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Title: Quantification of the risk of liver injury associated with flucloxacillin: a United Kingdom population-based cohort study

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Short running title: Risk of flucloxacillin-induced liver injury

Synopsis

Background

The antibiotic flucloxacillin is an established cause of liver injury. Despite this, there are a lack of published data on both the strength of association after adjusting for potential confounders, and the absolute incidence among different sub-groups of patients.

Objectives

To assess the relative and absolute risks of liver injury following exposure to flucloxacillin and identify subgroups at potentially increased risk.

Methods

A cohort study between the 1st January 2000 and the 1st January 2012 using the UK Clinical Practice Research Datalink, including 1046699 people with a first prescription for flucloxacillin (861962) or oxytetracycline (184737). Absolute risks of experiencing both symptom-defined (jaundice) and laboratory-confirmed liver injury within 1-45 and 46-90 days of antibiotic initiation were estimated. Multivariable logistic regression was used to estimate 1-45 day relative effects.

Results

There were 183 symptom-defined cases (160 prescribed flucloxacillin) and 108 laboratory-confirmed cases (102 flucloxacillin). The 1-45 day adjusted risk ratio for laboratory-confirmed injury was 5.22 (95% CI 1.64-16.62) comparing flucloxacillin with oxytetracycline use. The 1-45 day risk of laboratory-confirmed liver injury was 8.47 per 100000 people prescribed flucloxacillin (95% CI 6.64-10.65). People who received consecutive flucloxacillin prescriptions had a 1-45 day risk of jaundice of 39.00 per 100000 (95% CI 26.85- 54.77), while those aged over 70 receiving consecutive prescriptions had a risk of 110.57 per 100000 (95% CI 70.86-164.48).

Conclusions

The short-term risk of laboratory confirmed liver injury was more than 5-fold higher after a flucloxacillin prescription than an oxytetracycline prescription. The risk of flucloxacillin-induced liver injury is particularly high within those over the age of 70 and those who receive multiple flucloxacillin prescriptions. The stratified risk estimates from this study could help guide clinical care.

Introduction

Fucloxacillin is an antibiotic of the penicillin class, that has a broad range of uses in the treatment of Gram-positive bacterial infections, including skin and soft tissue infections, respiratory tract infections, urinary-tract infections, meningitis and prophylaxis during surgery.[1] First available in 1960, case reports appeared in the 1980's of an adverse drug reaction in which the patient developed serious liver injury, which in some cases could be fatal.[2] While commonly and increasingly prescribed in the UK,[3] flucloxacillin is not marketed in the U.S. and some European countries, where alternative therapies perceived to have a better safety profile are used (such as dicloxacillin).

Previous work has shown flucloxacillin to be associated with liver injury at a frequency of approximately eight per 100000 people exposed within the general population.[4-6] Liver injury may occur up to 45 days from initiation of treatment, can be prolonged and is characterised by a predominantly cholestatic pattern of liver test results, and symptoms including jaundice. A number of epidemiological studies have identified an association, with increased age, prolonged duration of use and female gender identified as possible risk factors.[6-8] Despite this, there are a lack of available data either in the literature or prescribing information on (1) the strength of association after adjusting for potential confounders or (2) the absolute risk of either laboratory-confirmed or symptom-defined liver injury associated with flucoxacillin within these potentially high-risk groups.

The aims of this study were (1) to measure the association between being prescribed flucloxacillin and liver-injury (compared with being prescribed oxytetracycline) after adjusting for potential confounders of the association and (2) to quantify the risk of both symptom-defined (jaundice) and laboratory-confirmed injury within both the general population and subgroups at potentially increased risk.

Materials and Methods

Study design

A cohort analysis of the association between flucloxacillin and liver injury, with oxytetracyline as a comparator drug. Oxytetracycline was selected as it is an antibiotic that is not considered to be hepatotoxic that, in the clinical context within which the study was set, is used for a number of the same conditions as flucloxacillin, including skin infections, respiratory tract infections and urinary tract infections (see supplementary data section 1).

Setting

The study was performed within the UK Clinical Practice Research Datalink (CPRD), which contains comprehensive anonymised diagnostic, prescribing and lifestyle records on patients from over 625 NHS primary care practices from across the UK (approximately 12 million total patients, broadly representative of the UK population).[9] Further information is provided in the supplementary data (section 1) and elsewhere.[9]

Participants

The cohort was selected from patients actively registered in the CPRD between the dates of 1st January 2000 and 1st January 2012. The exposed group was made up of people over the age of 18 with at least one prescription for flucloxacillin and at least 6 months of research-quality prescription history in CPRD prior to their first recorded prescription of flucloxacillin (see supplementary data, section 1).

Patients with diseases or conditions that were likely to cause liver-related symptoms in their CPRD record within 6 months prior to their first recorded flucloxacillin prescription were excluded (see supplementary data section 2), as were people with any liver test results that met the criteria for drug-induced-liver injury[10] (Table 1) within the previous 6 months. Women who were pregnant at the time of their first recorded flucloxacillin prescription were also excluded (in order to avoid liver symptoms caused by cholestasis in pregnancy).

People prescribed oxytetracycline were selected as the comparator group, as oxytetraycline is an antibiotic with a similar range of indications to flucloxacillin that is not considered to cause liver injury.[6] The exclusion criteria applied to the oxytetracycline group were the same as in the group exposed to flucloxacillin.

Ethics

Ethical approval was obtained from the Clinical Practice Research Datalink Independent Scientific Advisory Committee (approval number 12_049) and the LSHTM Research Ethics Committee (approval number 6215).

Exposures, outcomes and co-variates

Exposures

Exposures were determined from CPRD prescription records. Based on results from previous studies suggesting injury may occur within a period of 6 weeks after flucloxacillin initiation,[5, 6] a person was considered exposed and at risk for 45 days after the start of a first prescription for flucloxacillin or oxytetracycline. The date of the first prescription was the index date, and people receiving both drugs on the index date were included in the flucloxacillin group only. Anyone who received oxytetracycline on their index date but then received flucloxacillin within 45 days was reassigned to the flucloxacillin group, and their index date updated appropriately. A categorical *number of flucloxacillin prescriptions* variable was created, that recorded how many prescriptions for flucloxacillin an individual received between their index date and the earliest of: an outcome event, an exclusion event, transfer out of the database, death or day 45. For those in the exposed to flucloxacillin group, a (comparator) day 46-90 exposure period was also included for analysis.

