (0.34 – 4.06)		1.77 (0.63 – 5.01)		-	

Note 1: 95% confident interval

Note 2: p-value of test of homogeneity across categories of variable

Note 3: For this and all subsequent variables in the table, the overall RR controlled for the variable is presented on the first row. Stratum specific estimates are presented in addition to the overall estimate for 2 reasons: (1) important differences between strata can be observed (which could indicate effect modification, with p-value also being considered) (2) to see the effect of the different algorithm score cut-off on availability of data for each strata. Rows with a "-" indicate a lack of individuals with the outcome in both or either of the exposure groups for that variable category.

Chapter 5 Appendix Table 4

Reasons why people considered as cases of cholestatic liver injury were subsequently considered as exclusions, following close review of electronic health record (from two years prior to index until injury date) for (i) the complete CPRD cohort (ii) the subset of the cohort linked to HES (iii) the subset of the cohort not linked to HES

	Complete CPRD cohort N total exclusions=89 (100%)	HES-linked subset N=27 (30%)	Subset not linked to HES N=62 (70%)
Reason for exclusion[1]	**n (%)**	**n (%)**	**n (%)**
Abdominal pain pre-index[2]	1 (1)	0	1 (2)
Alcoholic	12 (13)	0	12 (19)
Cancer	18 (20)	4 (15)	14 (23)
Cholangitis	6 (7)	4 (15)	2 (3)
Data entry error	1 (1)	0	1 (2)
Gallstone	2 (2)	2 (7)	0
Heart failure	6 (7)	1 (4)	5 (8)
Hepatitis A	2 (2)	1 (4)	1 (2)
Unwell pre-index[3]	17 (19)	7 (26)	10 (16)
Infective hepatitis	4 (4)	2 (7)	2 (3)
Kidney disease	1 (1)	0	1 (2)
Lack of info	4 (4)	0	4 (6)
Pancreatitis	2 (2)	1 (4)	1 (2)
Same day[4]	13 (15)	5 (19)	8 (13)

Note 1: Reasons may include more than one CPRD diagnostic code that have been grouped under a more general heading in this table
Note 2: A code of abdominal pain was present leading up to the injury date, and before the index date (prescription with study drug)
Note 3: Codes indicating that the patient was generally unwell leading up to the injury date and before the index date were present
Note 4: Liver related diagnosis was on the index date (prescription date), so a cause other than the drug(s) under study considered more likely

Chapter 5 Appendix Table 5

Example questionnaire for obtaining verification from a patient's clinician in relation to their case status, when using a relatively non-specific, high sensitivity method for identifying cases of cholestatic liver injury in CPRD when recruiting to genetic association studies

Dear General Practitioner,

Re: confirmation of [drugsubstancename]-induced cholestatic liver injury

The Clinical Practice Research Database (CPRD) research group at the UK MHRA is researching the genetic predisposition to adverse drug reactions.

We are currently using CPRD records to identify individuals who have experienced cholestatic liver injury as a result of therapy with [drugsubstancename], so that these individuals might be recruited for genetic analysis of this adverse drug reaction.

Based upon codes in the medical record, the following patient from your practice has been identified as a potential case of [drugsubstancename]-induced liver injury:

Patient identifier: [*whatever patient identifier CPRD use for questionnaires*]

CPRD record of potential [drugsubstancename]-induced liver injury:

CPRD record	Description	Date
Therapy	*[drugsubstancename]*	*20/06/2010*
Clinical diagnosis	*Jaundice*	*02/07/2010*

We would be grateful if you could answer the following questions in order to help us confirm whether these records represent a true case of [drugsubstancename]-induced cholestatic liver injury for this patient.

1. Has this patient ever been referred to a liver specialist? *If yes, we would be grateful if you could provide any letters from the specialist.*	☐ Yes – *please provide letters* ☐ No
2. Based upon your knowledge of this patient, do you consider the events indicated by the above records to represent liver injury caused by [drugsubstancename] therapy?	☐ Yes ☐ No* ☐ Unsure*

***If "No or Unsure" to question 2., please indicate which of the following are likely to have led to (or may have possibly contributed to) the clinical and test records detailed above:**

☐ Alcoholism

☐ Cancer of the liver, pancreas or gallbladder

☐ Cholecystitis

☐ Cholelithiasis

☐ Cirrhosis

☐ Congestive heart failure

☐ Gallbladder disease

☐ HIV infection

☐ Inflammatory bowel disease

☐ Drug other than [drugsubstancename] (please specify):_____

☐ Pancreatic disease

☐ Pregnancy

☐ Pre-existing chronic liver disease

☐ Viral hepatitis

☐ Other reason (please specify):

_____

_____

_____

Please send the completed questionnaire back using the addressed envelope provided

Chapter 6 Appendix

Chapter 6 Appendix Table 1

Chapter 6 Appendix Table 1a: Association between drug exposures and cholestatic liver injury (low-specificity algorithm) by prescription with other hepatotoxic drugs

