Antibiotic Use and the Risk of Lung Cancer

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Abstract

Antibiotic use has been associated with an increased risk of cancer in epidemiologic studies. We evaluated the association between antibiotic use and the risk of primary lung cancer by conducting a prospective case-control study nested in a cohort of subjects who were 40 to 84 years old in 1995 to 2004, with at least 2 years of enrollment in The Health Improvement Network. There were 4,336 cases of primary lung cancer. A random sample of 10,000 controls was frequency matched to the cases for age, sex, and calendar year of diagnosis. Antibiotic exposure was measured by the total number of antibiotic prescriptions and the cumulative number of days on antibiotics since enrollment. We discounted exposure 1 year before the date of cancer diagnosis. Relative risks (RR) and 95% confidence intervals (95% CI) were estimated using conditional logistic regression. Variables such as smoking, chronic obstructive pulmonary disease, respiratory infections, body mass index, and health care utilization were introduced in the model. Compared with subjects with no prescription of antibiotics before the index date, the crude RR of lung cancer was 2.52 (95% CI, 2.25-2.83) among those who received 10 or more prescriptions. The corresponding RR was 1.31 (95% CI, 1.10-1.57) upon adjustment for confounders. We used directed acyclic graphs to illustrate that the observed higher risk of lung cancer among antibiotic users may be due to the increased frequency of infections in patients with subclinical cancer and to shared causes between cancer and infections. Current evidence is insufficient to support or refute a carcinogenic effect of antibiotics. (Cancer Epidemiol Biomarkers Prev 2008;17(6):1308–15)

Several epidemiologic studies have raised concern that antibiotic use may be associated with an increased risk of cancer, particularly of hematologic cancers (1-3) and possibly breast cancer (3-5). Others studies, however, have not been able to confirm such associations (6-9). Most later studies on antibiotic use and breast cancer suggested that the associations initially observed by Velicer in 2004 (5) are likely due to uncontrolled confounding (6-9).

Although lung cancer is the leading cause of death in Western countries (9), only two studies have evaluated the association between antibiotics and lung cancer. Selby et al. (10) in 1989 screened the carcinogenicity of all medications prescribed through the Kaiser Permanente health maintenance organization and found an association between several antibiotics and lung cancer risk. The ratio between observed and expected exposed cases (SMR) was 1.76 for ampicillin, 1.44 for erythromycin, and 1.29 for tetracycline ($P < 0.01$). The authors reported that the associations could not be explained by indications for antibiotic use or by differences in smoking habits between users and nonusers. However, only one third of the subjects had smoking data in this study and it was limited by the possibility of chance findings inherent to any screening design. In 2005, Didham et al. conducted a case-control study examining the use of major antibiotics and various cancer sites. The study found that both penicillins and macrolides may be associated with a 13% increased risk of lung and respiratory cancers, but it did not control for smoking and the authors concluded that the elevated RRs were likely due to confounding (3).

Studies on the association between antibiotic use and cancer have several methodologic limitations. Some failed to consider specific antibiotics, therefore assuming a carcinogenic class effect (4). Others were prone to recall bias due to retrospective data collection (1, 2) or were potentially biased from misclassification of etiologically relevant exposure (e.g., sufficient cumulative dose over time, sufficient lag time of effect (ref. 4); uncontrolled confounding, or reverse causation (ref. 3); that is, immunocompromised preclinical cancer patients might have more infections).

We approach the issue as follows. First, we use prospectively collected data from The Health Improvement Network (THIN) to evaluate the association between the use of specific antibiotics and the risk of primary lung cancer. To our knowledge, in contrast to previous screening studies, this is the first large epidemiologic study designed specifically to assess the association between the risk of lung cancer and use of antibiotics. Second, we consider some of the major methodologic challenges stated above and apply causal diagrams to propose mechanisms that may explain the association between antibiotics and cancer.

Materials and Methods

We conducted a nested case-control study within a population-based cohort using computerized medical record information from the THIN database in the United Kingdom (11, 12). THIN collects data on around

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3.9 million patients as recorded by participating general practitioners, including demographics, diagnoses from general practitioner's visits, specialist's referrals and hospital admissions, results of laboratory tests, and comments in a free-text section. All prescriptions issued by the general practitioner, including dose and indication, are recorded and directly generated from the computer to ensure a complete record. Data are sent anonymously to THIN. THIN collects and organizes the information to be used for research purposes (11). The READ classification is used to code specific diagnoses, and a drug dictionary based on data from the MULTILEX classification is used to code drugs. The current study was approved by the Multicenter Research Ethics Committee.