Outcomes

Diagnostic terms, codelists and laboratory parameters for the outcome were selected based upon a review of 12 studies[6, 11-21] identified by a systematic literature review performed for a previous study on liver injury.[22] Final review of outcome definitions was performed by a member of the study team who is a General Practitioner and Professor in Clinical Epidemiology (LS), and a list of final terms is provided in the supplementary data (section 3).

Assignment of outcome status was performed blinded to drug exposure status. Initially, potential cases were selected as people with any of a relatively broad list of liver-related diagnoses (supplementary data section 3) within the 90-day period after their index date (Figure 1). The 1-90 day period was searched (rather than just the 1-45 day risk period) because for those prescribed flucloxacillin, we wanted to compare the risk of injury in the 46-90 day period with that of the 1-45 day period. Any liver test results for Bilirubin (Bil), Alkaline Phosphatase (ALP) and Alanine Aminotransferase (ALT) recorded within the 1-90 day period were then identified for these potential cases. Blood levels of these enzymes taken from the same blood sample are standard parameters for indicating and classifying drug-induced liver injury based upon the R-value (a ratio of ALT to ALP, detailed in Table 1). Data management was performed to obtain R values as detailed previously.[22]

The R-values and Read codes were then used to define the following two potential liver injury case statuses:

(1) **Symptom-defined case**: people who had a liver-related diagnosis code within the 90-day period following the index date for any jaundice related diagnosis or symptom (see supplementary data section 3)

(2) **Laboratory-confirmed case**: people who had both of the following within the 90-day period following the index date (1) any of the liver-related diagnoses detailed in supplementary data section 3 and (2) a liver test result indicative of DILI (Table 1).

A symptom-only (jaundice) defined case definition was included due to the unavailibility of laboratory test results from secondary care within CPRD, meaning that reliance on only laboratory test results to define cases may underascertain the number of cases.[22]

The case-date for final symptom-defined cases was the date of jaundice, while for final laboratory-confirmed cases, it was the latest of the liver-related diagnosis or laboratory test result indicating DILI (Figure 1). The full electronic health record of all potential cases for the period from 6 months prior to the index date up until the case date was then reviewed by a clinician (AR), blinded to drug exposure status. Potential cases without any more likely causes of liver injury were designated as cases, while those with a more likely cause or liver-related symptoms occurring prior to the index date were considered to be exclusions, and either excluded from the analysis completely (if the exclusion event was prior to their index date) or were kept in the analysis but designated as non-cases (if the exclusion event happened after their index date but prior to their case date).

In order to assess the performance of our case detection method against an established method for assessing causality of drug-induced liver injury, we applied the RUCAM/CIOMS causality assessment method[23] to each of the laboratory-confirmed cases (see supplementary data section 3b).

Co-variates and risk factors

Results of previous studies and a causal diagram were used to assist with the selection of co-variates for the causal analysis. Age, gender, smoking, ethnicity, BMI, alcohol intake, socioeconomic status (SES), use of other drugs known to cause liver injury and calendar period were all included as potential measurable confounders. Further details are provided in supplementary data section 4a.

Potential risk factors for increased susceptibilty to flucloxacillin-induced liver injury were selected based on the results of previous studies[6-8] and included gender, age, and number of prescriptions.

Statistical analysis

Descriptive analysis

Co-variates were tabulated by exposure status, before the number of cases within the 1-90 day period within each drug-exposure group was calculated. For the flucloxacillin group, the proportion of type of liver injury (hepatocellular versus cholestatic), characteristic symptoms of cases and median time from first prescription until case assignment were also tabulated.

Overall risk of liver injury

The 1-45 day risk of liver injury for each drug was calculated by dividing the total number of events within the 45 day period after the index date by the number of patients in each exposure group (Figure 1). 95% confidence intervals were calculated on the basis of a Poisson distribution of injury events within each exposure group and the risk of liver injury occurring per 100000 people within each of the exposure groups was tabulated. The risk of liver injury in the 46-90 day period after exposure to flucloxacillin was also calculated (Figure 1).

Association between flucloxacillin and liver injury

For the analysis of the association between flucloxacillin and liver injury, all relative effects were calculated as odds ratios, which given the rarity of the outcomes under study were interpreted as risk ratios[24] (and will be referred to as such subsequently in this article).

Crude risk ratios comparing the risk of liver injury during the 1-45 day period after a first prescription of flucloxacillin to the risk during the 1-45 day after a first prescription of oxytetracycline (Figure 1) were obtained. A logistic regression model was then constructed, with potential confounders included as informed by the causal diagram, in order to estimate an overall adjusted risk ratio for the effect of flucloxacillin on liver injury.

Analysis of risk factors for flucloxacillin-induced liver injury

Risks per 100000 people exposed to flucloxacillin and multivariable adjusted risk ratios were calculated and tabulated across all categories of each potential risk factor, with tests-for-trend applied where appropriate. Graphs were plotted to illustrate the change in risk across categories for potential risk factors shown to increase susceptibility to injury.

Missing data and sensitivity analyses

A description of the handling of missing data is provided in the supplementary data (section 4b).

The following sensitivity analyses were performed: (1) removing those on co-fluampicil (2) removing those in the heaviest drinking category (3) removing people prescribed both flucloxacillin and oxytetracycline and (4) considering people with exclusion codes between drug prescription and an outcome event as cases.

All analysis was performed using STATA (StataCorp LP, version 14.0).

Results

Participants

Between the dates of 1st January 2000 and 1st January 2012 1073894 people aged 18 years and over were identified in CPRD who received a first prescription for either flucloxacillin or oxtetracycline and had been registered in the database for at least 6 months (Figure 2). 27156 people were subsequently removed as they did not meet the necessary eligibility criteria, leaving 1046738 patients in the cohort. An additional 39 were found to have reasons for exclusion during detailed potential case review, leaving a final cohort of 1046699 people for analysis.

Descriptive data

Background characteristics of participants are shown in Table 2. There were 861962 people prescribed flucloxacillin and 184737 prescribed oxytetracycline. 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 patients included a higher proportion of people on other drugs likely to cause liver injury than flucloxacillin patients (81% versus 52%). There was no difference in recorded ethnicity between the groups, and minimal differences in the distribution of all other characteristics between exposure groups. Ethnicity data was missing for 37% of the cohort.

Description of liver injury cases

Within 90 days from the index prescription, there were 183 symptom-defined cases (169 in the exposed to flucloxacillin group) and 108 laboratory-confirmed cases (102 in the exposed to flucloxacillin group). The type of liver injury within cases exposed to flucloxacillin was primarily (pure or mixed) cholestatic (69% of cases), and the median time from first flucloxacillin prescription until symptom-defined case assignment was 38 days (IQR 27–47), increasing to 40 days (IQR 32–48) for laboratory-confirmed cases (supplementary data section 5, Table S1).