		Multivariable RR[1] (95% CI[2]) by prescription with other hepatotoxic drugs		
		Not prescribed another cause of liver injury	Prescription for less common cause	Prescription for more common cause
Carbamazepine[3]	Never users	1	1	1
	Current users	1.66 (1.23 – 2.25)	1.03 (0.75 – 1.41)	0.40 (0.23 – 0.67)
	Recent users	1.35 (0.44 – 4.09)	1.66 (0.60 – 4.61)	0.51 (0.03 – 8.23)
	Past users	1.33 (1.06 – 1.69)	0.95 (0.81 – 1.11)	0.69 (0.48 – 0.98)
Celecoxib[4]	Never users	1	1	1
	Current users	1.60 (1.07 – 2.38)	0.59 (0.34 – 0.86)	0.84 (0.27 – 2.59)
	Recent users	3.57 (1.48 – 8.77)	1.47 (0.72 – 2.99)	1.14 (0.25 – 5.17)
	Past users	1.22 (1.00 – 1.49)	0.95 (0.84 – 1.06)	1.23 (0.89 – 1.72)
Ramipril[5]	Never users	1	1	1
	Current users	1.59 (1.41 – 1.79)	0.92 (0.83 – 1.02)	1.09 (0.82 – 1.45)
	Recent users	1.15 (0.51 – 2.61)	2.00 (1.30 – 3.07)	1.27 (0.25 – 6.38)
	Past users	1.51 (0.51 – 2.61)	0.91 (0.82 – 1.02)	0.76 (0.56 – 1.05)
Risperidone[6]	Never users	1	1	1
	Current users	3.22 (2.20 – 4.73)	1.38 (0.86 – 2.17)	1.14 (0.54 – 2.41)
	Recent users	3.57 (0.49 – 25.98)	0.55 (0.06 – 4.61)	1.54 (0.13 – 17.81)
	Past users	2.24 (1.39 – 3.59)	1.17 (0.85 – 1.61)	0.67 (0.36 – 1.22)
Flucloxacillin[7]	Never users	1	1	1
	Current users	3.73 (2.92 – 4.77)	1.73 (0.56 – 5.28)	1.47 (0.43 – 5.08)
	Recent users	6.25 (5.32 – 7.33)	1.89 (0.88 – 4.08)	0.96 (0.44 – 2.06)
	Past users	1.24 (1.17 – 1.32)	1.08 (1.03 – 1.13)	0.84 (0.71 – 1.00)

[1]Multivariable rate ratio, with adjustments as described in Table 2
[2]95% confidence interval
[3]p-value for Likelihood Ratio Test of interaction for carbamazepine:
[4]p-value for Likelihood Ratio Test of interaction for celecoxib:
[5]p-value for Likelihood Ratio Test of interaction for ramipril:
[6]p-value for Likelihood Ratio Test of interaction for risperidone:
[7]p-value for Likelihood Ratio Test of interaction for flucloxacillin:

Chapter 6 Appendix Table 1b: Association between drug exposures and cholestatic liver injury (low-specificity algorithm) by age at date of liver injury

		Multivariable RR[1] (95% CI[2]) by age at date of liver injury		
		18 - 49	50 - 69	70+
Celecoxib[1]	Never users	1	1	1
	Current users	3.72 (1.29 – 10.73)	0.74 (0.45 – 1.23)	0.89 (0.59 – 1.33)
	Recent users	18.50 (1.90 – 179.67)	2.13 (0.87 – 5.21)	1.34 (0.64 – 2.79)
	Past users	0.83 (0.53 – 1.30)	0.90 (0.75 – 1.08)	1.10 (0.98 – 1.24)
Duloxetine[2]	Never users	1	1	1
	Current users	3.54 (1.18 – 10.60)	1.94 (0.96 – 3.91)	0.37 (0.09 – 1.64)
	Recent users	1.57 (0.26 – 9.53)	1.45 (0.12 – 17.16)	1.57 (0.26 – 9.53)
	Past users	1.18 (0.51 – 2.74)	0.93 (0.46 – 1.90)	0.54 (0.26 – 1.12)
Ramipril[3]	Never users	1	1	1
	Current users	1.92 (1.32 – 2.79)	1.29 (1.13 – 1.47)	1.01 (0.91 – 1.12)
	Recent users	1.99 (0.54 – 7.29)	2.27 (1.19 – 4.34)	1.46 (0.91 – 2.36)
	Past users	1.52 (0.96 – 2.41)	1.04 (0.87 – 1.23)	0.94 (0.84 – 1.05)
Risperidone[4]	Never users	1	1	1
	Current users	4.79 (2.35 – 9.76)	2.91 (1.63 – 5.20)	1.38 (0.94 – 2.03)
	Recent users	-	1.91 (0.17 – 21.57)	0.83 (0.20 – 3.52)
	Past users	1.49 (0.87 – 2.54)	1.30 (0.76 – 2.22)	1.13 (0.80 – 1.59)
Flucloxacillin[5]	Never users	1	1	1
	Current users	4.20 (2.44 – 7.25)	5.01 (3.31 – 7.60)	2.48 (1.75 – 3.52)
	Recent users	2.75 (1.85 – 4.10)	7.07 (5.24 – 9.54)	5.89 (4.80 – 7.22)
	Past users	1.21 (1.11 – 1.31)	1.18 (1.11 – 1.26)	1.05 (0.99 – 1.11)

[1]Multivariable rate ratio, with adjustments as described in Table 2
[2]95% confidence interval
[3]p-value for Likelihood Ratio Test of interaction for celecoxib:
[4]p-value for Likelihood Ratio Test of interaction for ramipril:
[5]p-value for Likelihood Ratio Test of interaction for risperidone:
[6]p-value for Likelihood Ratio Test of interaction for flucloxacillin:

Chapter 6 Appendix Table 1c: Association between drug exposures and cholestatic liver injury (low-specificity algorithm) by ethnicity

