

1 **TITLE PAGE**

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

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24 **Short running title:** Risk of flucloxacillin-induced liver injury

25 **Synopsis**

26 **Background**

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

30 **Objectives**

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

33 **Methods**

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

39 **Results**

40 There were 183 symptom-defined cases (160 prescribed flucloxacillin) and 108 laboratory-confirmed
41 cases (102 flucloxacillin). The 1-45 day adjusted risk ratio for laboratory-confirmed injury was 5.22
42 (95% CI 1.64-16.62) comparing flucloxacillin with oxytetracycline use. The 1-45 day risk of
43 laboratory-confirmed liver injury was 8.47 per 100000 people prescribed flucloxacillin (95% CI 6.64-
44 10.65). People who received consecutive flucloxacillin prescriptions had a 1-45 day risk of jaundice of
45 39.00 per 100000 (95% CI 26.85- 54.77), while those aged over 70 receiving consecutive
46 prescriptions had a risk of 110.57 per 100000 (95% CI 70.86-164.48).

47 **Conclusions**

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

52 Introduction

53 Flucloxacillin is an antibiotic of the penicillin class, that has a broad range of uses in the treatment of
54 Gram-positive bacterial infections, including skin and soft tissue infections, respiratory tract
55 infections, urinary-tract infections, meningitis and prophylaxis during surgery.¹ First available in
56 1960, case reports appeared in the 1980's of an adverse drug reaction in which the patient
57 developed serious liver injury, which in some cases could be fatal.² While commonly and increasingly
58 prescribed in the UK,³ flucloxacillin is not marketed in the U.S. and some European countries, where
59 alternative therapies perceived to have a better safety profile are used (such as dicloxacillin).

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

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

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77 **Materials and Methods**

78 **Study design**

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

84 **Setting**

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

90 **Participants**

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

96 Patients with diseases or conditions that were likely to cause liver-related symptoms in their CPRD
97 record within 6 months prior to their first recorded flucloxacillin prescription were excluded (see
98 supplementary data section 2), as were people with any liver test results that met the criteria for
99 drug-induced-liver injury¹⁰ (Table 1) within the previous 6 months. Women who were pregnant at
100 the time of their first recorded flucloxacillin prescription were also excluded (in order to avoid liver
101 symptoms caused by cholestasis in pregnancy).

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

106 **Ethics**

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

110 **Exposures, outcomes and co-variates**

111 **Exposures**

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

123 **Outcomes**

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

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

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

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

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

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

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

160 In order to assess the performance of our case detection method against an established method for
161 assessing causality of drug-induced liver injury, we applied the RUCAM/CIOMS causality assessment
162 method²³ to each of the laboratory-confirmed cases (see supplementary data section 3b).

163 **Co-variates and risk factors**

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

168 Potential risk factors for increased susceptibility to flucloxacillin-induced liver injury were selected
169 based on the results of previous studies⁶⁻⁸ and included gender, age, and number of prescriptions.

170 **Statistical analysis**

171 **Descriptive analysis**

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

176 **Overall risk of liver injury**

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

183 **Association between flucloxacillin and liver injury**

184 For the analysis of the association between flucloxacillin and liver injury, all relative effects were
185 calculated as odds ratios, which given the rarity of the outcomes under study were interpreted as
186 risk ratios²⁴ (and will be referred to as such subsequently in this article).

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

192 **Analysis of risk factors for flucloxacillin-induced liver injury**

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

197 **Missing data and sensitivity analyses**

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

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

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

204 **Results**

205 **Participants**

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

212 **Descriptive data**

213 Background characteristics of participants are shown in Table 2. There were 861962 people
214 prescribed flucloxacillin and 184737 prescribed oxytetracycline. 56% of those prescribed
215 oxytetracycline were female, compared with 54% of those prescribed flucloxacillin, and a higher
216 proportion of those in the oxytetracycline group (55%) had an index date prior to 2006 than in the
217 flucloxacillin group (48%). Oxytetracycline patients included a higher proportion of people on other
218 drugs likely to cause liver injury than flucloxacillin patients (81% versus 52%). There was no
219 difference in recorded ethnicity between the groups, and minimal differences in the distribution of
220 all other characteristics between exposure groups. Ethnicity data was missing for 37% of the cohort.