Risk of liver injury associated with flucloxacillin

Table 3 shows absolute risk figures and both crude and multivariable adjusted results of the association between flucloxacillin and liver injury (compared with oxytetracycline).

There were 73 out of 861962 people prescribed flucloxacillin with laboratory-confirmed liver injury within the 45-days after prescription, giving a 1-45 day risk of flucloxacillin-induced liver injury of 8.47 cases per 100000 people (95% CI 6.64–10.65). The risk of laboratory-confirmed injury for those exposed to oxytetracycline within the same time period was 1.62 per 100000 people (95% CI 3.35–4.75), while the risk within those in the flucloxacillin group within the 46–90 day period from first

prescription was 3.45 per 100000 (95% CI 2.31–4.95) (data not shown). For the case definition requiring only a symptom or diagnosis of jaundice (symptom-defined), the risk of liver injury within the 1-45 day period for those prescribed flucloxacillin was almost double that of the laboratory confirmed case definition (14.15 per 100 000, 95% CI 11.75–16.92) (Table 3).

The crude risk ratio for the association between flucloxacillin and laboratory-confirmed liver injury was 5.22 (95% CI 1.65–16.57). There was no change in this estimate following multivariable adjustments (RR 5.22, 95% CI 1.64–16.62). The multivariable risk ratio for the symptom-defined outcome was lower than the laboratory-confirmed estimate, but had narrower confidence intervals (RR 3.73, 95% CI 1.73–8.03).

Risk factors for flucloxacillin-induced liver injury

There was strong evidence that increasing age was a risk factor for flucloxacillin-induced liver injury (p test-for-trend<0.001 for both symptom-based and laboratory confirmed outcomes), with a marked increase in the 1-45 day risk of injury in those over the age of 70 (e.g. multivariable-adjusted RR for laboratory-confirmed liver injury comparing those in the 70-79 year old age group with those aged 18-49: 23.26, 95% CI 7.88–68.67) (Table 4). There was also strong evidence for an increased 1-45 day risk of injury with increasing number of prescriptions (p test-for-trend<0.001), with people receiving 3 or more prescriptions within the 1-45 day risk period experiencing 9.37 times the 1-45 day risk of laboratory-confirmed injury (95% CI 4.40 – 19.95) than those receiving a single prescription within this period, after adjusting for age, gender and concomitant prescriptions for other causes of liver injury. For gender, there was a suggestion across both outcomes that females had a slightly increased risk of injury, although the 95% CI did not rule out a decreased risk (e.g. multivariable RR for symptom-based injury comparing females to males: 1.43, 95% CI 0.98–2.08).

Considering the absolute 1-45 day risk per 100000 people exposed to flucloxacillin, the risk of jaundice in the 18-49 year-old age group was 2.87, 95% CI 1.53-4.90, increasing to 14.71 (95% CI 8.86-22.98) in the 50-59 year old age group (Table 4 and Figure 3A). Within those over the age of 70, the absolute risk of jaundice was 45.30 per 100000 people (95% CI 35.69-56.69). In the overall population the risk of jaundice for those receiving a single prescription was 11.45 (95% CI 9.19-14.09), increasing to 78.60 per 100000 (95% CI 33.94–154.82) within people receiving three or more flucloxacillin prescriptions (Table 4 and Figure 3B). People over the age of 70 receiving three or more prescriptions had a risk of jaundice of 163.83 (95% CI 53.21-381.9) (Figure 3B), while over 70 yr olds receiving 2 or more had a risk of 110.57 per 100000 (95% CI 66.35-154.79). Risk figures for

laboratory-confirmed injury were generally smaller in magnitude but demonstrated similar changes by age group and increasing number of prescriptions (Table 4 and Figure 3A).

Performance of case definition compared to the RUCAM/CIOMS method

The RUCAM/CIOMS method[23] classified 63/73 (86%) of laboratory-confirmed cases from this study as "Probable (flucloxacillin) ADR" (see supplementary data section 3b for description of categories). The remaining 10/73 (14%) were classified as "Possible (flucloxacillin) ADR". Of these, 5 were under the RUCAM/CIOMS age risk factor cut-off of 55 years old, with the remaining 5 having a prescription record for another drug that may have been more likely to have caused the observed injury. Within 1-45 day laboratory-confirmed cases over the aged of 70, 91% (42/46) were classified as RUCAM/CIOMS probable.

Pattern of liver injury by age

We performed a post-hoc analysis of the 73 people exposed to flucloxacillin with laboratory-confirmed liver injury to investigate whether the pattern of liver injury associated with flucloxacillin use varied by age group. 38 of the 46 people aged 70 years or over had a cholestatic type of injury (83%, 95% CI 71% - 94%), compared with 15 out of 27 under the age of 70 (56%, 95% CI 35% - 76%, Mann Whitney test p=0.01).

Sensitivity analyses and missing data

None of the sensitivity analyses performed had anything other than a negligible impact on the results obtained. There was minimal difference between univariable analysis results obtained using complete records compared to the multiply imputed dataset (supplementary data section 5, Tables S2 and S3).

Discussion

In this study we have shown flucloxacillin to be associated with 5.22 (95% CI 1.64-16.62) times the 1-45 day risk of laboratory confirmed liver injury than oxytetracycline after multivariable adjustments, with an absolute 1-45 day risk of 8.47 (95% CI 6.64-10.65) per 100000 people prescribed the drug for the first time. There was strong evidence that increasing age and number of prescriptions were associated with increased flucloxacillin-induced liver injury, with those over the age of 70 who received at least one additional flucloxacillin prescription within 45 days of their initial prescription having a risk of jaundice of 110.57 per 100000 people (95% CI 66.35-154.79).

Comparison with previous studies

Our estimate of the overall risk of laboratory-confirmed liver injury is comparable to previously published risk estimates of 7.57 (95% CI 3.63-13.92)[7] and 8.48 (95% CI 5.43-12.61).[6] While previous studies have estimated the relative effect of age on risk to be between 18.61 (comparing over 55s versus under 30s)[8] and 6.1 (comparing over 60 versus under 60),[6] our large study is the first to estimate absolute risk figures by age categories, and has shown that those over 70 years of age experience the highest risk. We found a nine-fold increased risk in people given three or more flucloxacillin prescriptions compared with those given one prescription, also consistent with previous work showing that those with more than 14 consecutive days' use have 7.13 times the risk of injury than people using for less than this period (95% CI 2.90-17.58).[8] The size of our study has allowed us to demonstrate a dose(prescription)-response effect, and show that those over the aged of 70 who receive more than 1 prescription within the 1-45 day period have a particularly elevated risk.