		Multivariable RR[1] (95% CI[2]) by ethnicity						
		White	South Asian	Black	Other	Mixed	Not stated	Unknown
Duloxetine[3]	Never users	1	1	1	1	1	1	1
	Current users	1.69 (0.85 – 3.36)	-	1.69 (0.85 – 3.36)	1.69 (0.85 – 3.36)	1.69 (0.85 – 3.36)	0.26 (0.03 – 2.30)	1.92 (0.78 – 4.77)
	Recent users	0.61 (0.06 – 5.94)	0.61 (0.06 – 5.94)	0.61 (0.06 – 5.94)	0.61 (0.06 – 5.94)	0.61 (0.06 – 5.94)	0.61 (0.06 – 5.94)	5.11 (0.45 – 58.14)
	Past users	0.44 (0.22 – 0.88)	0.44 (0.22 – 0.88)	0.44 (0.22 – 0.88)	-	0.44 (0.22 – 0.88)	0.42 (0.04 – 4.03)	1.65 (0.89 – 3.07)
Ramipril[4]	Never users	1	1	1	1	1	1	1
	Current users	1.07 (0.97 – 1.19)	1.15 (0.50 – 2.61)	1.56 (0.57 – 4.27)	0.83 (0.22 – 3.11)	-	0.85 (0.64 – 1.11)	1.32 (1.15 – 1.50)
	Recent users	1.61 (1.01 – 2.55)	4.72 (0.61 – 36.51)	1.61 (1.01 – 2.55)	1.61 (1.01 – 2.55)	1.61 (1.01 – 2.55)	2.86 (0.85 – 9.77)	1.55 (0.74 – 3.24)
	Past users	0.91 (0.80 – 1.03)	0.67 (0.24 – 1.88)	0.96 (0.20 – 4.51)	-	1.30 (0.13 – 13.42)	0.88 (0.64 – 1.21)	1.20 (1.02 – 1.40)
Risperidone[5]	Never users	1	1	1	1	1	1	1
	Current users	1.93 (1.31 – 2.84)	26.06 (2.78 – 244.21)	7.60 (0.44 – 130.67)	1.93 (1.31 – 2.84)	1.93 (1.31 – 2.84)	4.11 (1.72 – 9.81)	1.40 (0.83 – 2.36)
	Recent users	-	-	-	-	-	0.64 (0.05 – 8.52)	3.01 (0.55 – 16.30)
	Past users	1.20 (0.84 – 1.71)	7.08 (1.02 – 49.02)	1.50 (0.14 – 16.39)	-	-	0.88 (0.36 – 2.08)	1.31 (0.87 – 1.98)

[1]Multivariable rate ratio, with adjustments as described in Table 2
[2]95% confidence interval
[3]p-value for Likelihood Ratio Test of interaction for duloxetine:
[4]p-value for Likelihood Ratio Test of interaction for ramipril:
[5]p-value for Likelihood Ratio Test of interaction for risperidone:

Chapter 6 Appendix Table 2

Chapter 6 Appendix Table 2a Characteristics of participants included in the case-control analysis by exposure to risperidone

		Not exposed to risperidone (N=96771) n (%)	Exposed to risperidone (N=596) n (%)
Age at index date	18 – 29	5283 (5)	19 (3)
	30 – 39	6738 (7)	28 (5)
	40 – 49	9922 (10)	56 (9)
	50 – 59	13917 (14)	51 (9)
	60 – 69	19300 (20)	79 (13)
	70 – 79	22286 (23)	122 (20)
	80+	19325 (20)	241 (40)
Gender	Male	51298 (53)	259 (43)
	Female	45473 (47)	337 (57)
Date of index prescription	1992-1993	4441 (5)	0 (0)
	1994-1996	7540 (8)	1 (0)
	1997-1999	9995 (10)	10 (2)
	2000-2002	14598 (15)	73 (12)
	2003-2005	17328 (18)	182 (31)
	2006-2008	17101 (18)	119 (20)
	2009-2011	15278 (16)	117 (20)
	2012-2014	10490 (11)	94 (16)
Prescriptions for other causes of cholestatic injury	None	55665 (58)	244 (41)
	Less common cause	38525 (40)	283 (47)
	More common cause	2581 (3)	69 (12)
Smoking status	Non-smoker	41294 (43)	296 (50)
	Ex-smoker	31836 (33)	162 (27)
	Current smoker	18342 (19)	112 (19)
	Unknown	5288 (5)	26 (4)
BMI	<20	5237 (5)	51 (9)
	20 - 25	28682 (30)	150 (25)
	25+	48295 (50)	274 (46)
	Unknown	14557 (15)	121 (20)
Alcohol intake	Non-drinker	11372 (12)	120 (20)
	Ex-drinker	3805 (4)	72 (12)
	Current NOS	2316 (2)	21 (4)
	2 or less u/d	16785 (17)	110 (18)
	3/6 u/d	41581 (43)	128 (21)
	>6 u/d	7819 (8)	40 (7)
	Unknown	13093 (14)	105 (18)
Socioeconomic status	1 (Highest SES)	17205 (18)	73 (12)
	2	17259 (18)	101 (17)
	3	16732 (17)	85 (14)
	4	14072 (15)	93 (16)
	5 (Lowest SES)	12924 (13)	102 (17)
	Unknown	18579 (19)	142 (24)
Ethnicity	White	47229 (49)	320 (54)

		Not exposed to risperidone (N=96771) n (%)	Exposed to risperidone (N=596) n (%)
	South Asian	849 (1)	10 (2)
	Black	448 (0)	7 (1)
	Other	410 (0)	2 (0)
	Mixed	98 (0)	1 (0)
	Not Stated	7598 (8)	52 (9)
	Unknown	40139 (41)	204 (34)

Chapter 6 Appendix Table 2b Characteristics of participants included in the case-control analysis by exposure to celecoxib