221 **Description of liver injury cases**

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

228 **Risk of liver injury associated with flucloxacillin**

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

231 There were 73 out of 861962 people prescribed flucloxacillin with laboratory-confirmed liver injury
232 within the 45-days after prescription, giving a 1-45 day risk of flucloxacillin-induced liver injury of
233 8.47 cases per 100000 people (95% CI 6.64–10.65). The risk of laboratory-confirmed injury for those
234 exposed to oxytetracycline within the same time period was 1.62 per 100000 people (95% CI 3.35–
235 4.75), while the risk within those in the flucloxacillin group within the 46–90 day period from first

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

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

245 **Risk factors for flucloxacillin-induced liver injury**

246 There was strong evidence that increasing age was a risk factor for flucloxacillin-induced liver injury
247 (p test-for-trend<0.001 for both symptom-based and laboratory confirmed outcomes), with a
248 marked increase in the 1-45 day risk of injury in those over the age of 70 (e.g. multivariable-adjusted
249 RR for laboratory-confirmed liver injury comparing those in the 70-79 year old age group with those
250 aged 18-49: 23.26, 95% CI 7.88–68.67) (Table 4). There was also strong evidence for an increased 1-
251 45 day risk of injury with increasing number of prescriptions (p test-for-trend<0.001), with people
252 receiving 3 or more prescriptions within the 1-45 day risk period experiencing 9.37 times the 1-45
253 day risk of laboratory-confirmed injury (95% CI 4.40 – 19.95) than those receiving a single
254 prescription within this period, after adjusting for age, gender and concomitant prescriptions for
255 other causes of liver injury. For gender, there was a suggestion across both outcomes that females
256 had a slightly increased risk of injury, although the 95% CI did not rule out a decreased risk (e.g.
257 multivariable RR for symptom-based injury comparing females to males: 1.43, 95% CI 0.98–2.08).

258 Considering the absolute 1-45 day risk per 100000 people exposed to flucloxacillin, the risk of
259 jaundice in the 18-49 year-old age group was 2.87, 95% CI 1.53-4.90, increasing to 14.71 (95% CI
260 8.86-22.98) in the 50-59 year old age group (Table 4 and Figure 3A). Within those over the age of 70,
261 the absolute risk of jaundice was 45.30 per 100000 people (95% CI 35.69-56.69). In the overall
262 population the risk of jaundice for those receiving a single prescription was 11.45 (95% CI 9.19-
263 14.09), increasing to 78.60 per 100000 (95% CI 33.94–154.82) within people receiving three or more
264 flucloxacillin prescriptions (Table 4 and Figure 3B). People over the age of 70 receiving three or more
265 prescriptions had a risk of jaundice of 163.83 (95% CI 53.21-381.9) (Figure 3B), while over 70 yr olds
266 receiving 2 or more had a risk of 110.57 per 100000 (95% CI 66.35-154.79). Risk figures for

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

269 **Performance of case definition compared to the RUCAM/CIOMS method**

270 The RUCAM/CIOMS method²³ classified 63/73 (86%) of laboratory-confirmed cases from this study
271 as “Probable (flucloxacillin) ADR” (see supplementary data section 3b for description of categories).
272 The remaining 10/73 (14%) were classified as “Possible (flucloxacillin) ADR”. Of these, 5 were under
273 the RUCAM/CIOMS age risk factor cut-off of 55 years old, with the remaining 5 having a prescription
274 record for another drug that may have been more likely to have caused the observed injury. Within
275 1-45 day laboratory-confirmed cases over the aged of 70, 91% (42/46) were classified as
276 RUCAM/CIOMS probable.

277 **Pattern of liver injury by age**

278 We performed a post-hoc analysis of the 73 people exposed to flucloxacillin with laboratory-
279 confirmed liver injury to investigate whether the pattern of liver injury associated with flucloxacillin
280 use varied by age group. 38 of the 46 people aged 70 years or over had a cholestatic type of injury
281 (83%, 95% CI 71% - 94%), compared with 15 out of 27 under the age of 70 (56%, 95% CI 35% - 76%,
282 Mann Whitney test p=0.01).