Implications and further work

Current flucoxacillin prescribing information relating to hepatic side-effects[1] states that (1) jaundice affects less than 1 in 10000 people and (2) the drug should be used cautiously in people over 50 years of age. Our results suggest that flucloxacillin causes jaundice at a frequency closer to 1 in 7000 people in the overall population, that prolonged use is likely to increase the risk further, and those over the age of 70 have an approximately 15-fold higher risk than those under the age of 50. This is a particular concern when considering recent flucloxacillin prescribing trends showing that people over the age of 70 have both the highest prescribing rates and largest increase in rates.[3] We would therefore hope that these findings could help physicians gain a greater understanding of the nature of the risk involved with prescribing flucloxacillin, and exercise caution in prescribing particularly long treatment courses to those over the age of 70. In a clinical setting the choice may be between flucloxacillin and another drug with known adverse effects on the liver – the absolute risk figures provided in our study would help inform clinicians' prescribing decisions in this situation.

In terms of a mechanism for an age-dependent increase in the risk of flucloxacillin-induced liver injury, it is plausible that impaired renal function in the elderly could increase drug concentrations.[25] Not all drugs associated with liver injury demonstrate a similar age-dependent increase in risk, however,[26] suggesting an alternative mechanism. An increased use of concomitant hepatotoxins amongst the elderly has also been suggested as contributing to the observed increased risk,[26] but in our study we adjusted for use of a large number of known hepatotoxins. We did observe that patients over the age of 70 had a higher proportion of cholestatic (versus hepatocellular) injury than those under 70 (consistent with previous studies on drug-induced liver injury),[27, 28] and we hope this could help inform studies on the mechanism of flucloxacillin-induced liver injury in the future.

We would also hope that our findings might help further development of a predictive genetic test and/or elucidation of mechanism via genetic association studies. Genetic analysis has demonstrated the *HLA-B*5701* genotype to be a major determinant of drug-induced liver injury due to flucloxacillin.[29] Despite this finding, subsequent consideration of clinical utility[30] showed that (based on an overall population prevalence of 8.5 per 100000) predictive genetic testing for the reaction would be unfeasible, as 13513 people would need screened in order to prevent 1 case. Assuming that all of the cases of jaundice attributed to flucloxacillin in this study fulfil the criteria for DILI (which we consider a fair assumption, given how clear an indicator jaundice is of a serious liver problem), calculating the number needed to test within those over 70 using the drug reduces this number to 2512 (see supplementary data section 6). Although still likely to be prohibitively high, further elucidation of characteristics associated with increased risk may allow the number needed to test to be reduced further for specific groups in the future.

Limitations

It is likely that older people will have more liver tests performed, meaning that ascertaiment bias could have affected our results. We found comparable results for jaundice-defined cases, however, making this an unlikely explanation for our results. There is no specific Read code or term to allow a clinician to record a case of drug-induced liver injury within CPRD, meaning that there was an element of clinical uncertainty around assigning case status. We attempted to overcome this by using a detailed algorithm based upon a literature search of diagnostic terms, defined standards for laboratory test patterns indicative of drug-induced liver injury and applying multiple case definitions. We were also able to demonstrate that 86% of the cases of liver injury that we attributed to flucloxacillin would have been assigned as “Probable” flucloxacillin-induced liver injury by the RUCAM/CIOMS causality assessment method (91% of those in the over 70 year-old age group). Improved coding and linkages with (e.g.) liver pathology databases could simplify this process in the future. Utilising existing linkages between CPRD and the UK Hospital Episodes Statistics database and Office of National Statistic mortality data could have allowed biopsy, scan and mortality data to be

considered, which if combined with laboratory results can be used to support the diagnosis of drug-induced liver injury.[10] In a previous study, however, we found that an algorithm for detecting liver injury that included information on death and 11 different biopsy/scan procedure terms from these data sources provided only very limited improvement on the ability to detect cases (when compared to the use of diagnostic and biochemical criteria from CPRD alone).[22] The use of our very broad definition (i.e. just jaundice) means that a small degree misclassification of outcome is possible. We used a very thorough process of review to rule out other causes of injury, however, and considered jaundice to be a clear marker of a serious liver problem. Furthermore, the choice not to use the linked datasets meant we had a larger sample size within which our stratified analyses had better power.

Our causal analysis could have been impacted by confounding by indication. In order to assess the potential for this to occur, we tabulated the ten most common diagnostic terms entered on the index date for each drug (supplementary sata section 7). For both drugs the predominant diagnosis was a skin condition – acne for oxytetracycline, cellulitis/skin and subcutaneous tissue infections for flucloxacillin. As cirrhosis is a recognised risk factor for cellulitis,[31] it is plausible that some of the liver injury observed in flucloxacillin users could be attributed to underlying cirrhosis. We consider this to be highly unlikely, however, due to the fact that (1) cirrhosis was included as an exclusion term in our study and (2) we performed a detailed (blinded) clinician review of medical records in the 6 months prior to index date in order to rule out non-drug causes of injury. We also believe that the strength of the association we observe is too large to be explained by confounding by indication. Finally, although we aimed to include participants based upon first-time use of the drugs under study, it is possible that patients may have been prescribed the drugs prior to registration with a General Practice (GP) contributing to CPRD, which could mean that our risk estimates are an underestimation of the true frequency within those prescribed flucloxacillin for the first time.

Conclusions

In the largest study of flucloxacillin-induced liver injury to date, we have provided new absolute risk estimates by age, number of prescriptions and gender for both laboratory-confirmed injury and jaundice, providing insight into groups particularly susceptible to harm, especially those aged over 70 years receiving multiple prescriptions. These results should help guide clinical care decisions and support further work on predictive genetic test implementation.

Acknowledgements

None.

Funding

Kevin Wing's work on this study was funded by PROTECT (http://www.imi-protect.eu/). 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. Additional funding sources are as follows:

- Krishnan Bhaskaran holds a Sir Henry Dale fellowship jointly funded by the Wellcome Trust and the Royal Society
- Louise Pealing is funded by a National Institute of Health Research Collaboration for Leadership in Applied Health Research and Care (grant n° BZR00180/P2-503/BZ35.17)
- Liam Smeeth is funded by a Wellcome Trust Senior Clinical Fellowship (grant n° WT - 098504/Z/12/Z)
- Adrian Root is funded by an MRC Population Health Scientist Fellowship (grant n° MR/M014649/1)
- Ian Douglas is funded by an unrestricted grant from GlaxoSmithKline.