		Not exposed to celecoxib (N=94174) n (%)	Exposed to celecoxib (N=3193) n (%)
Age at index date	18 – 29	5297 (6)	5 (0)
	30 – 39	6730 (7)	36 (1)
	40 – 49	9859 (10)	119 (4)
	50 – 59	13674 (15)	294 (9)
	60 – 69	18698 (20)	681 (21)
	70 – 79	21405 (23)	1003 (31)
	80+	18511 (20)	1055 (33)
Gender	Male	50253 (53)	1304 (41)
	Female	43921 (47)	1889 (59)
Date of index prescription	1992-1993	4440 (5)	1 (0)
	1994-1996	7538 (8)	3 (0)
	1997-1999	9999 (11)	6 (0)
	2000-2002	14554 (15)	117 (4)
	2003-2005	16672 (18)	838 (26)
	2006-2008	16301 (17)	919 (29)
	2009-2011	14569 (15)	826 (26)
	2012-2014	10101 (11)	483 (15)
Prescriptions for other causes of cholestatic injury	None	54757 (58)	1152 (36)
	Less common cause	36889 (39)	1919 (60)
	More common cause	2528 (3)	122 (4)
Smoking status	Non-smoker	40269 (43)	1321 (41)
	Ex-smoker	30596 (32)	1402 (44)
	Current smoker	18001 (19)	453 (14)
	Unknown	5297 (6)	17 (1)
BMI	<20	5100 (5)	188 (6)
	20 - 25	27950 (30)	882 (28)
	25+	46656 (50)	1913 (60)
	Unknown	14468 (15)	210 (7)
Alcohol intake	Non-drinker	11097 (12)	395 (12)
	Ex-drinker	3641 (4)	236 (7)
	Current NOS	2250 (2)	87 (3)
	2 or less u/d	16168 (17)	727 (23)
	3/6 u/d	40361 (43)	1348 (42)
	>6 u/d	7646 (8)	213 (7)
	Unknown	13011 (14)	187 (6)
Socioeconomic status	1 (Highest SES)	16726 (18)	552 (17)
	2	16800 (18)	560 (18)
	3	16316 (17)	501 (16)
	4	13665 (15)	500 (16)
	5 (Lowest SES)	12674 (13)	352 (11)

		Not exposed to celecoxib (N=94174) n (%)	Exposed to celecoxib (N=3193) n (%)
	Unknown	17993 (19)	728 (23)
Ethnicity	White	45639 (48)	1910 (60)
	South Asian	827 (1)	32 (1)
	Black	448 (0)	7 (0)
	Other	401 (0)	11 (0)
	Mixed	98 (0)	1 (0)
	Not Stated	7432 (8)	218 (7)
	Unknown	39329 (42)	1014 (32)

Chapter 6 Appendix Table 2c Characteristics of participants included in the case-control analysis by exposure to ramipril

		Not exposed to ramipril (N=89365) n (%)	Exposed to ramipril (N=8002) n (%)
Age at index date	18 – 29	5297 (6)	5 (0)
	30 – 39	6730 (8)	36 (0)
	40 – 49	9768 (11)	210 (3)
	50 – 59	13239 (15)	729 (9)
	60 – 69	17715 (20)	1664 (21)
	70 – 79	19686 (22)	2722 (34)
	80+	16930 (19)	2636 (33)
Gender	Male	46973 (53)	4584 (57)
	Female	42392 (47)	3418 (43)
Date of index prescription	1992-1993	4433 (5)	8 (0)
	1994-1996	7516 (8)	25 (0)
	1997-1999	9876 (11)	129 (2)
	2000-2002	14190 (16)	481 (6)
	2003-2005	16276 (18)	1234 (15)
	2006-2008	15219 (17)	2001 (25)
	2009-2011	13034 (15)	2361 (30)
	2012-2014	8821 (10)	1763 (22)
Prescriptions for other causes of cholestatic injury	None	52904 (59)	3005 (38)
	Less common cause	34083 (38)	4725 (59)
	More common cause	2378 (3)	272 (3)
Smoking status	Non-smoker	38659 (43)	2931 (37)
	Ex-smoker	27850 (31)	4148 (52)
	Current smoker	17562 (20)	892 (11)
	Unknown	5283 (6)	31 (0)
BMI	<20	4938 (6)	350 (4)
	20 - 25	26801 (30)	2031 (25)
	25+	43323 (48)	5246 (66)
	Unknown	14303 (16)	375 (5)
Alcohol intake	Non-drinker	10656 (12)	836 (10)
	Ex-drinker	3134 (4)	743 (9)
	Current NOS	2188 (2)	149 (2)
	2 or less u/d	15011 (17)	1884 (24)
	3/6 u/d	38297 (43)	3412 (43)
	>6 u/d	7266 (8)	593 (7)
	Unknown	12813 (14)	385 (5)
Socioeconomic status	1 (Highest SES)	15919 (18)	1359 (17)
	2	15963 (18)	1397 (17)
	3	15429 (17)	1388 (17)
	4	12894 (14)	1271 (16)
	5 (Lowest SES)	12011 (13)	1015 (13)
	Unknown	17149 (19)	1572 (20)

		Not exposed to ramipril (N=89365) n (%)	Exposed to ramipril (N=8002) n (%)
Ethnicity	White	42689 (48)	4860 (61)
	South Asian	774 (1)	85 (1)
	Black	414 (0)	41 (1)
	Other	385 (0)	27 (0)
	Mixed	90 (0)	9 (0)
	Not Stated	7039 (8)	611 (8)
	Unknown	37974 (42)	2369 (30)

Chapter 6 Appendix Table 2d Characteristics of participants included in the case-control analysis by exposure to flucloxacillin