283 **Sensitivity analyses and missing data**

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

288

289 Discussion

290 In this study we have shown flucloxacillin to be associated with 5.22 (95% CI 1.64-16.62) times the 1-
291 45 day risk of laboratory confirmed liver injury than oxytetracycline after multivariable adjustments,
292 with an absolute 1-45 day risk of 8.47 (95% CI 6.64-10.65) per 100000 people prescribed the drug for
293 the first time. There was strong evidence that increasing age and number of prescriptions were
294 associated with increased flucloxacillin-induced liver injury, with those over the age of 70 who
295 received at least one additional flucloxacillin prescription within 45 days of their initial prescription
296 having a risk of jaundice of 110.57 per 100000 people (95% CI 66.35-154.79).

297 Comparison with previous studies

298 Our estimate of the overall risk of laboratory-confirmed liver injury is comparable to previously
299 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
300 studies have estimated the relative effect of age on risk to be between 18.61 (comparing over 55s
301 versus under 30s)⁸ and 6.1 (comparing over 60 versus under 60),⁶ our large study is the first to
302 estimate absolute risk figures by age categories, and has shown that those over 70 years of age
303 experience the highest risk. We found a nine-fold increased risk in people given three or more
304 flucloxacillin prescriptions compared with those given one prescription, also consistent with previous
305 work showing that those with more than 14 consecutive days' use have 7.13 times the risk of injury
306 than people using for less than this period (95% CI 2.90-17.58).⁸ The size of our study has allowed us
307 to demonstrate a dose(prescription)-response effect, and show that those over the age of 70 who
308 receive more than 1 prescription within the 1-45 day period have a particularly elevated risk.

309 Implications and further work

310 Current flucloxacillin prescribing information relating to hepatic side-effects¹ states that (1) jaundice
311 affects less than 1 in 10000 people and (2) the drug should be used cautiously in people over 50
312 years of age. Our results suggest that flucloxacillin causes jaundice at a frequency closer to 1 in 7000
313 people in the overall population, that prolonged use is likely to increase the risk further, and those
314 over the age of 70 have an approximately 15-fold higher risk than those under the age of 50. This is a
315 particular concern when considering recent flucloxacillin prescribing trends showing that people
316 over the age of 70 have both the highest prescribing rates and largest increase in rates.³ We would
317 therefore hope that these findings could help physicians gain a greater understanding of the nature
318 of the risk involved with prescribing flucloxacillin, and exercise caution in prescribing particularly
319 long treatment courses to those over the age of 70. In a clinical setting the choice may be between
320 flucloxacillin and another drug with known adverse effects on the liver – the absolute risk figures
321 provided in our study would help inform clinicians' prescribing decisions in this situation.

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

331 We would also hope that our findings might help further development of a predictive genetic test
332 and/or elucidation of mechanism via genetic association studies. Genetic analysis has demonstrated
333 the *HLA-B*5701* genotype to be a major determinant of drug-induced liver injury due to
334 flucloxacillin.²⁹ Despite this finding, subsequent consideration of clinical utility³⁰ showed that (based
335 on an overall population prevalence of 8.5 per 100000) predictive genetic testing for the reaction
336 would be unfeasible, as 13513 people would need screened in order to prevent 1 case. Assuming
337 that all of the cases of jaundice attributed to flucloxacillin in this study fulfil the criteria for DILI
338 (which we consider a fair assumption, given how clear an indicator jaundice is of a serious liver
339 problem), calculating the number needed to test within those over 70 using the drug reduces this
340 number to 2512 (see supplementary data section 6). Although still likely to be prohibitively high,
341 further elucidation of characteristics associated with increased risk may allow the number needed to
342 test to be reduced further for specific groups in the future.

343 **Limitations**

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

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

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

381 **Conclusions**

382 In the largest study of flucloxacillin-induced liver injury to date, we have provided new absolute risk
383 estimates by age, number of prescriptions and gender for both laboratory-confirmed injury and
384 jaundice, providing insight into groups particularly susceptible to harm, especially those aged over
385 70 years receiving multiple prescriptions. These results should help guide clinical care decisions and
386 support further work on predictive genetic test implementation.