Study Population. We identified all patients who were 40 to 84 years old between January 1995 and December 2004. Patients became members of the study population when they met the criteria of at least 2 years of enrollment with the general practitioner and had been using the computerized prescriptions system for at least 1 year (therefore having had at least one recorded prescription in the database). The date patients satisfied all the eligibility criteria and first became members of the study population is designated as their starting date. Cohort members were followed until one of the following end points: diagnosis of primary lung cancer or any other cancer, 85th birthday, death, or end of study period, whichever came first. Members with any cancer before the start date were excluded. Patients 70 years and older at start date with a follow-up greater than 1 year and no recording of any data during their follow-up time were also excluded. This was done to exclude people whose data completeness is most likely seriously deficient. The final cohort consisted of 1,078,299 persons.

Cases. We identified a total of 4,419 patients with newly diagnosed primary lung cancer during the study period. Computerized patient profiles, which included demographic and clinical data but not drug exposure information, were reviewed. We excluded 81 cases whose diagnosis was uncertain based on the available information. We selected a random sample of 80 cases and sent a brief questionnaire to their general practitioners for validation. The questionnaire included specific questions on the histologic type of the tumor and first date of diagnosis. Out of the 79 responses received, 75 (95%) confirmed the diagnosis of primary lung cancer. The four nonconfirmed cases were excluded from the study (two of which having been excluded in the systematic review of computerized profile). We were left with a total of 4,336 cases for our final analysis.

Controls. We used density sampling to select the controls. This was done by generating at random a date encompassed within the study period for each of the members of the cohort. If the random date for a study member was included in his/her eligible person-time (follow-up period), we marked that person-day as an eligible control. The same exclusion criteria were applied to controls as to cases. A group of 10,000 controls were then randomly selected from the list of eligible person-days and frequency matched to the cases on sex, age within 1 year, and calendar year of the cancer diagnosis.

Exposure Definition. Early cancer symptoms or increased susceptibility to infection in the subclinical phase (latent period), or after clinical suspicion but before the final diagnosis of lung cancer, might affect the use of antibiotics. Therefore, we incorporated a lag time of 1 year into our definition of index date for exposure status assessment. This is done by discounting a period of 1 year from the date of diagnosis for cases and from the random date for controls. We chose 1 year as our lag time because the average time between potential initial symptoms and clinical diagnosis was 66 days in the computerized records, and none of the patients had warning symptoms recorded more than 365 days before diagnosis. We selected two measures of antibiotic exposure: the total number of antibiotic prescriptions and the cumulative number of days on antibiotics according to the prescription. Each of these variables was calculated for two different periods: from enrollment to the index date (cumulative exposure) and during the year before the index date (recent exposure). Subjects who had a prescription for antibiotics within 1 year of the index date were defined as recent users and those who had the last prescription more than 1 year before the index date were defined as past users. We divided the exposure into four categories according to increasing days of antibiotic use (never use and 1-50, 51-100, and >101 days). We also ascertained the treatment indication for the last antibiotic used before the index date and the total number of respiratory, urinary tract, and skin infections from enrollment to the index date. Duration of exposure information recorded in the database was similar between the cases and the controls (median of 7.5 years).

Analysis. We estimated the relative risk (RR) and 95% confidence intervals (95% CI) for lung cancer associated with use of antibiotics compared with nonuse by means of conditional logistic regression models stratified for age, sex, and calendar year. Other potential risk factors, such as smoking status (current, past, or never smoker by index date), smoking cessation interventions, episodes of respiratory and urinary tract infection and other types of infection, history of chronic obstructive pulmonary disease (COPD), asthma, body mass index (weight in kilograms divided by height in meters squared), use of other drugs, alcohol intake, and indicators of health care utilization (e.g., frequency of visits to the general practitioner), were introduced into the multivariate models.

Given the importance of tobacco smoking on the occurrence of lung cancer, we conducted a separate analysis using only patients with smoking status recorded in the database. Specific analyses were performed for smokers and nonsmokers separately. Smoking classification was limited to never, recent, and former as a more detailed lifetime smoking history was unavailable for most subjects. As a surrogate for smoking severity, we used information on smoking cessation interventions, including general practitioner's advice and specific treatments, such as bupropion or nicotine patch, gum, nasal spray, or sublingual preparations prescribed to facilitate smoking cessation.