		Not exposed to flucloxacillin (N=89365) n (%)	Exposed to flucloxacillin (N=8002) n (%)
Age at index date	18 – 29	5297 (6)	5 (0)
	30 – 39	6730 (8)	36 (0)
	40 – 49	9768 (11)	210 (3)
	50 – 59	13239 (15)	729 (9)
	60 – 69	17715 (20)	1664 (21)
	70 – 79	19686 (22)	2722 (34)
	80+	16930 (19)	2636 (33)
Gender	Male	46973 (53)	4584 (57)
	Female	42392 (47)	3418 (43)
Date of index prescription	1992-1993	4433 (5)	8 (0)
	1994-1996	7516 (8)	25 (0)
	1997-1999	9876 (11)	129 (2)
	2000-2002	14190 (16)	481 (6)
	2003-2005	16276 (18)	1234 (15)
	2006-2008	15219 (17)	2001 (25)
	2009-2011	13034 (15)	2361 (30)
	2012-2014	8821 (10)	1763 (22)
Prescriptions for other causes of cholestatic injury	None	52904 (59)	3005 (38)
	Less common cause	34083 (38)	4725 (59)
	More common cause	2378 (3)	272 (3)
Smoking status	Non-smoker	38659 (43)	2931 (37)
	Ex-smoker	27850 (31)	4148 (52)
	Current smoker	17562 (20)	892 (11)
	Unknown	5283 (6)	31 (0)
BMI	<20	4938 (6)	350 (4)
	20 - 25	26801 (30)	2031 (25)
	25+	43323 (48)	5246 (66)
	Unknown	14303 (16)	375 (5)
Alcohol intake	Non-drinker	10656 (12)	836 (10)
	Ex-drinker	3134 (4)	743 (9)
	Current NOS	2188 (2)	149 (2)
	2 or less u/d	15011 (17)	1884 (24)
	3/6 u/d	38297 (43)	3412 (43)
	>6 u/d	7266 (8)	593 (7)
	Unknown	12813 (14)	385 (5)
Socioeconomic status	1 (Highest SES)	15919 (18)	1359 (17)
	2	15963 (18)	1397 (17)
	3	15429 (17)	1388 (17)
	4	12894 (14)	1271 (16)
	5 (Lowest SES)	12011 (13)	1015 (13)

		Not exposed to flucloxacillin (N=89365) n (%)	Exposed to flucloxacillin (N=8002) n (%)
	Unknown	17149 (19)	1572 (20)
Ethnicity	White	42689 (48)	4860 (61)
	South Asian	774 (1)	85 (1)
	Black	414 (0)	41 (1)
	Other	385 (0)	27 (0)
	Mixed	90 (0)	9 (0)
	Not Stated	7039 (8)	611 (8)
	Unknown	37974 (42)	2369 (30)

Drug Discovery Today • Volume 00, Number 00 • December 2013 PERSPECTIVE

feature

Development of predictive genetic tests for improving the safety of new medicines: the utilization of routinely collected electronic health records

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Serious adverse drug reactions are an important cause of hospitalization and can result in the withdrawal of licensed drugs. Genetic variation has been shown to influence adverse drug reaction susceptibility, and predictive genetic tests have been developed for a limited number of adverse drug reactions. The identification of patients with adverse drug reactions, obtaining samples for genetic analysis and rigorous evaluation of clinical test effectiveness represent significant challenges to predictive genetic test development. Using the example of serious drug-induced liver injury, we illustrate how a database of routinely collected electronic health records (EHRs) could be used to overcome these barriers by facilitating rapid recruitment to genome-wide association studies and supporting efficient randomized controlled trials of predictive genetic test effectiveness.

Adverse drug reactions are estimated to be responsible for over 5% of hospital admissions [1,2]. Approximately 150 drugs have been withdrawn from the market since 1960 owing to safety issues [3], in some cases many years after initial approval [4]. Evidence is increasing for a genetic predisposition to several serious adverse drug events [5]. For a limited number of drugs, observational genetic studies of association and subsequent randomized controlled trials (RCTs) of effectiveness of genotype-guided treatment have enabled predictive genetic tests to be developed that have allowed valuable medicines to remain on the market with greatly improved risk:benefit profiles [6].

Serious drug-induced liver injury is a leading cause of drug withdrawals [3]. Associations between specific genes and susceptibility to drug-induced liver injury caused by several drug therapies have been identified, although predictive genetic test development has been minimal and many genes conferring susceptibility are yet to be identified [7,8]. A major challenge is the time and cost associated with finding patients who have had a reaction of interest, and recruiting sufficient numbers for initial genome-wide association studies (GWAS) and subsequent replication studies [9]. Following predictive genetic test development, an RCT to evaluate effectiveness of the genotype-guided treatment versus standard care might be required [10], introducing further logistical challenges. The possibility of using databases of routinely collected EHRs to support pharmacogenomics has been discussed elsewhere (Yasmina *et al.* unpublished; [11–13]). In this article, we provide further detail by illustrating how an EHR database could be used to: (i) identify patients who have had serious adverse reactions linked to a newly licensed drug to invite them to provide genetic samples for GWAS; and (ii) test the efficacy of any developed genetic test in a cluster RCT. We illustrate these ideas using drug-induced liver injury as an example of an adverse drug reaction.

Identification of drug-induced liver injury within routinely collected EHRs

Routinely collected EHRs provide the potential for low-cost, efficient epidemiological cohort

 http://dx.doi.org/10.1016/j.drudis.2013.11.003

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identification and analysis [14]. Although database specific, an EHR for an individual patient typically includes a unique patient id, clinical diagnoses (as standardized diagnostic codes), drug prescriptions, laboratory test results and, in some cases, lifestyle information, such as smoking, drinking and body mass index. Coverage of the underlying population is likely to be broad, and new linkages between databases are enhancing the ability to ascertain disease status. For example, the UK Clinical Practice Research Datalink (CPRD) primary care database contains anonymized health information for approximately 8% of the total UK population and can be linked to the UK Hospital Episode Statistics database [15].

