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Original article

Association of antibiotic use with risk of lung cancer: A nationwide cohort study

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ABSTRACT

Background: Although recent studies indicated that antibiotics may be a risk factor for lung cancer, further understanding is needed. We investigated the association of long-term antibiotic exposure with lung cancer risk.
Methods: This population-based retrospective cohort study investigated 6,214,926 participants aged ≥ 40 years who underwent health screening examinations (2005–2006) from the Korean National Health Insurance Service database. The date of the final follow-up was December 31, 2019. Exposures were the cumulative days of antibiotics prescription and the number of antibiotics classes. The adjusted hazard ratios (aHRs) and 95% confidence intervals (CIs) for lung cancer risk according to antibiotic use were assessed using multivariable Cox proportional hazards regression.
Results: Compared with the antibiotic non-user group, participants with ≥ 365 days of antibiotics prescribed had a significantly increased risk of lung cancer (aHR, 1.21; 95% CI, 1.16–1.26). Participants with ≥ 365 days of antibiotics prescribed also had a significantly increased risk of lung cancer (aHR, 1.21; 95% CI, 1.17–1.24) than 1–14 days of the antibiotic user group. The results were also consistent in competing risk analyses and adjusted Cox regression models that fitted restricted cubic spline. Compared with the antibiotic non-user group, ≥ 5 antibiotic classes prescribed group had a higher lung cancer risk (aHR, 1.15; 95% CI, 1.10–1.21).
Conclusion: The long-term cumulative days of antibiotic use and the increasing number of antibiotics classes were associated with an increased risk of lung cancer in a clear duration-dependent manner after adjusting for various risk factors.

Introduction

Lung cancer is the greatest cause of cancer death, responsible for nearly 25% of all cancer-related deaths. Lung cancer has a five-year survival rate of 18.6%, which is lower than other prevalent cancers such as prostate and breast cancer [1]. More than half of lung cancer patients die within a year of being diagnosed [2]. Although cigarette smoking is projected to be the major cause of lung cancer, evidence

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regarding other drivers of lung cancer remains uncertain [3]. Moreover, identifying risk factors for lung cancer, especially among non-smokers, is of importance from a public health perspective. Recent studies have revealed that not only environmental and genetic factors but also microbiota is associated with lung cancer [4].

The gut microbiota plays a substantial role in essential human biological functions and is especially involved in disease states, including inflammation and carcinogenesis [5,6]. The gut-lung axis is currently understood to be linked to alterations in immune pathways and pulmonary diseases [7]. Dysbiosis of the gut microbiota leads to increased susceptibility to lung cancer by inhibiting the role of immune cells and inducing pro-inflammatory cytokines [4,8,9]. Therefore, the presence of microbiome dysbiosis caused by antibiotic use and the interplay between gut and lung trigger the development of lung cancer [4,9,10].

Although the relationship between antibiotics and lung cancer is currently being unraveled, previous studies are insufficient to fully confirm the association. Several studies have reported significant changes in the composition of lung and intestinal microbiota in lung cancer patients [11,12]. Moreover, antibiotic treatment alters the gut microbial diversity, specifically depleting the gut microbiota, and consequently causes impairment of the microbial anti-tumor activity within the lung [13,14]. A case-control study has shown that lung cancer risk was related to antibiotic use; however, they concluded that this association might be due to confounding effects and reverse causation [15]. Thus, a large population-based study considering possible confounders is needed to show whether antibiotic exposure leads to increased lung cancer risk.

In this longitudinal retrospective study, we explored the association between antibiotic exposure, such as cumulative antibiotic days from 2002 to 2006, and lung cancer incidence from 2007 to 2019 by analyzing data from the Korean National Health Insurance Service database between 2002 and 2019 using survival analysis.

Material and methods

Study cohort

This nationwide population-based retrospective cohort study included participants from the Korean National Health Insurance Service (NHIS) database (NHIS-2023–1–161). The NHIS provides a diverse range of mandatory health insurance coverage to approximately 97% of South Korea's population [16]. Individuals ≥ 20 years of age are eligible to undergo biannual health screening examinations, which include self-reported questionnaires about lifestyle behaviors, anthropometric measurements, and blood and urine laboratory findings [17]. The NHIS database consists of sociodemographic characteristics, health screening results, all inpatient and outpatient usage, and drug prescriptions [16]. A variety of research on epidemiology has used the NHIS database [18–20], and its validity has been thoroughly explained elsewhere [16,17].