387

388

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409

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411 All authors except Kevin Wing, Olaf Klungel, Robert Reynolds declare no conflicts of
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422 Robert Reynolds declared he that he is an employee and shareholder of Pfizer, Inc. and that
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Table 1: Classification of drug-induced liver injury based on liver test results¹⁰

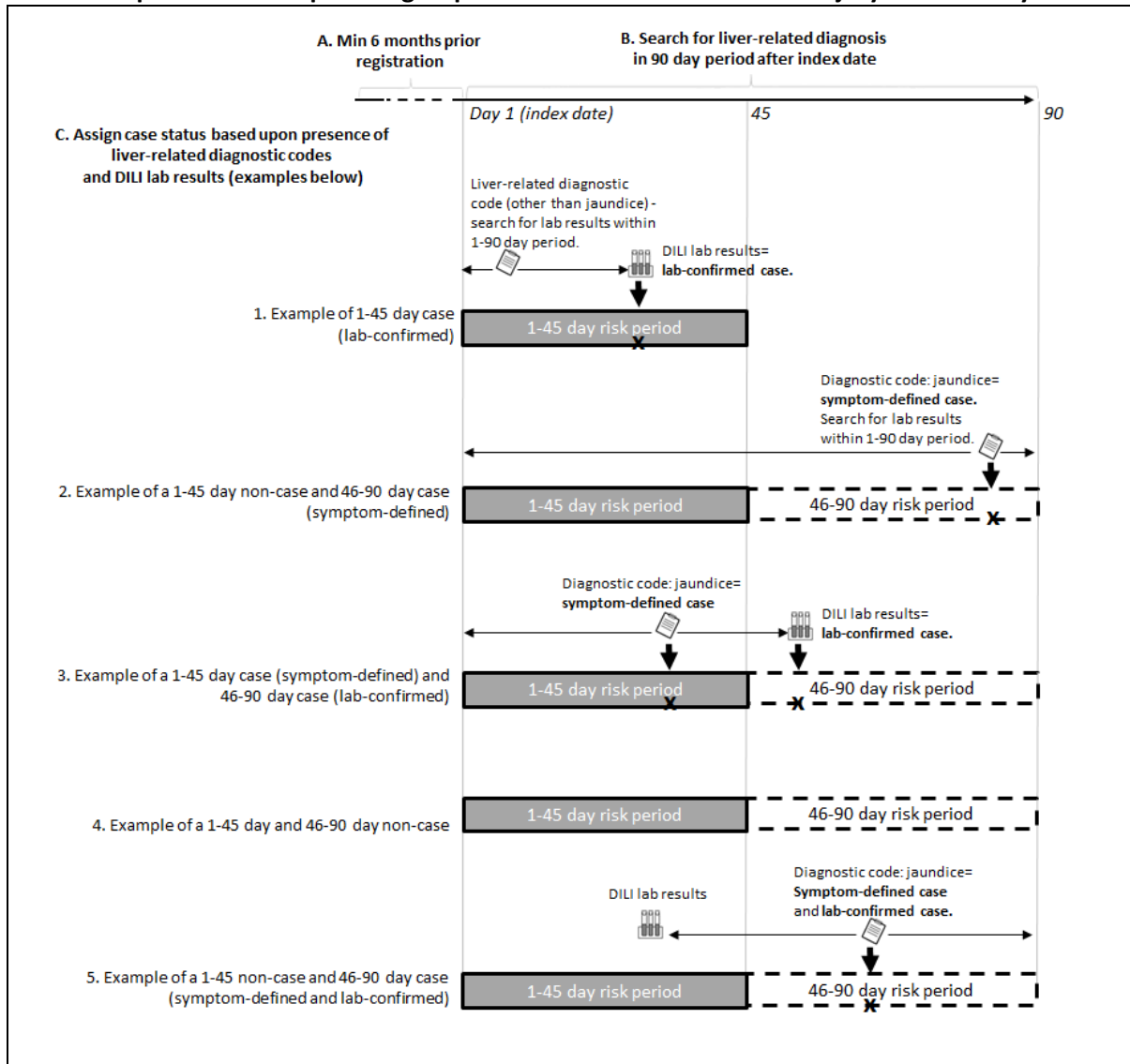
Type of liver injury	Liver test result
Characteristic of any DILI	ALT \geq 5 x ULN or ALP \geq 2 x ULN or ALT \geq 3 x ULN and Bil > 2 x ULN
Characteristic of hepatocellular type of DILI	R* \geq 5
Characteristic of mixed type of DILI (=cholestatic hepatitis)	R > 2 and < 5
Characteristic of pure cholestatic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