Effective case identification algorithms can be developed that utilize EHR databases to identify cases of drug-induced liver injury. Work has been performed demonstrating the portability of such algorithms across different institutions [16] and work is ongoing to facilitate standardized implementation across databases in different countries (Ruigomez and Brauer, unpublished, for the IMI PROTECT group [17]). An example algorithm is provided in Fig. 1. Potential drug-induced liver injury cases are identified based upon specific diagnostic codes (routinely inputted by clinicians). The database is searched for liver test results within a specific time period

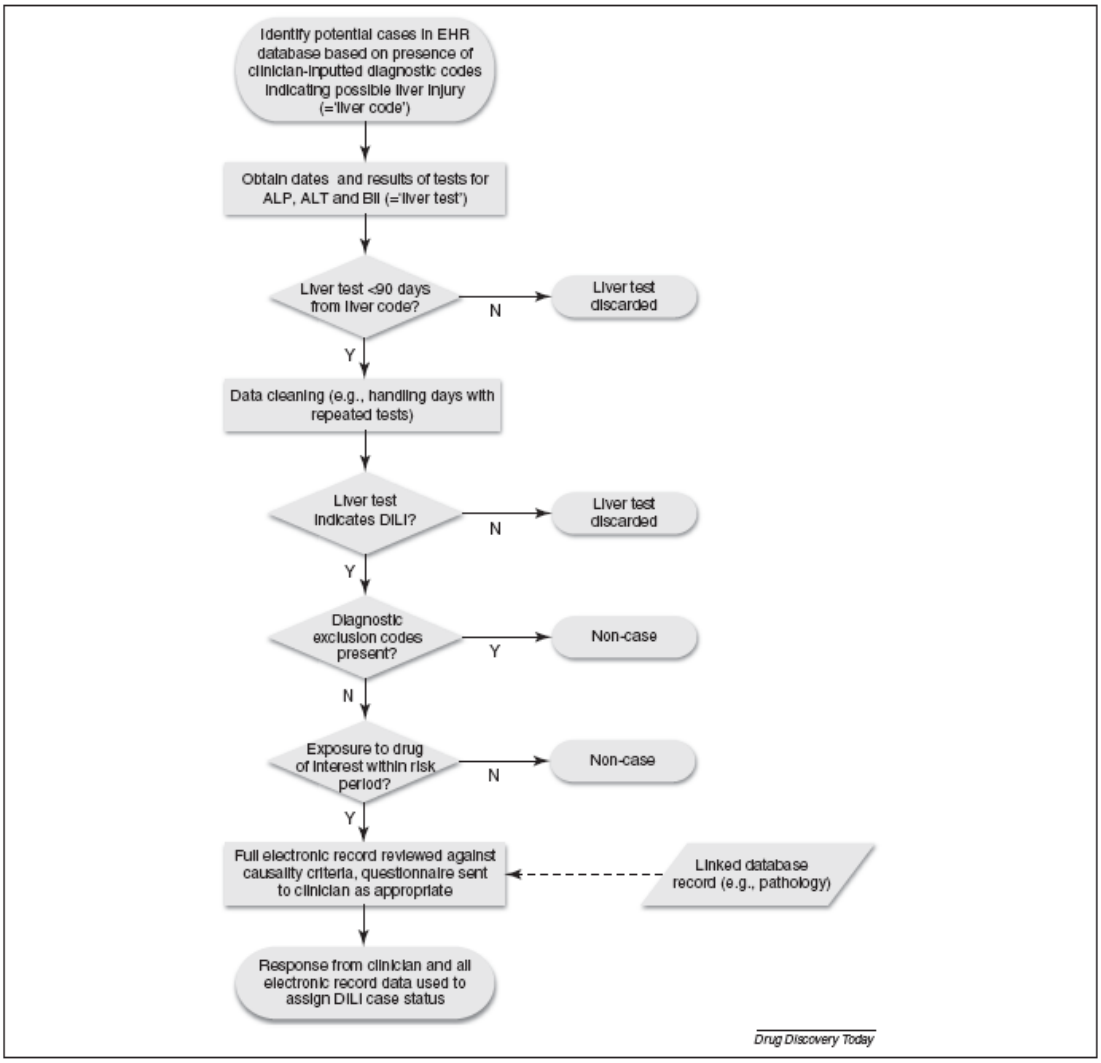

FIGURE 1

Example algorithm for identifying cases of drug-induced liver injury using a database of electronic health records. Drug-induced liver injuries (DILI) are injuries defined as ALT≥5 × ULN, ALP≥2 × ULN, or ALT≥3 × ULN with Bil>2 × ULN [13]. Causality criteria are as determined by international consensus [17]. *Abbreviations:* ALP, alkaline phosphatase; ALT, alanine aminotransferase; Bil, bilirubin; EHR, electronic health record.

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from the diagnostic code indicating possible liver injury. Patient records with no liver test results or results not indicative of drug-induced liver injury are removed, and the remaining patients are considered to be characteristic of drug-induced liver injury. The type of liver injury can be determined as required [13], before a set of exclusion codes is applied to remove individuals with other underlying (nondrug) causes of their liver symptoms. Verification of the cohort of potential cases can be performed by analyzing the association between being an algorithm-selected case and having recently been advised to take drugs that are well-known causes of drug-induced liver injury (such as flucloxacillin or amoxicillin-clavulanate [8]).

For potential cases with a prescription for the drug of interest within a specified risk period, a history of prescriptions and diagnoses (within a defined period) can be extracted from the database, enabling information on other potential causes of the liver injury to be obtained. Additional data on whether the patient was referred to hospital, and from linked databases could also be considered at this point (such as pathology databases or general hospital statistics databases). Brief questionnaires can then be sent to the responsible clinician as appropriate, to obtain any referral letters from liver specialists, and to ascertain whether the responsible clinician considers the events to represent drug-induced liver injury as a result of the drug in question. The totality of the health data obtained for each patient can then be reviewed by medically trained professionals and considered against international causality criteria [18], to identify probable cases.

Active monitoring of EHRs for recruitment to genetic association studies

Drug-induced liver injury algorithms as described above have typically been applied to database study populations at a single time point following drug registration, to retrospectively identify cases for inclusion in epidemiological studies [19,20]. For recruitment to genetic association studies, we propose an alternative 'active monitoring' model. Genetic sampling kits would be sent directly to the clinician of individuals identified as cases by continuous database surveillance, and the clinician would obtain consent and take a blood sample to be sent to the study coordinator.