To estimate the sensitivity of our results to the length of the lag time period, and indirectly evaluate the role of reverse causation, we repeated the analyses defining the index date as the date of diagnosis and as 2 years before the diagnosis. To indirectly evaluate the role of earlier diagnosis of cancer among antibiotic users, who might

Table 1. Baseline characteristics of lung cancer cases and controls at index date (1 y lag time with date of diagnosis) THIN database, UK, 1995-2004

Characteristics	Cases (%)* n = 4,336	Controls (%)* n = 10,000	Adjusted RR† (95% CI)
Age			
<50	150 (3.5)	427 (4.3)	NA‡
50-59	613 (14.1)	1,477 (14.8)	
60-69	1,275 (29.4)	2,901 (29.0)	
70-79	1,855 (42.8)	4,177 (41.8)	
>79	443 (10.2)	1,018 (10.2)	
Sex			
Male	2,658 (61.3)	6,053 (60.5)	NA‡
Female	1,678 (38.7)	3,947 (39.5)	
Smoking status			
Never	764 (17.6)	5,092 (50.9)	Reference
Recent	2,200 (50.7)	1,742 (17.4)	7.58 (6.82-8.41)
Smoking cessation:			
None	1,752 (40.4)	1,555 (15.6)	6.89 (6.19-7.68)
Advice only	156 (3.6)	74 (0.74)	15.78 (11.58-21.51)
Recent treatment	150 (3.5)	41 (0.41)	21.63 (14.60-31.26)
Past treatment	142 (3.3)	72 (0.72)	10.89 (7.95-14.86)
Former	848 (19.6)	1,503 (15.0)	3.34 (2.96-3.76)
Unknown	524 (12.1)	1,663 (16.6)	1.86 (1.56-2.22)
BMI (kg/cm^2)			
<18.5	141 (3.3)	119 (1.2)	1.66 (1.24-2.21)
18.5-<25	1,452 (33.5)	2,869 (28.7)	Reference
25-<30	1,112 (25.7)	3,196 (32.0)	0.79 (0.71-0.87)
30-35	378 (8.8)	952 (9.5)	0.87 (0.75-1.02)
>35	96 (2.2)	308 (3.1)	0.73 (0.56-0.96)
Unknown	1,157 (26.7)	2,556 (25.6)	1.27 (1.11-1.44)
Alcohol (units per week)			
None	1,452 (33.5)	3,372 (33.7)	Reference
1-9	1,027 (23.7)	2,529 (25.3)	1.06 (0.95-1.18)
10-19	429 (9.9)	918 (9.2)	1.08 (0.93-1.25)
≥20	448 (10.3)	740 (7.4)	1.21 (1.03-1.41)
Unknown	980 (22.6)	2,441 (24.4)	1.02 (0.88-1.19)
Number of respiratory infections§			
None	1,602 (37.7)	5,039 (50.6)	Reference
1-5	1,958 (46.1)	4,047 (40.7)	1.23 (1.12-1.34)
6-10	429 (10.1)	598 (6.0)	1.29 (1.08-1.53)
≥11	263 (6.2)	267 (2.7)	1.50 (1.19-1.88)
Number of GU infections§			
None	3,814 (88.1)	8,974 (89.9)	Reference
1-2	482 (11.1)	944 (9.5)	0.97 (0.84-1.11)
≥3	32 (0.7)	61 (0.6)	0.86 (0.52-1.42)
Number of skin infections§			
None	2,767 (67.6)	6,845 (71.5)	Reference
1-2	1,167 (28.5)	2,363 (24.7)	0.98 (0.89-1.08)
≥3	157 (3.8)	360 (3.8)	0.81 (0.65-1.01)
COPD	848 (19.6)	507 (5.1)	2.70 (2.34-3.12)
Asthma	703 (16.2)	1,031 (10.3)	1.01 (0.88-1.14)
Number of medical visits			
0-10	725 (16.7)	2,374 (23.7)	Reference
11-30	1,477 (33.4)	3,592 (35.9)	1.15 (1.02-1.30)
31-50	881 (20.3)	1,803 (18.0)	1.21 (1.04-1.40)
>50	1,283 (29.6)	2,231 (22.3)	1.19 (1.00-1.41)
Number of referrals			
0	1,155 (26.6)	3,231 (32.3)	Reference
1-4	1,814 (41.8)	4,266 (42.7)	0.99 (0.89-1.10)
>4	1,367 (31.5)	2,503 (25.0)	1.08 (0.95-1.24)
Hospitalizations			
No	3,055 (70.5)	7,679 (76.8)	Reference
Yes (≥1)	1,281 (29.5)	2,321 (23.2)	1.12 (1.02-1.24)

Abbreviations: BMI, body mass index (kg/m^2); GU, genitourinary; CI, Confidence Interval.
*The total percentage may not add up to 100% due to rounding.
†Stratified for age, sex, and calendar year, and adjusted for all other variables in the table.
‡Due to the matching, there were no differences between cases and controls with respect to age and sex.
§Numbers of infections are from enrollment date to index date.