Transparency declarations

All authors except Kevin Wing, Olaf Klungel, Robert Reynolds declare no conflicts of interests.

Kevin Wing and Olaf Klungel specified receiving grants from PROTECT during the study, with the following additional explanatory comment:

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 (*Kevin*)/Department of Pharmacoepidemiology, Utrecht University (*Olaf*), received direct financial contributions from Pfizer.

Robert Reynolds declared he that he is an employee and shareholder of Pfizer, Inc. and that the views expressed are those of the authors and not necessarily those of Pfizer, Inc.

References

1. Pharmaceuticals K. Flucloxacillin 250mg capsules - Summary of Product Characteristics. In: Pharmaceuticals K, ed. *electronic Medicines Compendium (eMC)*, 2013.
2. (ADRAC) AADRAC. Fatal hepatic reactions to flucloxacillin. 1994.
3. Francis NA, Hood K, Lyons R et al. Understanding flucloxacillin prescribing trends and treatment non-response in UK primary care: a Clinical Practice Research Datalink (CPRD) study. *Journal of Antimicrobial Chemotherapy* 2016; **71**: 2037-46.
4. Andrews E, Daly AK. Flucloxacillin-induced liver injury. *Toxicology* 2008; **254**: 158-63.
5. Devereaux BM, Crawford DH, Purcell P et al. Flucloxacillin associated cholestatic hepatitis. An Australian and Swedish epidemic? *European journal of clinical pharmacology* 1995; **49**: 81-5.
6. Russmann S, Kaye JA, Jick SS et al. Risk of cholestatic liver disease associated with flucloxacillin and flucloxacillin prescribing habits in the UK: Cohort study using data from the UK General Practice Research Database. *British Journal of Clinical Pharmacology* 2005; **60**: 76-82.
7. Derby LE, Jick H, Henry DA et al. Cholestatic hepatitis associated with flucloxacillin. *Medical Journal of Australia* 1993; **158**: 596-600.
8. Fairley CK, McNeil JJ, Desmond P et al. Risk factors for development of flucloxacillin associated jaundice. *Bmj* 1993; **306**: 233-5.
9. Herrett E, Gallagher AM, Bhaskaran K et al. Data Resource Profile: Clinical Practice Research Datalink (CPRD). *International journal of epidemiology* 2015; **44**: 827-36.
10. Aithal GP, Watkins PB, Andrade RJ et al. Case Definition and Phenotype Standardization in Drug-Induced Liver Injury. *Clin Pharmacol Ther* 2011; **89**: 806-15.
11. Cheetham TC, Lee J, Hunt CM et al. An automated causality assessment algorithm to detect drug-induced liver injury in electronic medical record data. *Pharmacoepidemiology and Drug Safety* 2014; **23**: 601-8.
12. De Abajo FJM, D., Madurga M, Garcia Rodriguez LA. Acute and clinically relevant drug-induced liver injury: A population case-control study. *British Journal of Clinical Pharmacology* 2004; **58**: 71-80.
13. García Rodríguez LA, Duque A, Castellsague J et al. A cohort study on the risk of acute liver injury among users of ketoconazole and other antifungal drugs. *British Journal of Clinical Pharmacology* 1999; **48**: 847-52.
14. García Rodríguez LA, Stricker BH, Zimmerman HJ. Risk of acute liver injury associated with the combination of amoxicillin and clavulanic acid. *Archives of Internal Medicine* 1996; **156**: 1327-32.
15. García Rodríguez LA, Wallander MA, Stricker BH. The risk of acute liver injury associated with cimetidine and other acid-suppressing anti-ulcer drugs. *British Journal of Clinical Pharmacology* 1997; **43**: 183-8.
16. García Rodríguez LA, Williams R, Derby LE et al. Acute liver injury associated with nonsteroidal anti-inflammatory drugs and the role of risk factors. *Archives of Internal Medicine* 1994; **154**: 311 - 6.
17. Huerta C, Zhao SZ, García Rodríguez LA. Risk of acute liver injury in patients with diabetes. *Pharmacotherapy* 2002; **22**: 1091-6.
18. Jick H, Derby L, Dean A. Flucloxacillin and cholestatic hepatitis. *The medical journal of Australia* 1994; **160**: 525.
19. Jick H, Derby LE. A large population-based follow-up study of trimethoprim-sulfamethoxazole, trimethoprim, and cephalexin for uncommon serious drug toxicity. *Pharmacotherapy* 1995; **15**: 428-32.
20. Li L, Jick H, Jick SS. Updated study on risk of cholestatic liver disease and flucloxacillin: Letter to the Editors. *British Journal of Clinical Pharmacology* 2009; **68**: 269-70.
21. Shin J, Hunt CM, Suzuki A et al. Characterizing phenotypes and outcomes of drug-associated liver injury using electronic medical record data. *Pharmacoepidemiol Drug Saf* 2013; **22**: 190-8.
22. Wing K, Bhaskaran K, Smeeth L et al. Optimising case detection within UK electronic health records: use of multiple linked databases for detecting liver injury. *BMJ Open* 2016; **6**.

23. Danan G, Benichou C. Causality assessment of adverse reactions to drugs—I. A novel method based on the conclusions of international consensus meetings: Application to drug-induced liver injuries. *Journal of Clinical Epidemiology* 1993; **46**: 1323-30.
24. Kirkwood BR, Sterne JAC. 16.6 Odds Ratios. In: Blackwell, ed. *Essential Medical Statistics*. Oxford: Blackwell, 2005; 161.
25. Wynne HA, Cope LH, Mutch E et al. The effect of age upon liver volume and apparent liver blood flow in healthy man. *Hepatology* 1989; **9**: 297-301.
26. Chalasani N, Bjornsson E. Risk factors for idiosyncratic drug-induced liver injury. *Gastroenterology* 2010; **138**: 2246-59.
27. Lucena MI, Andrade RJ, Kaplowitz N et al. Phenotypic characterization of idiosyncratic drug-induced liver injury: the influence of age and sex. *Hepatology* 2009; **49**: 2001-9.
28. Andrade RJ, Lucena MI, Fernandez MC et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. *Gastroenterology* 2005; **129**: 512-21.
29. Daly AK, Donaldson PT, Bhatnagar P et al. HLA-B[ast]5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet* 2009; **41**: 816-9.
30. Alfirevic A, Pirmohamed M. Predictive genetic testing for drug-induced liver injury: considerations of clinical utility. *Clin Pharmacol Ther* 2012; **92**: 376-80.
31. Hamza RE, Villyoth MP, Peter G et al. Risk factors of cellulitis in cirrhosis and antibiotic prophylaxis in preventing recurrence. *Annals of Gastroenterology : Quarterly Publication of the Hellenic Society of Gastroenterology* 2014; **27**: 374-9.

