Associations of antibiotic use with risk of primary liver cancer in the Clinical Practice Research Datalink

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Background: Use of antibiotics could alter human microbiota composition and decrease bacterial diversity. Such microbial dysbiosis may have implications in hepatocarcinogenesis; however, the association between antibiotic use and liver cancer risk has been minimally examined in humans.

Methods: We performed a nested case–control study (1195 primary liver cancer cases and 4640 matched controls) within the United Kingdom's Clinical Practice Research Datalink. Antibiotic use was obtained from prescription records. Multivariable-adjusted odds ratio (OR) and 95% confidence interval (CI) were estimated using conditional logistic regression.

Results: Ever-use of prescription antibiotics was associated with a slightly increased risk of liver cancer, compared to non-use (OR = 1.22, 95% CI = 1.03 − 1.45). However, there was no clear dose–response relationship by the number of prescriptions or cumulative dose of antibiotic use, suggesting a non-causal association.

Conclusions: Our results do not support a role of antibiotic use in liver cancer development.

Antibiotics are widely used to treat or prevent bacterial infection. However, they can also disturb the normal composition of gut microbiota, resulting in microbial dysbiosis including decreased bacterial diversity and increased antibiotic-resistant pathogens (Clemente *et al*, 2012). Such dysbiosis could increase hepatic exposure to cancer-promoting microbial products and metabolites that reach the liver through the portal vein (Schwabe and Jobin, 2013). Thus, we hypothesised that antibiotic use may be associated with increased risk of liver cancer. To our knowledge, only two population-based studies have investigated this hypothesis, with inconsistent results (Kilkkinen *et al*, 2008; Boursi *et al*, 2015). It is important to expand this evidence base due to the wide use of antibiotics in clinical settings.

Herein, we investigated the association of prescription antibiotic use with the risk of primary liver cancer within the Clinical Practice Research Datalink (CPRD) in the UK.

MATERIALS AND METHODS

Data source. This nested case–control study was based in the CPRD, a large, population-based, electronic medical record database with information on ∼8.5% of the UK population (Jick *et al*, 1991; Lawson *et al*, 1998; Jick *et al*, 2003). This study was approved by the National Institutes of Health Human Research Protection Program and the Independent Scientific Advisory Committee of the CPRD (Protocol 12_127R2A).

Study population. Cases and controls were drawn from persons enrolled in the CPRD from 1988 through 2011 who were between the ages of 10 and 90 years. Cases met the following criteria: (1) had a first time diagnosis of primary liver cancer, (2) had no prior diagnosis of cancers most likely to metastasise to the liver (lung, stomach, breast, colon, or pancreatic cancer) and no code of

liver metastases, and (3) had no diagnosis of any other cancer (except for non-melanoma skin cancer) in 3 years before the index date. The index date of cases was defined as 1 year before liver cancer diagnosis, and all cases were required to have at least 2 years of history in the CPRD before the index date. For each case, controls were selected from individuals who were in the CPRD at the case's index date and had no cancer diagnosis (except non-melanoma skin cancer) before that date. Controls were matched to cases at a four-to-one ratio on age (same year of birth), sex, general practice, and number of years in the CPRD before the case's index date. We then defined the controls' index date to be the same as the matched case's index date. We only identified three eligible controls for 59 of the cases, two for 24 cases, and one for 11 cases, thus the number of controls totalled 4640.

In addition to the full case–control match, we completed an additional match based on the presence of diabetes, at a four-to-one ratio using the same matching factors as in the primary match. Overall, 1379 controls with diabetes were matched to the 346 cases

with diabetes and 3396 controls without diabetes were matched to the 849 cases without diabetes.

Exposure definition. We identified all antibiotic prescriptions (Supplementary Table 1) recorded before the index date from the electronic records. Ever-use of antibiotics was defined as having two or more antibiotic prescriptions before the index date, and non-use was defined as having none or one prescription before the index date. We additionally examined antibiotic use by the total number of prescriptions, cumulative dose (the number of pills multiplied by the dose per pill, summed from first entry into CPRD through the index date), and recency of use (current use was defined as use that ended within 1 year before the index date, whereas past use was defined as use that ended more than 1 year before the index date). Furthermore, to assess the intensity of antibiotic use, we calculated the time between the first and last use (categorised as <2 years, 2–5 years, and >5 years) and examined