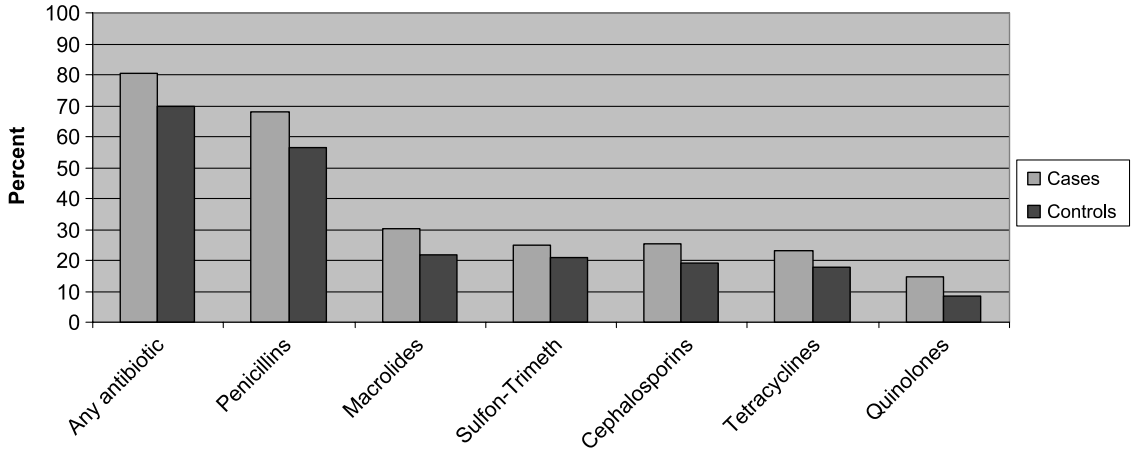

Figure 1. Percentage of patients among cases and controls receiving at least one prescription of antibiotics from enrollment to index date (1 y lag time). THIN database, United Kingdom (1995-2004).

attend health services more frequently, we restricted the analyses to 2,501 patients who died within 6 months of diagnosis (fatal lung cancer cases).

Causal Diagrams. Investigators can propose various hypothetical causal networks linking antibiotic use, cancer, and other relevant factors. Diagrams known as directed acyclic graphs can be used to represent those networks as shown in Figs. 2A to D (13, 14). The diagrams link variables (nodes) by arrows (directed edges) that represent direct causal effects (protective or causative) of one variable on another. Directed acyclic graphs are acyclic because the arrows never point from a given variable to any other variable in its past (i.e., causes precede their effects) and, thus, one can never start from one variable and, following the direction of the arrows, end up at the same variable. The absence of an arrow between two variables indicates that the investigator believes that there is no direct effect (i.e., a causal effect not mediated through other variables in the directed acyclic graphs) of one variable on the other (13, 14).

Results

Table 1 presents characteristics of cases and controls: recent and former smokers, heavy drinkers with more than 20 drinks per week, and subjects with COPD, body mass index less than 18.5 kg/m^2, and respiratory infections had an elevated risk of lung cancer. Overall, cases had higher percentage use of antibiotics (for all classes of antibiotics) compared with controls (Fig. 1).

Among controls, 69.9% had received at least one prescription for antibiotics during the period from enrollment to index date. Not surprisingly, antibiotic use was strongly associated with increasing number of infections, especially respiratory infections, which was also associated with asthma and COPD. Smoking was associated with a slightly higher frequency of both respiratory infections and prescriptions for antibiotics. The prevalence of use of penicillins was 64% among recent smokers, 62% among ex-smokers, and 58% among nonsmokers. The main indications for penicillins were respiratory (22.5%) and upper respiratory (9.0%) infections. Other types of infections were not associated with smoking. More frequent health services utilization was also associated with both infections and antibiotic prescriptions.

For antibiotics overall, a higher number of prescriptions since enrollment with a general practitioner was associated with an increased risk of lung cancer (Table 2); the crude RR was 2.52 (95% CI, 2.25-2.83) for those with 10 or more prescriptions of antibiotics compared with those with none. However, with adjustment for smoking status, smoking cessation interventions, episodes of different types of infection, history of COPD, asthma, body mass index, alcohol intake, and indicators of health care utilization (e.g., frequency of visits to the general practitioner), the adjusted RR was 1.31 (95% CI, 1.10-1.57). This association was 1.29 (95% CI, 1.02-1.62) for men and 1.30 (95% CI, 0.97-1.74) for women. In general, the RR estimates were attenuated after adjusting for potential confounders, particularly smoking, utilization of health services, number of respiratory infections, and COPD. Restricting the analysis to those subjects with known smoking status did not change the results. However, the association was 1.41 (95% CI, 0.99-2.02) among recent smokers and 0.89 (95% CI, 0.65-1.21) among never smokers.