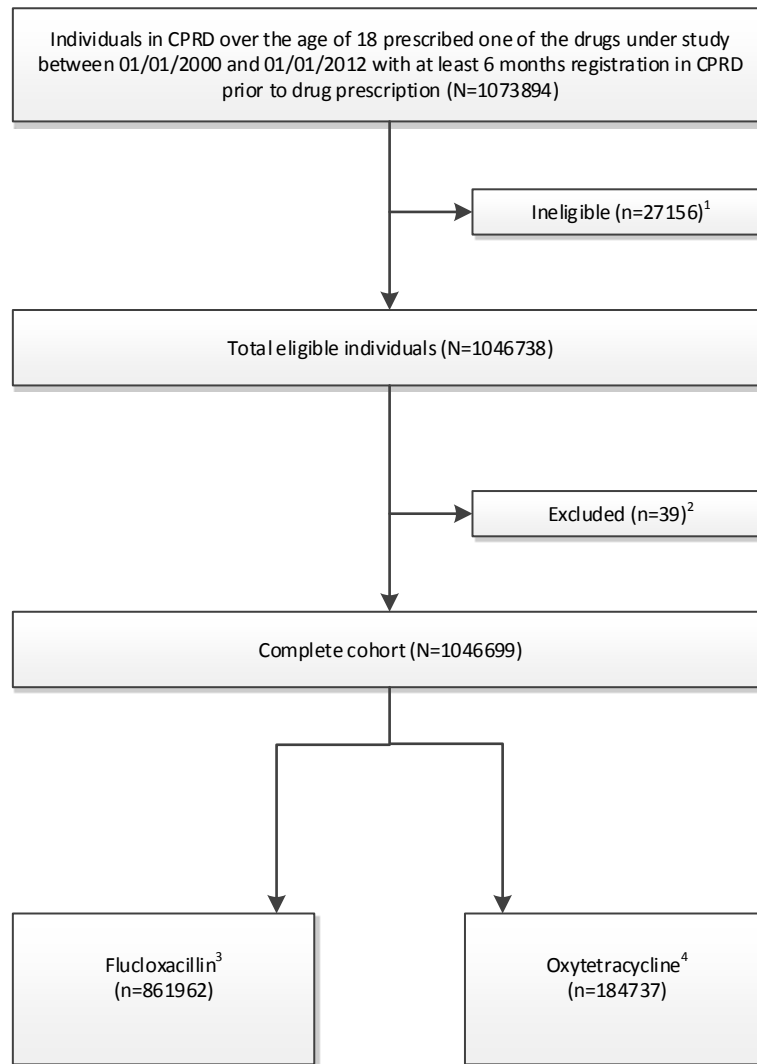
Estimation of absolute and relative effects

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.

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



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509 ¹**Ineligible:** had a diagnostic exclusion code or test result within 6 months prior to their index date, made up of: (i) 11089
510 individuals with pregnancy codes but no subsequent end of pregnancy code before index date (ii) 13139 individuals with liver
511 pathology codes as defined in the supplementary data (iii) 2928 individuals with liver test results that qualified as DILI
512 as defined in the supplementary data.

513 ²**Excluded:** individuals identified as cases of liver injury, but on clinician review of record from 6 months prior to index
514 date, an underlying cause other than a prescription with either of the drugs of interest was identified (and the date was prior to
515 the index date).

516 ³**Flucloxacillin:** Number of people prescribed flucloxacillin on their index date. 47370/861959 were prescribed the flucloxacillin-
517 ampicillin combination (co-fluampicil).

518 ⁴**Oxytetracycline:** Number of individuals prescribed oxytetracycline on their index date who were not also prescribed
519 flucloxacillin before the end of the 1-45 day risk period. Individuals who were also prescribed flucloxacillin before day 45 were
520 assigned to the flucloxacillin group.

521

522 **Table 2: Characteristics of participants included in the cohort analysis of the association between**
523 **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 injury¹	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.

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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.