500 **Table 1: Classification of drug-induced liver injury based on liver test results[10]**

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

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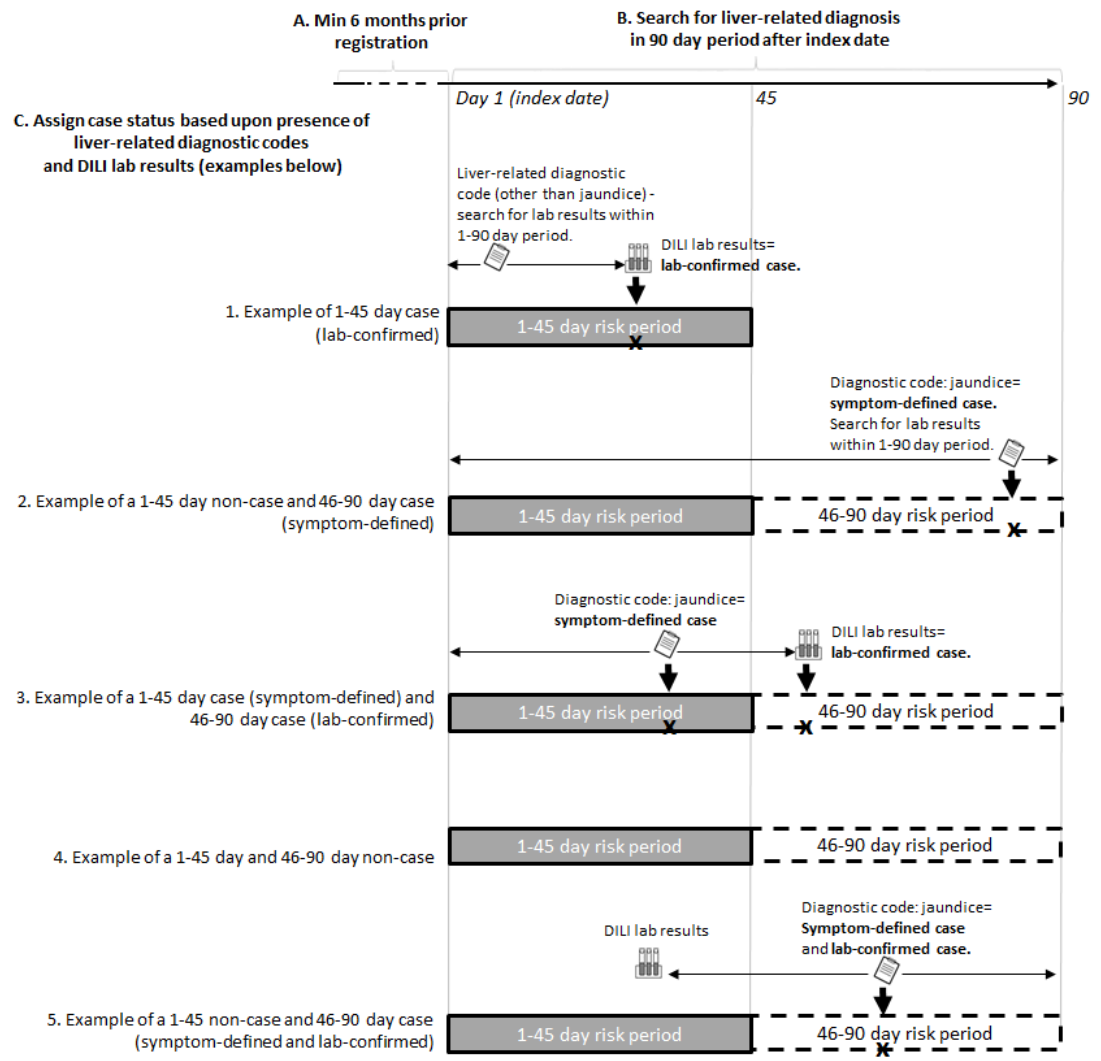
Figure 1: Overview of time windows used for case assignment and analysis performed for the exposed and comparator groups of the flucloxacillin and liver injury cohort study



Overview of time windows applied for case assignment

A. Minimum 6 months prior registraton required before Day 1 (=the index date, i.e. prescription for flucloxacillin or oxytetracycline)

B. Within the 90 day period after the index date, all participants had their records searched for a liver-related diagnosis

C. Case status assigned based upon presence of liver-related diagnostic codes and DILI lab results. Examples shown as follows:

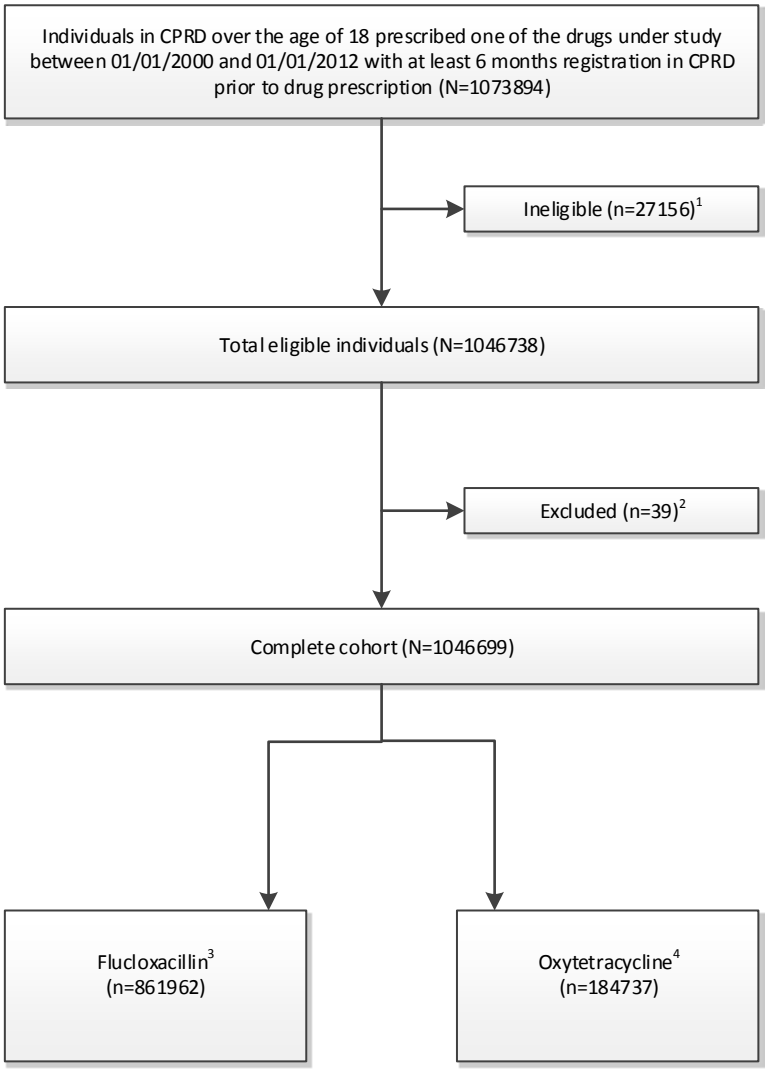
1. **Lab-confirmed 1-45 day case.** Liver related diagnostic code other than jaundice found in the 1-45 period, DILI lab results found before day 46 so person is a lab-confirmed 1-45 day case. Case date=date of DILI lab results (as this occurs after the liver-related diagnostic code). Diagnostic code ≠ jaundice, therefore is not a symptom-defined case.
2. **1-45 day non-case, symptom-defined 46-90 day case.** No liver-related codes during day 1-45, diagnostic code recording jaundice in the 46-90 day period so person is a symptom-defined 46-90 day case. No lab test result indicating DILI within the 1-90 day period, so person is not a lab-confirmed case.
3. **Symptom-defined 1-45 day case, lab-confirmed 46-90 day case.** Person has a diagnostic code recording jaundice within the 1-45 day period so is a symptom-defined 1-45 day case. Lab results indicating DILI also found within person's record within 46-90 day period, so person is a lab-defined 46-90 day case. Dates of the two separate case classifications are different.
4. **1-45 day and 46-90 day non-case.** Person does not have any liver-related diagnostic codes within the 1-90 day period.
5. **1-45 day non-case, symptom-defined and lab-confirmed 46-90 day case**. Person does not have a liver-related diagnostic code in the 1-45 day period so is a 1-45 day non-case. Person has a diagnostic code for jaundice in the 46-90 day period so is a symptom-defined 46-90 day case. Subsequent search of 90 day period for DILI lab results finds lab results qualifing as DILI in the 1-45 day period so person is also a 46-90 day lab-confirmed case (with date of lab-confirmed case assignment being the latest of the DILI result date and diagnostic code dates).

Estimation of absolute and relative effects

Risk: 1-45 day risk calculations were performed by dividing the total number of 1-45 day cases by the total number of people in each group. Within those prescribed flucloxacillin who did not experience the specific classification of liver injury under study (symptom-defined or lab-confirmed) in the 1-45 day period, the **46-90 day risk** was also calculated.

Risk ratio: Given the rarity of the outcome under study, an odds ratio was calculated and interpreted as a **risk ratio** [24]. Risk ratios comparing the risk of liver injury during the 1-45 day period after a first prescription of flucloxacillin to the risk during the 1-45 day after a first prescription of oxytetracycline were calculated and presented.

Figure 2: Flow of number of individuals included in the cohort study of the association between flucloxacillin (compared with oxytetracycline) and liver injury



[1]**Ineligible:** had a diagnostic exclusion code or test result within 6 months 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 the supplementary data (iii) 2928 individuals with liver test results that qualified as DILI as defined in the supplementary data.

[2]**Excluded:** individuals identified as cases of liver injury, but on clinician review of record from 6 months 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 the end of the 1-45 day risk period. Individuals who were also prescribed flucloxacillin before day 45 were assigned to the flucloxacillin group.

Table 2: Characteristics of participants included in the cohort analysis of the association between flucloxacillin (compared with oxytetracycline) and liver injury, by exposure status

		Oxytetracycline (N = 184737) n (%)	Flucloxacillin (N = 861962) n (%)
Age at index date	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 liver 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)
	Missing	3551 (2)	18203 (2)
BMI	<20	10923 (6)	48451 (6)
	20 – 25	55689 (30)	247583 (29)
	25+	95215 (52)	447203 (52)
	Missing	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)
	Missing	24760 (14)	121750 (15)
Socioeconomic status (SES)	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)
	Missing	47163 (26)	188450 (22)
Ethnicity	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)
	Missing	70630 (37)	318780 (37)

Note 1: Prescription counted if it occurred anytime from 1 month prior to index date or between index and before end of follow-up. Less or more common in relation to flucloxacillin, as reported in the literature. **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). **Note 3:** Obtained from CPRD, unless none found, in which case from HES if patient from a linked practice.

Table 3: (1) 1-45 day risk of liver injury by exposure to flucloxacillin or oxytetracycline and (2) crude and multivariable adjusted risk ratios (RR) (comparing the flucloxacillin 1-45 day period with the oxytetracycline 1-45 day period)

Case definition[1]	Exposure group	# with outcome	People	45-day risk (CI[2]) (per 100 000 patients prescribed the drug)	Crude RR (CI)	Multivariable RR[3] (CI)
Symptom-based only	Oxytetracycline 1 - 45 days	7	184737	3.79 (1.52 – 7.81)	1	1
	Flucloxacillin 1 - 45 days	122	861962	14.15 (11.75 – 16.92)	3.74 (1.74 – 8.00)	3.73 (1.73 – 8.03)
Laboratory-confirmed	Oxytetracycline 1 - 45 days	<5[3]	184737	1.62 (3.35 – 4.75)	1	1
	Flucloxacillin 1 - 45 days	73	861962	8.47 (6.64 – 10.65)	5.22 (1.65 – 16.57)	5.22 (1.64 – 16.62)