For specific classes of antibiotics, a higher number of prescriptions for penicillins and quinolones were associated with a borderline significant increased risk. We also examined the association of lung cancer with some specific antibiotics such as amoxicillin, amoxicillin-clavulanic acid, erythromycin, floxacillin, trimethoprim, cephalexin, penicillin V, and oxytetracycline, and found no significantly increased risk (data not shown).

When we examined duration of use of different classes of antibiotics, we found similar results. That is, cumulative duration of antibiotic use overall and penicillins in

Table 2. Risk of lung cancer according to cumulative number of prescription of major classes of antibiotics from enrollment date to index date (THIN Database, United Kingdom, 1995-2004)

Antibiotics	Cases (%) n = 4,336	Controls (%) n = 10,000	Crude RR (95% CI)	Adjusted RR* (95% CI)
Total				
0	843 (19.4)	3,019 (30.2)	Reference	Reference
1-4	1,767 (40.8)	4,204 (42.0)	1.51 (1.37-1.65)	1.45 (1.31-1.60)
5-9	828 (19.1)	1,503 (15.0)	1.97 (1.76-2.21)	1.87 (1.65-2.12)
≥10	898 (20.7)	1,274 (12.7)	2.52 (2.25-2.83)	2.14 (1.89-2.44)
Penicillins				
0	1,391 (32.1)	4,337 (43.4)	Reference	Reference
1-4	1,945 (44.9)	4,298 (43.0)	1.42 (1.31-1.54)	1.34 (1.22-1.46)
5-9	635 (14.6)	973 (9.7)	2.04 (1.82-2.29)	1.78 (1.57-2.03)
≥10	365 (8.4)	392 (3.9)	2.91 (2.50-3.40)	2.26 (1.89-2.68)
Tetracyclines				
0	3,337 (76.9)	8,205 (82.0)	Reference	Reference
1-4	870 (20.1)	1,595 (16.0)	1.34 (1.23-1.47)	1.25 (1.13-1.38)
≥5	129 (3.0)	200 (2.0)	1.57 (1.25-1.96)	1.61 (1.25-2.07)
Macrolides				
0	3,031 (69.9)	7,819 (78.2)	Reference	Reference
1-4	1,153 (26.6)	2,001 (20.0)	1.49 (1.37-1.62)	1.38 (1.26-1.52)
≥5	152 (3.5)	180 (1.8)	2.17 (1.74-2.71)	1.91 (1.48-2.44)
Cephalosporins				
0	3,234 (74.6)	8,102 (81.0)	Reference	Reference
1-4	924 (21.3)	1,709 (17.1)	1.35 (1.24-1.48)	1.29 (1.17-1.43)
≥5	178 (4.1)	189 (1.9)	2.36 (1.91-2.91)	1.94 (1.53-2.46)
Quinolones				
0	3,702 (85.4)	9,135 (91.4)	Reference	Reference
1-4	579 (13.4)	809 (8.1)	1.77 (1.58-1.99)	1.66 (1.47-1.89)
≥5	55 (1.3)	56 (0.5)	2.38 (1.64-3.46)	2.57 (1.69-3.91)
Sulfonamides				
0	3,250 (75.0)	7,898 (79.0)	Reference	Reference
1-4	954 (22.0)	1,836 (18.3)	1.28 (1.17-1.39)	1.21 (1.09-1.33)
≥5	132 (3.0)	266 (2.7)	1.22 (0.98-1.51)	1.12 (0.88-1.45)

NOTE: Index Date is defined as one year lag time from the date of diagnosis.
*Adjusted for smoking. All analyses are stratified for age, sex, and calendar year.
†Adjusted for smoking and health care utilization measures.
‡Adjusted for smoking, health care utilization measures, and number of respiratory infections.
§Adjusted for smoking, health care utilization measures, number of respiratory infections, and other types of infections.
‖Adjusted for smoking, health care utilization measures, number of respiratory infections, and other type of infections, body mass index, alcohol, and COPD.

particular was associated with a slightly increased risk of lung cancer, compared with never users. Upon examining specific antibiotics, cumulative use of amoxicillin-clavulanic acid for more than 100 days since enrollment was associated with a RR of 4.35 (95% CI, 1.78-10.67). Of note, this antibiotic is frequently indicated for lower respiratory infections. Similar results were found when we focused on exposure within the 1 year preceding the index date rather than on cumulative exposure since enrollment.