528

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

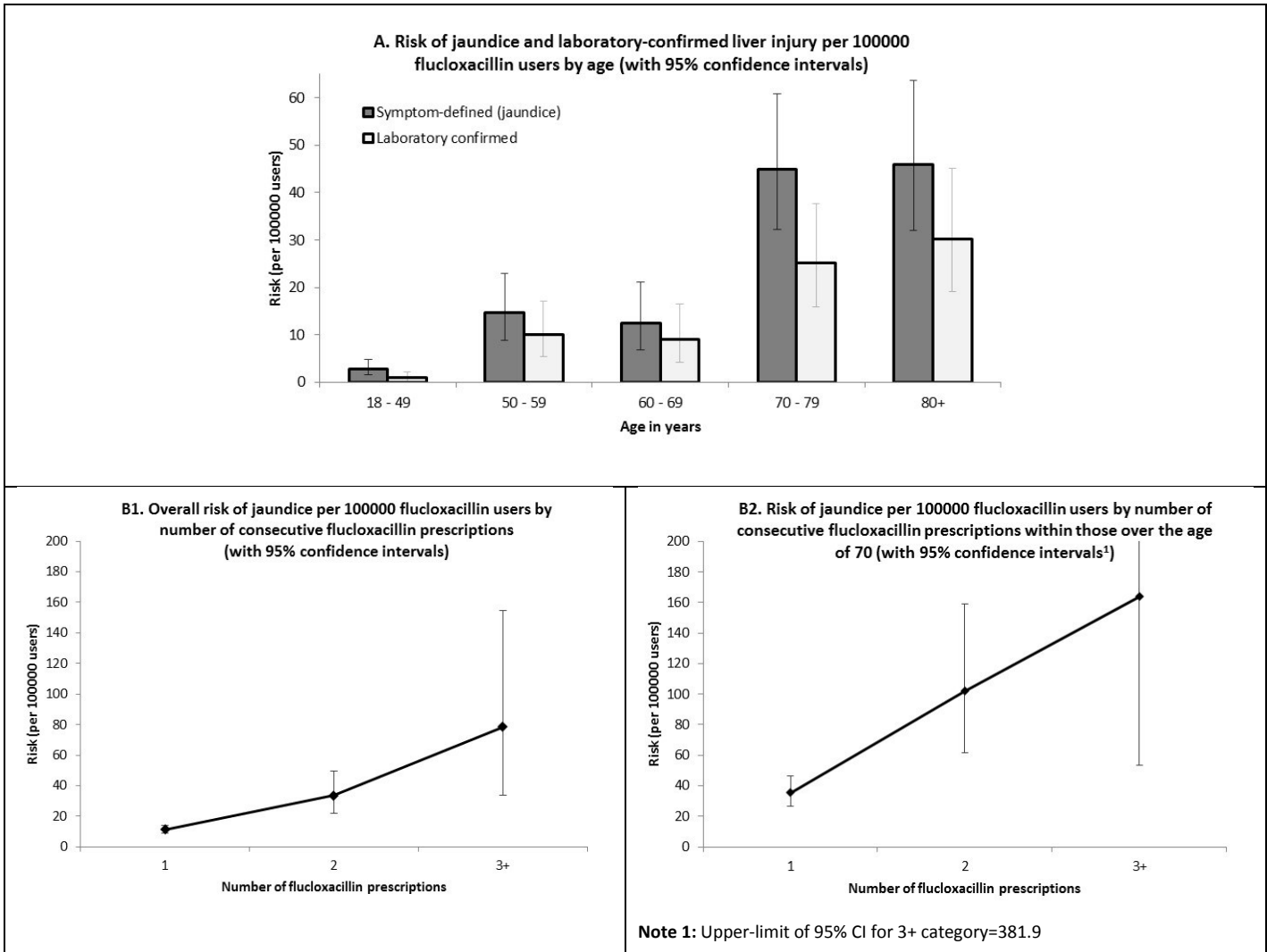
Case definition ¹	Risk factor		# with outcome	People	Risk ² (CI ³)	Multivariable RR ⁴ (CI)
Symptom-based only (n=122)	Age	18 – 49	13	453636	2.87 (1.53 – 4.90)	1 ⁶
		50 – 59	19	129179	14.71 (8.86 – 22.97)	5.02 (2.47 – 10.19)
		60 – 69	14	111368	12.57 (6.87 – 21.09)	4.18 (1.95 – 8.99)
		70 – 79	41	91443	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 prescrrs	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 prescrrs	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

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



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