Two possible information-processing approaches could support this model. The first approach would utilize the regular (e.g. monthly) download of EHRs from contributing health centers to the central database. The drug-induced liver injury algorithm could be run against the database following each download, and the clinicians of selected potential cases could then be invited to complete the short questionnaire described above, before a genetic sampling kit is sent to them if appropriate (Fig. 2). The second approach would be to utilize technology comparable to that installed on clinician desktops for performing pragmatic randomized trials within the UK CPRD database [21]: patients advised to take the drug of interest would be identified at the EHR database center and added to lists of eligible patients. The algorithm would be run locally on the clinician's desktop when an eligible patient had their health record updated and, if potential drug-induced liver injury was suggested, the patient would be flagged as an eligible potential case. The clinician would make the final decision on case status, and obtain and provide genetic samples using pre-provided genetic sampling kits (Fig. 3).

EHR-based RCTs of predictive genetic test effectiveness

Following development of a potential predictive test, an RCT would provide rigorous evidence that genotype-guided treatment improves health outcomes [10,22]. Existing EHR database infrastructure could enable a cluster RCT to be set up in which contributing health centers are randomly assigned to implement use of the predictive test or not, an approach that is already being applied for other disease areas [23]. In the intervention arm, patients with an indication for the drug of interest would be tested for the gene of susceptibility and the results could be used to inform subsequent treatment and monitoring; in non-intervention-arm health centers, treatment would be based on information available according to standard care only. Follow-up of the participating health centers could then be performed by using the algorithm to monitor EHR data on a monthly basis, to assess whether the rate of adverse drug reaction for the drug of interest differed between health centers randomized to use of the predictive test and those randomized to give standard care. An evaluation of subsequent changes in treatment approach based upon predictive test result could also be performed in this way.

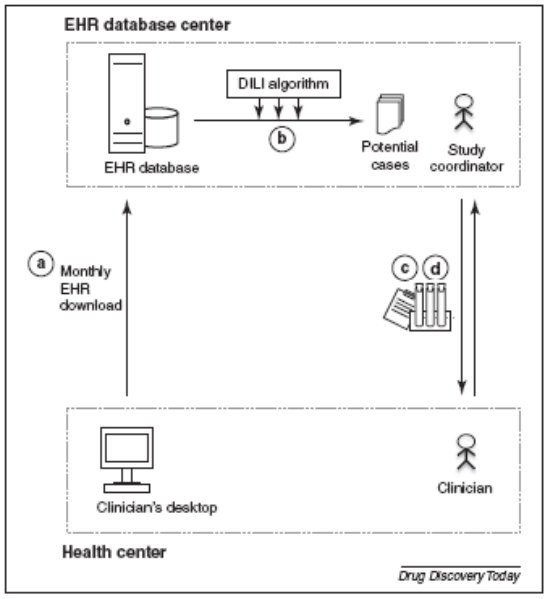

FIGURE 2

Actively monitoring an electronic health record (EHR) database to recruit to drug-induced liver injury (DILI) genetic association studies: approach one. **(a)** Updated EHRs are uploaded from the clinician's desktop to the EHR database according to the usual protocol (e.g. monthly); **(b)** the drug-induced liver injury algorithm is applied to the database of EHRs, to identify potential cases; **(c)** the study coordinator sends a short questionnaire to the responsible clinician of all the potential cases, and the coordinator uses this response and all the electronic record information to identify probable cases; **(d)** the study coordinator sends a genetic sampling kit to the clinician, who obtains consent from the probable case before taking genetic samples and sending back to the study coordinator.

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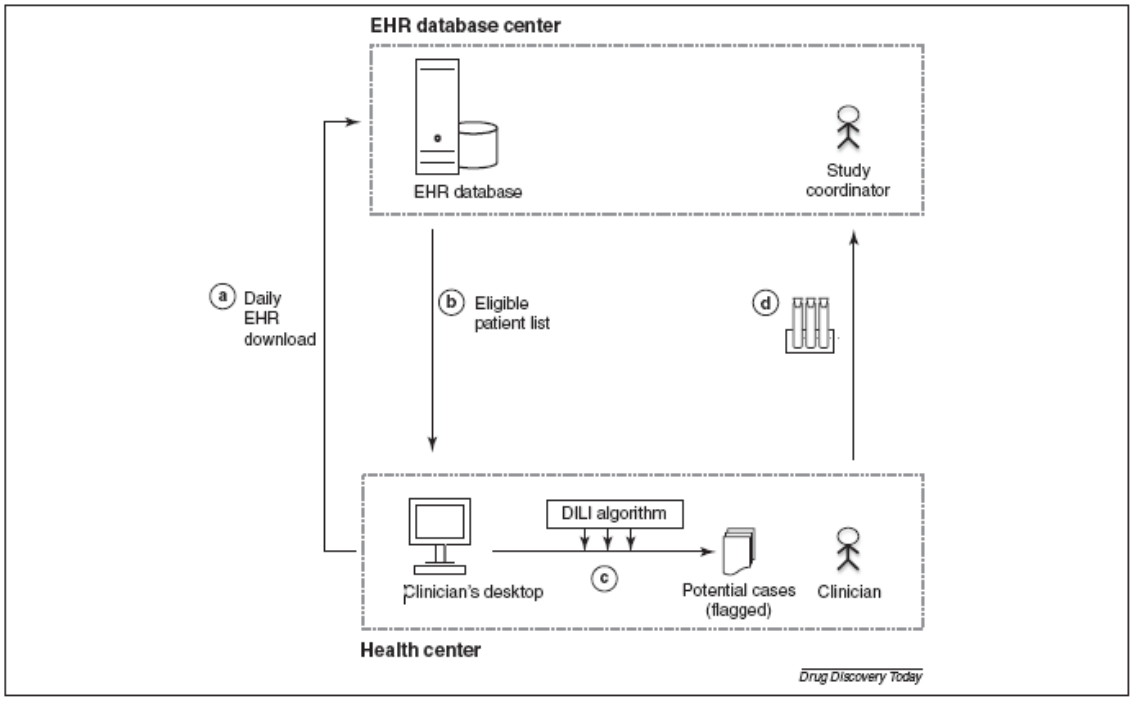

FIGURE 3

Actively monitoring an electronic health record (EHR) database to recruit to drug-induced liver injury (DILI) genetic association studies: approach two. **(a)** Daily downloads of information on drug prescriptions are transferred between the clinician's desktop and the EHR database. **(b)** This updates an eligible patient list within the EHR database, which is sent to the clinician's desktop each time it is updated. **(c)** The algorithm is run on the clinician's desktop for eligible patients. **(d)** Potential cases are flagged to the clinician, who makes a decision on case status. **(e)** Genetic samples from probable cases are provided by the clinician to the study coordinator.