Table 1. Characteristics of cases and controls and univariate associations with the risk of liver cancer, CPRD					
	Cases (n = 1195)		Controls (n = 4640)		Univariate OR[a]
	n	%	n	%	OR (95% CI)
Index year[b]					
1991–1994	59	4.9	230	4.9	—
1995–1999	140	11.7	546	11.8	—
2000–2004	306	25.6	1190	25.7	—
2005–2010	690	57.7	2674	57.6	—
Age at index (years)[b]					
<40	28	2.3	112	2.4	—
40–49	63	5.3	252	5.4	—
50–59	217	18.2	850	18.3	—
60–69	304	25.4	1188	25.6	—
70–79	407	34.1	1591	34.3	—
80–89	176	14.7	647	13.9	—
Mean ± SD	67.2 ± 12.1		67.0 ± 12.1		
Sex[b]					
Male	856	71.6	3322	71.6	—
Female	339	28.4	1318	28.4	—
Length of history before index date (years)[b]					
Mean ± SD	10.9 ± 5.3		11.1 ± 5.3		—
BMI (Kg/m^2)					
<18.5 (underweight)	20	1.7	52	1.1	1.62 (0.94–2.80)
18.5–24.9 (normal)	308	25.8	1302	28.1	1.00 (ref)
25.0–29.9 (overweight)	372	31.1	1609	34.7	0.99 (0.84–1.17)
30.0+ (obese)	320	26.8	817	17.6	1.73 (1.44–2.07)
Unknown	175	14.6	860	18.5	0.79 (0.63–0.99)
Mean ± SD	27.7 ± 5.3		27.0 ± 4.8		—
Smoking status					
Non-smoker	384	32.1	1942	41.9	1.00 (ref)
Current smoker	304	25.4	815	17.6	1.98 (1.65–2.36)
Former smoker	425	35.6	1458	31.4	1.56 (1.32–1.84)
Unknown	82	6.9	425	9.2	0.86 (0.63–1.16)
Alcohol-related disorders	189	15.8	189	4.1	5.28 (4.16–6.70)
Hepatitis B or C infection	74	6.2	5	0.1	70.2 (25.7–192.2)
Chronic liver disease	170	14.2	23	0.5	32.8 (20.6–52.1)
Rare metabolic disorders[c]	26	2.2	9	0.2	12.5 (5.65–27.7)
Diabetes	346	29.0	463	10.0	3.85 (3.27–4.55)
Type 1	36	3.0	31	0.7	5.76 (3.46–9.58)
Type 2	265	22.2	398	8.6	3.44 (2.87–4.13)
Type unspecified	45	3.8	34	0.7	6.34 (3.97–10.1)
Statin use (2+ prescriptions)	302	25.3	1242	26.8	0.91 (0.77–1.07)
Anti-diabetic medication use (2+ prescriptions)	208	17.4	277	6.0	3.47 (2.84–4.24)
Paracetamol use (2+ prescriptions)	616	51.6	2030	43.8	1.46 (1.27–1.68)

Abbreviations: BMI = body mass index; CI = confidence interval; CPRD = Clinical Practice Research Datalink; OR = odds. ratio; SD = standard deviation.
[a]Using conditional logistic regression to account for matching.
[b]Matching variables.
[c]Rare metabolic disorders include haemochromatosis, Wilson disease, porphyrias, and alpha-1 antitrypsin deficiency.

the association between total number of prescriptions and liver cancer risk within each time period category.

Statistical analysis. We used conditional logistic regression to calculate the crude and adjusted odds ratio (OR) and 95% confidence intervals (CI). We adjusted for the following factors in multivariable models selected *a priori* based on previous literature: body mass index (BMI), smoking status, alcohol-related disorders, hepatitis B or C virus (HBV or HCV) infection, diabetes, rare metabolic disorders, and use of anti-diabetic medications, paracetamol, and statins. All analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

As shown in Table 1, cases ($n = 1195$) were more likely to be obese, be current or former smokers, have HBV and/or HCV infection, chronic liver disease, rare metabolic disorders, alcohol-related

disorders, or diabetes, and to take anti-diabetic medication or paracetamol, compared to their matched controls ($n = 4640$).

Table 2 shows that ever-use of antibiotics was associated with a 22% higher risk of liver cancer, compared to non-use (OR = 1.22, 95% CI: 1.03 − 1.45). However, there was no clear dose–response relationship by the number of prescriptions or by cumulative dose. When we examined major classes of antibiotics, the inhibitors of nucleic acid synthesis yielded the highest OR, but sample size was limited (Supplementary Table 2). In analyses based on additional matching according to the presence of diabetes, antibiotic use was associated with increased risk of liver cancer in both the diabetes match and the non-diabetes match (Table 3).