The study population consisted of 8,105,760 participants aged more than 40 years who underwent health screening examinations from 2005 to 2006 from the NHIS. Among them, 23,006 individuals who were dead and 1,561,563 individuals with a cancer diagnosis before the index date of January 1, 2007, were excluded, respectively. 306,265 individuals with missing values for covariates were also excluded. Finally, a total of 6,214,926 participants were included in this study (Fig. 1). All participants were followed up until the date of cancer event, death, or December 31, 2019, whichever came earliest.

Exposures

Antibiotic use was assessed by the cumulative prescription days of antibiotics and the number of antibiotic classes. In the NHIS database, the antibiotic cumulative days and the number of antibiotic classes were collected independently. All analyses were performed separately in distinct models, excluding the potential interaction between these two variables. The cumulative days of antibiotic use were defined by adding all antibiotic subscription days from 2002 to 2006, the first five years in the database. We categorized antibiotic use ranging from none to ≥ 365 days (none, 1–14, 15–59, 60–179, 180–364, and ≥365 days). The number of antibiotic classes prescribed was defined as the sum of all classes of antibiotic subscriptions from 2002 to 2006. Antibiotic classes were defined by the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) classification of drugs as macrolides, penicillins, cephalosporins, fluoroquinolones, sulfonamides, lincosamides, tetracyclines, vancomycin, carbapenems, monobactams, and linezolid (Supplementary Table S1). We categorized the number of antibiotic classes as ranging from none to five or more classes (none, 1, 2, 3, 4, and 5 or more).

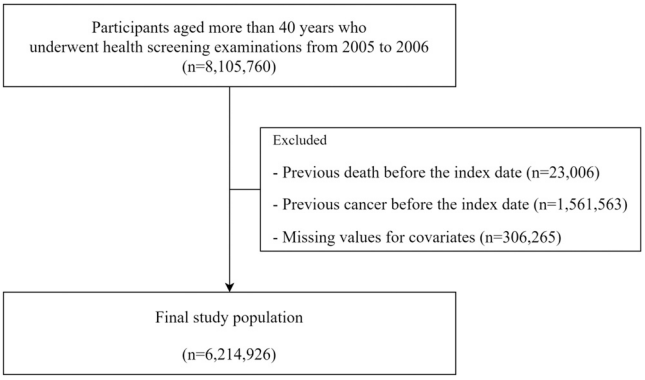

Fig. 1. Selection of cohort study participants.

Outcomes

The primary outcome was a lung cancer diagnosis. Diagnosis codes for lung cancer were based on the International Classification of Diseases, Tenth Revision (ICD-10) codes. We identified lung cancer events from 2007 through 2019 using ICD-10 codes (C33-C34) with the critical condition codes for cancer (V193-V194) simultaneously based on the database [21,22]. In the NHIS database, the critical condition code for cancer has a high degree of reliability since the patients are confirmed only after a diagnosis of cancer is completed [23,24].

Covariates

On multivariate analysis, the covariates which are considered potential confounding factors included age (continuous; years), sex (categorical; men and women), household income (categorical; 1st, 2nd, 3rd, and 4th quartile), smoking status (categorical; never smoker, past smoker, and current smoker), alcohol intake (categorical; 0, 1–2, 3–4, and ≥5 times per week), physical activity (categorical; 0, 1–2, 3–4, and ≥5 times per week), body mass index (BMI; continuous; kg/m^2), total cholesterol (TC; continuous; mg/dL), systolic blood pressure (SBP; continuous; mmHg), fasting serum glucose (FSG; continuous; mg/dL), Charlson comorbidity index (CCI; continuous), acid suppressants use (categorical; yes and no), statin use (categorical; yes and no), non-steroidal anti-inflammatory drugs use (NSIADs; categorical; yes and no), aspirin use (categorical; yes and no), chronic obstructive pulmonary disease (COPD; categorical; yes and no), tuberculosis (categorical; yes and no), asthma (categorical; yes and no), respiratory diseases (categorical; yes and no), urinary tract infections (UTI; categorical; yes and no), skin, soft tissue, bone, and joint infections (SSTBJ; categorical; yes and no), intra-abdominal infections (IAI; categorical; yes and no), intestinal

infectious diseases (categorical; yes and no), and other infectious diseases (categorical; yes and no).