When we restricted the cases to lung cancer patients who died within 6 months of diagnosis (fatal cases), the adjusted RR for those with 10 or more prescriptions of antibiotics since enrollment compared with those with none was 1.41 (95% CI, 1.14-1.75); the corresponding adjusted RR was 1.20 (95% CI, 0.94-1.54) for the remaining cases.

We also conducted sensitivity analyses with a 2-year lag time and with no lag time (index date equals to the date of diagnosis). Results did not change with the 2-year lag time analysis. However, with no lag time, the adjusted RR of lung cancer for those with 10 or more prescriptions of antibiotics since enrollment was 3.46 (95% CI, 2.78-4.30) and the corresponding adjusted RR was 4.74 (95% CI, 3.48-6.46) among fatal cases.

Discussion

We have conducted a population-based nested case-control study to evaluate the association between antibiotic use and the risk of lung cancer using data from the THIN database. The present study has several strengths compared with earlier studies (3, 10). First, in contrast to previous screening studies, this study was designed specifically to assess the association of antibiotic use and risk of lung cancer. Second, the data were collected prospectively. Third, we had more information on smoking compared with previous studies and excluded antibiotic use during the year immediately before the date of cancer diagnosis.

The incidence rate of primary lung cancer in our study population was 7.6 per 10,000 person-years, which is similar to the overall incidence of lung cancer in the United Kingdom (15). Similar to previous studies (3, 10), our study shows an increased risk of lung cancer associated with prescription of antibiotics. Specifically, we found subjects prescribed penicillins and quinolones to have an increased risk. However, after adjustment for smoking, respiratory infections, COPD, and health services utilization, the strength of the association decreased notably. We use below causal diagrams to illustrate how, under plausible causal networks, a

Table 2. Risk of lung cancer according to cumulative number of prescription of major classes of antibiotics from enrollment date to index date (THIN Database, United Kingdom, 1995-2004) (Cont'd)

Adjusted RR[†] (95% CI)	Adjusted RR[‡] (95% CI)	Adjusted RR[§] (95% CI)	Adjusted RR[‖] (95% CI)
Reference	Reference	Reference	Reference
1.39 (1.25-1.55)	1.34 (1.20-1.50)	1.35 (1.21-1.51)	1.31 (1.17-1.47)
1.72 (1.50-1.97)	1.59 (1.37-1.84)	1.62 (1.40-1.88)	1.48 (1.28-1.73)
1.92 (1.65-2.23)	1.60 (1.35-1.90)	1.62 (1.37-1.93)	1.31 (1.10-1.57)
Reference	Reference	Reference	Reference
1.26 (1.15-1.38)	1.19 (1.08-1.31)	1.20 (1.09-1.32)	1.16 (1.05-1.28)
1.59 (1.38-1.83)	1.40 (1.21-1.63)	1.42 (1.22-1.65)	1.28 (1.10-1.50)
1.96 (1.62-2.36)	1.65 (1.34-2.02)	1.68 (1.36-2.06)	1.23 (1.00-1.53)
Reference	Reference	Reference	Reference
1.13 (1.02-1.25)	1.03 (0.92-1.14)	1.03 (0.92-1.15)	1.02 (0.91-1.14)
1.38 (1.07-1.78)	1.21 (0.93-1.57)	1.22 (0.94-1.58)	1.15 (0.88-1.50)
Reference	Reference	Reference	Reference
1.25 (1.14-1.38)	1.16 (1.05-1.28)	1.17 (1.05-1.29)	1.00 (0.90-1.12)
1.60 (1.24-2.07)	1.46 (1.13-1.89)	1.45 (1.12-1.88)	1.20 (0.93-1.56)
Reference	Reference	Reference	Reference
1.15 (1.04-1.28)	1.06 (0.95-1.18)	1.07 (0.96-1.18)	1.13 (1.03-1.26)
1.62 (1.27-2.06)	1.43 (1.11-1.83)	1.42 (1.11-1.83)	1.19 (0.92-1.56)
Reference	Reference	Reference	Reference
1.48 (1.30-1.69)	1.38 (1.21-1.58)	1.39 (1.21-1.59)	1.24 (1.08-1.43)
2.20 (1.44-3.36)	2.07 (1.35-3.18)	2.07 (1.35-3.17)	1.59 (1.03-2.46)
Reference	Reference	Reference	Reference
1.07 (0.97-1.19)	1.03 (0.93-1.15)	1.04 (0.93-1.15)	1.02 (0.92-1.13)
0.93 (0.73-1.19)	0.91 (0.71-1.16)	0.91 (0.71-1.15)	0.87 (0.68-1.12)

positive relationship between antibiotic use and cancer risk can be found even in the absence of any carcinogenic effect of antibiotics.