None of the results materially changed when we conducted the following sensitivity analyses: (1) using an index date of 2 years before the case's date of diagnosis, rather than 1 year; (2) restricting to patients with 5 or more years of information in their medical record before the index date; and (3) using 0 prescriptions (rather than 0–1 prescriptions) as the reference group.

Table 2. Association of antibiotic use with the risk of liver cancer, CPRD				
	Cases ($n = 1195$) No.	Controls ($n = 4640$) No.	Crude OR[a] (95% CI)	Adjusted OR[b] (95% CI)
Any antibiotic use				
0–1 prescriptions	331	1607	1.0 (ref)	1.0 (ref)
2+ prescriptions	864	3033	1.50 (1.28–1.75)	1.22 (1.03–1.45)
Number of prescriptions				
0–1	331	1607	1.0 (ref)	1.0 (ref)
2–4	324	1276	1.32 (1.10–1.57)	1.19 (0.98–1.44)
5–9	259	875	1.61 (1.32–1.97)	1.25 (1.00–1.57)
10–19	178	497	2.03 (1.61–2.55)	1.37 (1.06–1.78)
20+	103	385	1.55 (1.18–2.05)	1.08 (0.79–1.48)
P_{trend}			<0.01	0.73
Cumulative dose[c]				
None	182	928	1.0 (ref)	1.0 (ref)
Q1 (1–<11935)	226	928	1.32 (1.06–1.65)	1.28 (1.00–1.64)
Q2 (11935–<27285)	225	928	1.36 (1.08–1.71)	1.23 (0.96–1.59)
Q3 (27285–<64272.5)	260	928	1.61 (1.28–2.02)	1.27 (0.98–1.63)
Q4 (64272.5–9918500)	302	928	2.00 (1.58–2.53)	1.35 (1.03–1.77)
P_{trend}			<0.01	0.18
Recency of antibiotic prescription				
0–1 prescriptions	331	1607	1.0 (ref)	1.0 (ref)
Current antibiotic use	433	1364	1.66 (1.40–1.98)	1.26 (1.04–1.53)
Past antibiotic use	431	1669	1.36 (1.14–1.61)	1.18 (0.98–1.44)
Intensity of antibiotic use[d]				
0–1 prescriptions	331	1607	1.0 (ref)	1.0 (ref)
Time between first and last prescription: <2 years				
2–4 prescriptions	134	483	1.38 (1.10–1.73)	1.22 (0.95–1.57)
5+[e] prescriptions	24	50	2.30 (1.39–3.82)	1.55 (0.85–2.83)
P_{trend}			<0.01	0.10
Time between first and last prescription: 2–5 years				
2–4 prescriptions	93	384	1.24 (0.95–1.61)	1.12 (0.83–1.49)
5–9 prescriptions	74	201	1.87 (1.39–2.53)	1.35 (0.96–1.89)
10+[e] prescriptions	27	67	1.97 (1.22–3.16)	1.42 (0.85–2.38)
P_{trend}			<0.01	0.02
Time between first and last prescription: >5 years				
2–4 prescriptions	97	409	1.26 (0.96–1.65)	1.17 (0.87–1.58)
5–9 prescriptions	166	628	1.44 (1.13–1.82)	1.19 (0.91–1.55)
10–19 prescriptions	154	440	1.94 (1.51–2.49)	1.33 (1.00–1.76)
20+ prescriptions	95	371	1.43 (1.07–1.91)	0.98 (0.71–1.37)
P_{trend}			0.05	0.51

Abbreviations: CI = confidence interval; CPRD = Clinical Practice Research Datalink; OR = odds ratio; Q = quartile.
[a]Using conditional logistic regression to account for matching.
[b]Using conditional logistic regression to account for matching, and additionally adjusted for body mass index, smoking status, alcohol-related disorders, hepatitis B or C virus infection, diabetes, rare metabolic disorders, and use of anti-diabetic medications, paracetamol, and statins.
[c]Quartiles created based on the distribution among controls who had a cumulative dose above zero.
[d]To assess the intensity of antibiotic use, we calculated the time between the first and last use (categorised as <2 years, 2–5 years, and >5 years) and examined the association between total number of prescriptions and liver cancer risk within each time period category.
[e]Higher categories combined due to small sample sizes.