Discussion

In an ideal world, one might investigate genetic determinants of drug-induced liver injury owing to a newly licensed drug by setting up a multicenter prospective observational study at hospital liver clinics, in which genetic samples are taken rapidly after a patient with a drug reaction is identified. However, such studies require a dedicated infrastructure and are costly to perform. Biobanks represent an alternative source of genetic information. Typically, these are repositories of prospectively stored genetic information for large numbers of patients [24] that provide valuable resources for studying (relatively common) complex gene–disease genetic associations. Unfortunately, the typically low frequency of adverse drug reactions means that recruiting sufficient patients from biobanks to carry out an adequately powered GWAS study within a short timeframe following drug registration would be difficult [25]. Further disadvantages include the high financial and environmental cost of each repository, and the possibility that a lack of inter-biobank standardization could result in measurement error within studies across biobanks [26].

One possible approach for addressing these challenges is the formation of collaborative groups, such as the International Serious Adverse Event Consortium (iSAEC), a large-scale private–public biomedical consortium [27,28]. Genetic samples obtained from large prospective studies and biobanks are shared between stakeholders, with an indication of the resource applied to this effort provided by the fact that six of the collaborators are top ten-ranked pharmaceutical companies [29]. This level of collaboration has contributed significantly to recent progress in the identification of adverse drug reaction–gene associations [30].

Our proposed model provides an alternative to an iSAEC-type approach, could be implemented at relatively low cost, and would enable active monitoring and recruitment for newly licensed drugs. By continuous retrospective selection of recent drug-induced liver injury from a population-based database of routinely collected EHRs, the beneficial characteristics of a large prospective multicenter prospective study are conferred, but with the most costly components already in place (i.e. multiple study centers, on-site study staff and individual patient records). Furthermore, the same infrastructure could be utilized for multiple drugs, the population being screened for the reaction is inherently large, detection does not rely on a hospital referral, and a source of population-based controls for case–control studies of genetic association is provided. Data are also routinely collected that would enable analysis of some coexisting clinical and environmental determinants of susceptibility to adverse drug reactions (another factor that might have contributed to a lack of progress in this area [9]). A comparable approach has enabled recruitment of over 700 patients from the UK CPRD for a statin-induced

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myopathy genetic association study, demonstrating the feasibility of this approach [31]. The ongoing work within the IMI-PROTECT consortium to standardize definitions across databases opens up the possibility of rapid recruitment for a single gene association study across multiple databases (such as the UK CPRD, Dutch Mondrian and Spanish BIFAP databases) [17]. As EHR databases start to become linked to biobanks [11], genetic samples collected in this way could be stored for use in future studies of specific adverse drug reactions.

We also propose a role for EHRs in performing RCTs of genetic test effectiveness [21]. The costs of a conventional multicenter RCT are likely to represent a serious obstacle to progress, and results might have limited generalizability [21]. Adoption of a recently proposed model for cluster RCTs performed within an EHR database [23] could address both of these challenges: whole health centers could be randomized to the use of a newly developed predictive genetic test, with minimum set-up costs, maximum reusability and a focus on test effectiveness in real-world settings.

Although this article focuses on the development of predictive genetic tests, we feel that the active monitoring approach described compares favorably with the current passive yellow card system for pharmacovigilance, and would also support adaptive licensing [32]. Spontaneous reporting systems suffer from reporting bias, missing denominator information and vague or incomplete case definitions, problems that would be minimized by applying real-time detection to one or multiple EHR databases. Targeted detection of adverse drug reactions observed during drug development could begin immediately following product launch (from day 1), and could be included as part of iterative data gathering within an adaptive licensing framework.

Concluding remarks

It is important to try and ensure that innovative products are not removed from the market because of risks associated with detectable genetic variation in the population, as has occurred previously [33]. In a climate where urgently needed new drugs (such as antibiotics) could have a worldwide impact on public health, unnecessary withdrawals could have severe consequences. Databases of EHRs can facilitate the development of rapid and low-cost predictive genetic tests, which could in turn prevent avoidable withdrawals, and reduce the number of patients unnecessarily exposed to serious adverse drug reactions.

Acknowledgments

The research leading to these results was conducted as part of the PROTECT consortium (Pharmacoepidemiological Research on Outcomes of Therapeutics by a European ConsorTium, http://www.imi-protect.eu), which is a public-private partnership coordinated by the European Medicines Agency. The PROTECT project has received support from the Innovative Medicine Initiative Joint Undertaking (http://www.imi.europa.eu) under Grant Agreement n° 115004, resources of which comprise financial contribution from the European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies' in kind contribution. In the context of the IMI Joint Undertaking (IMI JU), the London School of Hygiene and Tropical Medicine and the Department of Pharmacoepidemiology, Utrecht University, received direct financial contributions from Pfizer.

I.D. is funded by an MRC methodology fellowship; K.B. is funded by a post-doctoral fellowship from the National Institute of Health Research; L.S. is funded by a Wellcome Trust Senior Clinical Fellowship; and M.P. and L.S. are NIHR senior investigators. The views expressed are those of the authors only and not of their respective institutions, companies, or other bodies/committees that they might be associated with.

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