Causal Effect of Antibiotics. Figure 2A depicts the simplest scenario in which antibiotics affect the risk of cancer. Some types of antibiotics can cause genotoxicity (16), cytotoxicity (17), or leukopenia (18, 19). Even if there were a causal association between antibiotics and lung cancer, because different classes of antibiotics usually act through different biological mechanisms, it is unlikely that all classes of antibiotics increased the risk of lung cancer. Animal data has shown different antibiotics to have different biological effects on cancer initiation or promotion. Prolonged high-dose exposure of mice to metronidazole led to an elevated incidence of lung tumors and to a suggestive increase in lymphoreticular neoplasia in female animals (20). On the other hand, macrolides such as erythromycin and clarithromycin have been shown to suppress human lung cancer cells *in vitro* (21, 22) and tumors in animal models (23). Thus, the elevated risk found for multiple major classes of antibiotics examined suggests a noncausal association.

Confounding. Figure 2B adds potential confounders to the previous diagram (e.g., smoking and infection). Despite the fact that smoking is the strongest risk factor for lung cancer, the amount of confounding due to smoking was relatively modest because of the weak association between smoking and antibiotic use. We adjusted for smoking cessation interventions, which may be a marker of smoking severity as well as of health problems leading to smoking cessation treatments and counseling. However, we lacked detailed information on pack-years smoked and smoking duration, and there might still be some residual confounding by smoking severity in our study. In fact, the association between antibiotics and lung cancer was 0.89 among nonsmokers compared with 1.41 among smokers, suggesting that better control for confounding by smoking severity might have resulted in a RR closer to the null among smokers, and thus overall.

Respiratory infections are one of the most common indications for antibiotic use in our data and were associated with a higher risk of lung cancer. Thus, the observed increased risk of lung cancer among antibiotic users might be a consequence of the underlying association between respiratory infections and lung cancer. The classes of antibiotics that were associated with an increased risk of lung cancer in ours and previous studies (i.e., penicillins, macrolides, and quinolones) are all commonly prescribed for respiratory infections, bronchitis, and/or community-acquired pneumonia (24, 25). Although smokers had a slightly higher frequency of respiratory infections in our data, infection may also independently play a carcinogenic role.

Infectious agents are known to initiate or promote carcinogenesis in other type of tumors (26, 27); for example, *Helicobacter pylori* infection in stomach cancer, hepatitis B or C in liver cancer, human papillomavirus, mainly 16 and 18, in cervical cancer, and human herpesvirus −8 in Kaposi's sarcoma among HIV patients. Evidence of infection and lung cancer in the literature

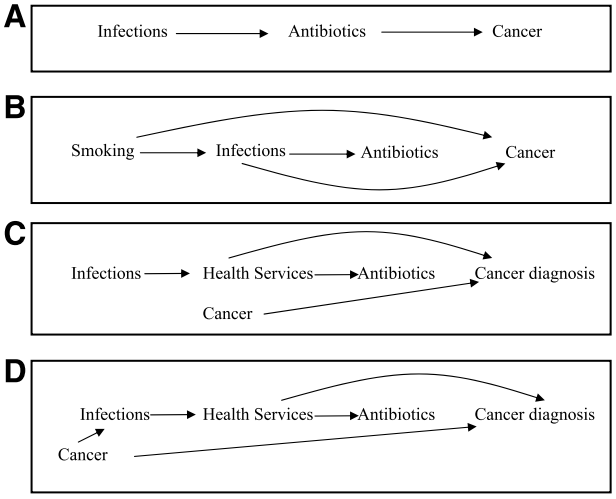

Figure 2. Proposed causal structure (directed acyclic graph) to represent the association between use of antibiotics and the risk of lung cancer.

trace back to 1772 when *Mycobacterium tuberculosis* was implicated as a cause of lung cancer (28-31), although other epidemiologic studies were not able to confirm the association (32, 33). Studies have also suggested an increased risk of lung cancer associated with pneumonia (34, 35), *Chlamydia pneumoniae* (36, 37), and pulmonary human papillomavirus infection (38). Further, chronic colonization of *Haemophilus influenzae* and *Streptococcus pneumoniae* in the lower respiratory tract has been observed in patients with COPD (1), which is an established risk factor for lung cancer (32, 33).