Table 3. Association of antibiotic use with the risk of liver cancer stratified by the presence of diabetes, CPRD

	Among individuals with diabetes (the diabetes match)			Among individuals without diabetes (the non-diabetes match)		
	Cases (n = 346) No.	Controls (n = 1379) No.	Adjusted OR (95% CI)[a]	Cases (n = 849) No.	Controls (n = 3396) No.	Adjusted OR (95% CI)[a]
Any antibiotic use						
0–1 prescriptions	66	382	1.0 (ref)	265	1276	1.0 (ref)
2+ prescriptions	280	997	1.79 (1.25–2.57)	584	2120	1.22 (1.00–1.47)
Number of prescriptions						
0–1	66	382	1.0 (ref)	265	1276	1.0 (ref)
2–4	90	355	1.74 (1.17–2.59)	234	937	1.16 (0.94–1.44)
5–9	87	285	1.97 (1.28–3.03)	172	613	1.24 (0.96–1.60)
10–19	65	217	1.77 (1.10–2.85)	113	342	1.43 (1.06–1.92)
20+	38	140	1.58 (0.91–2.74)	65	228	1.22 (0.85–1.77)
P_{trend}			0.43			0.22
Cumulative dose[b]						
None	38	216	1.0 (ref)	144	759	1.0 (ref)
Q1 (1–<11 935)	45	229	1.42 (0.84–2.42)	181	692	1.35 (1.03–1.76)
Q2 (11 935–<27 285)	68	262	1.80 (1.09–2.99)	157	704	1.18 (0.90–1.57)
Q3 (27 285–<64 272.5)	80	307	1.69 (1.00–2.84)	180	654	1.34 (1.01–1.79)
Q4 (64 272.5–9 918 500)	115	365	1.99 (1.19–3.34)	187	587	1.50 (1.10–2.04)
P_{trend}			0.05			0.06

Abbreviations: CI = confidence interval; CPRD = Clinical Practice Research Datalink; OR = odds ratio; Q = quartile.
[a]Using conditional logistic regression to account for matching, and additionally adjusted for body mass index, smoking status, alcohol-related disorders, hepatitis B or C virus infection, rare metabolic disorders, and use of anti-diabetic medications, paracetamol, and statins.
[b]Quartiles created based on the distribution among controls in the original match who had a cumulative dose above zero.

DISCUSSION

In this nested case–control study, we observed an OR of 1.22 (95% CI: 1.03 − 1.45) of liver cancer for antibiotic ever-use compared to non-use. Our results do not support a causal association as there was no clear pattern of dose–response by the number of prescriptions or cumulative dose.

Before our study, only two population-based studies have examined the association of antibiotic use and risk of liver cancer, with inconsistent results (Kilkkinen et al, 2008; Boursi et al, 2015). In our study, ever-use of prescription antibiotics was associated with slightly increased risk of liver cancer. One possible mechanism underlying this association is antibiotic-induced disturbance of commensal microbiota and subsequent dysbiosis, which may result in increased hepatic exposure to bacterial products and metabolites that could be carcinogenic (Schwabe and Jobin, 2013). Alternatively, given the lack of dose–response, confounding may at least partially explain the slightly elevated risk of liver cancer in our study. Several important risk factors of liver cancer, such as cirrhosis and diabetes, are associated with increased risk of bacterial infection (Navasa et al, 1997; Joshi et al, 1999), which may subsequently require antibiotic treatments. Thus, liver cancer cases may have used antibiotics to treat bacterial infections that arose from these clinical conditions before cancer diagnosis. To explore this possibility, we created a relatively 'clean' population using the non-diabetes match, and further excluded individuals with chronic liver disease; however the results were similar to what we observed in the main analysis (OR 1.23, 95% CI: 0.99–1.51). It is possible that among this relatively 'clean' population, residual confounding by other unmeasured variables still exists. Thus, whether the slightly elevated risk of liver cancer in our study is explained by confounding warrants further investigation.

A major strength of this study is that the analysis was conducted using the CPRD, a large, well-established, validated, longitudinal primary-care database known for diagnostic accuracy of cancer outcomes and complete outpatient prescription pharmaceutical data. Antibiotic use was obtained from prescription records before cancer diagnosis, which minimised recall bias. A possible limitation is that liver cancer diagnosis was not confirmed by linkage to a cancer registry; previous validation studies have shown that cancer diagnoses within the CPRD are reasonably complete (Jick et al, 1991), however we cannot rule out the possibility that a proportion of liver cancer diagnoses were missed. As discussed above, we do not have comprehensive information on risk factors of liver cancer that may require antibiotic treatments; thus we are unable to sufficiently evaluate whether the observed excess risk was due to residual confounding. In addition, prescription records may not reflect the actual usage of antibiotics, and antibiotic use before entry into CPRD was unavailable, thus exposure misclassification is possible.

In conclusion, our results do not support a role of antibiotics in the development of liver cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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