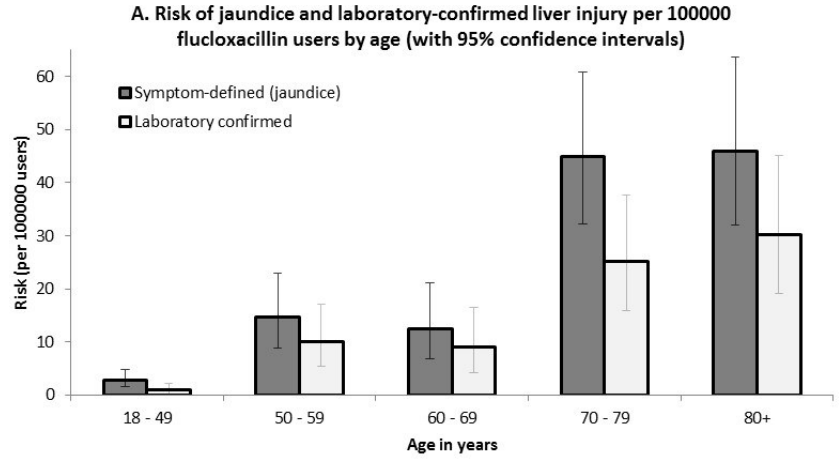
Note 1: Symptom based only: diagnostic code for jaundice present within the 45-day risk period being analysed. Laboratory-confirmed: both of the following present within the 45-day risk period being analysed: (1) any of the diagnostic codes listed in supplementary data section 3 and (2) liver test results indicating drug-induced liver injury (according to Aithal et al). Both definitions: all other more likely causes of the liver symptoms ruled out by clinician review of full electronic health record in the 6-month period before the case date **Note 2:** 95% confidence interval. **Note 3:** Adjusted for age, gender, date of index prescription, prescriptions for other drugs likely to cause liver injury, smoking status, BMI, alcohol intake, socioeconomic status and ethnicity. Missing covariate data taken account of using multiple imputation by chained equations, with all available variables included in the multiple imputation model.

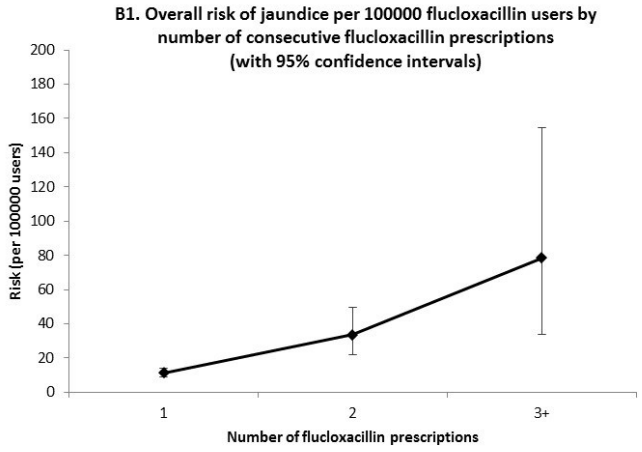
Table 4: Risks and multivariable adjusted risk ratios (RR) for liver injury within those exposed to flucloxacillin (for the 1-45 day period after exposure) for laboratory and symptom-based cases by potential risk factors age, gender and number of prescriptions

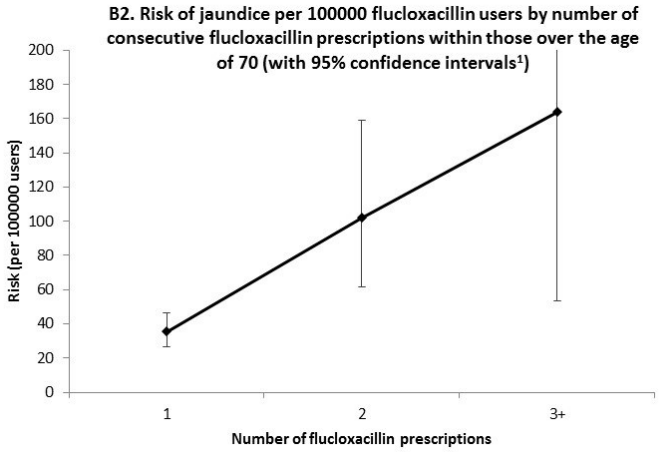
Case definition[1]	Risk factor		# with outcome	People	Risk[2] (CI[3])	Multivariable RR[4] (CI)
Symptom-based only (n=122)	Age	18 – 49	13	453636	2.87 (1.53 – 4.90)	1[6]
		50 – 59	19	129179	14.71 (8.86 – 22.97)	5.02 (2.47 – 10.19)
		60 – 69	14	111368	12.57 (6.87 – 21.09)	4.18 (1.95 – 8.99)
		70 – 79	41	91443	44.84 (32.18 – 60.82)	14.31 (7.51 – 27.26)
		80+	35	76336	45.85 (31.94 – 63.76)	13.87 (7.16 – 26.86)
	Gender	Male	43	394126	10.91 (7.90 – 14.70)	1
		Female	79	467836	16.89 (13.37 – 21.04)	1.43 (0.98 – 2.08)
	No. of prescrs	1	88	777353	11.45 (9.19 – 14.09)	1[6]
		2	26	74431	33.59 (21.74 – 49.58)	2.45 (1.57 – 3.82)
		3+	8	10178	78.60 (33.94 – 154.82)	5.06 (2.44 – 10.46)
Laboratory-confirmed (n=73)	Age	18 – 49	4	453636	0.89 (0.24 – 2.26)	1[6]
		50 – 59	13	129179	10.06 (5.36 – 17.21)	10.79 (3.50 – 33.19)
		60 – 69	10	111368	8.97 (4.31 – 16.51)	8.83 (2.74 – 28.50)
		70 – 79	23	91443	25.15 (15.95 – 37.74)	23.26 (7.88 – 68.67)
		80+	23	76336	30.13 (19.10 – 45.21)	25.42 (8.58 – 75.33)
	Gender	Male	24	394126	6.09 (3.90 – 9.06)	1
		Female	49	467836	10.47 (7.75 – 13.85)	1.61 (0.98 – 2.65)
	No. of prescrs	1	46	777353	5.92 (4.33 – 7.89)	1[6]
		2	19	74431	25.53 (15.37 – 39.86)	3.50 (2.05 – 6.00)
		3+	8	10178	78.60 (33.94 – 154.82)	9.37 (4.40 – 19.95)

Note 1: Symptom based only: diagnostic code for jaundice present within 1-45 day risk period. Laboratory-confirmed: both of the following present within the 1-45 day risk period: (1) any of the diagnostic codes listed in supplementary data section 3 and (2) liver test results indicating drug-induced liver injury (according to Aithal et al). Both definitions: all other more likely causes of the liver symptoms ruled out by clinician review of full electronic health record in the 6 months period before the case date. **Note 2:** Per 100000 people prescribed flucloxacillin. **Note 3:** 95% confidence interval. **Note 4:** Adjusted for date of index prescription, concomitant therapies for drugs considered to be causes of liver injury and all other variables in this table. **Note 5:** p-value(test for trend)<0.001

Figure 3: Illustration of change in absolute risk of flucloxacillin-induced liver injury by (a) increasing age (for both jaundice and laboratory confirmed outcomes) and (b) increasing number of prescriptions (for jaundice, showing B1: overall risk and B2: risk within those over the age of 70)







Note 1: Upper-limit of 95% CI for 3+ category=381.9