We only found an association between antibiotics and lung cancer among smokers. Such apparent effect modification might be due to residual confounding by smoking severity, as suggested above. Alternatively, there may be a synergistic inflammatory effect between smoking and respiratory infections: smoking results in chronic airway inflammation and immunologic stimulation by infection may aggravate the inflammation. Infection and inflammation together can trigger the production of DNA-damaging free radicals and reactive oxygen species (39), which promote carcinogenesis. Proinflammatory and Th-1 cytokines up-regulate the expression of cyclooxygenase-2 enzyme, which plays a key role in the regulation of the immune system and in angiogenesis (40, 41). Cyclooxygenase-2 expression leads to Th2 cytokines production, which inhibits the synthesis of Th1 cytokines and suppress cell-mediated immunity; the proangiogenic, Th-2 predominant environment, with an up-regulated humoral immunity and a suppressed cell-mediated immunity becomes very favorable to the development of malignancy (41).

Selection Bias. Figure 2C depicts the scenario in which antibiotics do not affect the risk of cancer but are associated with a more frequent utilization of health services, which results in an earlier diagnosis of cancer. Our data indicate that infections, especially respiratory infections, were strongly associated with increased number of visits to the general practitioners and increased number of referrals. Cases had more respiratory infections than controls and patients who have respiratory illnesses are frequently evaluated with a chest X-ray, which would detect early lung cancer. This would lead to ascertainment bias because those without a respiratory illness would not receive a chest X-ray and thus are more likely to have lung cancer detected at a later stage. However, a preferential diagnosis of early cancer stages would induce a stronger spurious association for cases with longer survival postdiagnosis (referred to as nonfatal cases in our study), which is inconsistent with our findings.

Reverse Causation. Rather, as illustrated in Fig. 2D, the stronger association of antibiotics with diseases diagnosed at an advanced stage (i.e., with worse prognosis) is consistent with the existence of preclinical cancer leading to infections, utilization of health services, and prescription of antibiotics. Patients with undiagnosed cancer may use antibiotics more often due to impaired immune systems and increase susceptibility to infections, or even due to tumor-induced postobstructive pneumonias. In this case, the temporal relationship is reversed and the positive association between antibiotics and cancer would be due to increased infection rates as a consequence of having cancer rather than antibiotics increasing the risk of cancer.

For our primary analysis, we attempted to minimize this reverse causation bias by excluding antibiotic use during the year before diagnosis. Unfortunately, it is difficult to know the precise onset of a patient's cancer. Despite the 1-year lag time, our results may still be affected by reverse causation bias, as suggested by the stronger RR found upon restriction of the analyses to fatal cases and the increased risk of lung cancer associated with being underweight.

The association between antibiotics and lung cancer was strongest when we repeated the analyses with no lag time. The sensitivity of the results to different lag times suggests that reverse causation might explain the increased risk of cancer found in studies that included any use of antibiotics during the months immediately preceding diagnosis (3). If the latency period is longer, as suggested by the carcinogenic effect of asbestos or smoking, perhaps future studies should consider a lag time of 2 or more years.

Misclassification of Exposure and Other Limitations. One of the major strengths of our study is that information on drug prescriptions was prospectively collected; thus, differential misclassification of antibiotic use between cases and controls is unlikely. Underestimation of exposure due to over-the-counter preparations should not be of a concern in this study because antibiotics require prescriptions by physicians in the United Kingdom. However, we could not account for either antibiotic use before enrollment or patient compliance; in addition, our exposure window and follow-up time might not be biologically meaningful. The latter sources of misclassification would tend to attenuate any potential effect.

Regarding outcome classification, our validation study confirmed 95% of the lung cancer cases. However, we did not have adequate pathologic information as to evaluate different histologic subtypes of lung cancer. Therefore, we can not exclude the possibility of antibiotics being preferentially associated with specific histologic types of lung cancer.

Conclusion. Using causal diagrams, we conclude that the apparent carcinogenic effect of antibiotics observed in the epidemiologic studies could be the result of residual confounding and reverse causation (42-44). The higher risk of lung cancer among users of antibiotics may be due to common causes (e.g., smoking, respiratory infections, or impaired immune functions) and to the increased risk of infections in those with subclinical cancer. Overall, current epidemiologic evidence is insufficient to support or refute a carcinogenic effect of antibiotics on lung cancer. Due to the multifactorial nature of cancer and the strong correlation of antibiotic use with respiratory infections, future studies should consider the role of respiratory infections in the etiology of lung cancer and try to find good serologic markers of chronic or persistent infection.

Disclosure of Potential Conflicts of Interest

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