Household income was estimated according to the insurance premium, and body mass index was calculated by dividing the weight in kilograms by the height in meters squared. CCI was calculated by means of a previous study [25]. Acid suppressants were defined as histamine-2-receptor antagonists and proton pump inhibitors [18]. COPD (J44), tuberculosis (A15-A19), and asthma (J45-J46) were defined as diagnoses before the index. Infectious diseases, which are the reason for antibiotic use, were considered six systems by diagnosis before the index date (Supplementary Table S2) [18].

Statistical analysis

The χ^2 test for categorical variables and the analysis of variance for continuous variables were used to determine the significance of the difference in distribution for covariates among the study population. Multivariable Cox proportional hazards regression after adjusting for various covariates was used to calculate the adjusted hazard ratios (aHRs) and 95% confidence intervals (CIs) for lung cancer according to the cumulative prescription days of antibiotics and the number of antibiotic classes prescribed, respectively. Furthermore, in order to account for the competing risk of death, we performed additional analyses utilizing the competing risk model. We used various multivariate models to assess lung cancer risk. Covariates of model 1 included age, sex, household income, and lifestyle behaviors (smoking status, alcohol intake, and physical activity). We additionally and cumulatively included the results of health screening examinations (BMI, TC, SBP, and FSG), and CCI in model 2; other medication use (acid suppressants, statin, and NSIADs) in model 3; other diseases, which are known as risk factors for lung cancer (COPD [26], tuberculosis [27], and asthma [28]) in model 4. Furthermore, we included the source of infection (respiratory diseases, UTI, SSTBJ, IAI, intestinal infectious diseases, and other infectious diseases) as covariates in model 5, which is used for groups with antibiotic prescription records. Sensitivity analyses were performed by excluding participants with cancer diagnosis after the index date within 1, 3, and 5 years. We also performed analyses to assess the risk of lung cancer by excluding participants with COPD, tuberculosis, and asthma before the index date. Furthermore, we performed analyses by shifting the index date to 2 years later. That is, the antibiotic exposure period and the follow-up period were redefined from 5 years to 7 years and from 13 years to 11 years, respectively. Stratified analyses on the association of antibiotic use with lung cancer risk according to subgroups of age, smoking status, CCI, COPD, tuberculosis, and asthma were conducted. Statistical significance was defined as a two-sided $P < 0.05$. P_{trend} was calculated independently using the cumulative days of antibiotic use and the number of antibiotic classes, as continuous variables. Restricted cubic spline of lung cancer risk according to cumulative antibiotic days was presented to graphically assess the non-linear association between cumulative antibiotic exposure and aHRs (95% CIs) of lung cancer [29]. Data collection, data mining, and all analyses were performed using SAS Enterprise Guide 8.2 (SAS Institute, Cary, NC, USA).

Results

Table 1 and Supplementary Table S3 present the baseline characteristics of the study population. The median follow-up duration

Table 1
Baseline characteristics of study population.

Characteristics	Cumulative days of antibiotics prescribed for 5 years before the index date						*P* value
	None	1–14 days	15–59 days	60–179 days	180–364 days	≥ 365 days	
Participants, n	327 314	1 011 786	1 479 213	1 501 651	818 532	1 076 430	
Lung cancer events, n	3 601	10 984	16 916	18 457	10 874	16 940	
Age, years, mean (SD)	53.82 (10.14)	53.65 (9.82)	54.11 (9.89)	54.72 (10.10)	55.36 (10.34)	56.55 (10.75)	< 0.001
Sex, n (%)							< 0.001
Men	215 343 (65.79)	615 273 (60.81)	836 869 (56.58)	792 694 (52.79)	405 331 (49.52)	517 441 (48.07)	
Women	111 971 (34.21)	396 513 (39.19)	642 344 (43.42)	708 957 (47.21)	413 201 (50.48)	558 989 (51.93)	
Household income, n (%)							< 0.001
1st quartile (highest)	111 977 (34.21)	368 112 (36.38)	553 503 (37.42)	570 905 (38.02)	313 282 (38.27)	407 220 (37.83)	
2nd quartile	76 829 (23.47)	247 772 (24.49)	367 133 (24.82)	375 928 (25.03)	206 161 (25.19)	273 590 (25.42)	
3rd quartile	62 805 (19.19)	185 946 (18.38)	265 014 (17.92)	262 928 (17.51)	141 082 (17.24)	186 594 (17.33)	
4th quartile (lowest)	75 703 (23.13)	209 956 (20.75)	293 563 (19.85)	291 890 (19.44)	158 007 (19.30)	209 026 (19.42)	
Smoking status, n (%)							< 0.001
Never smoker	200 335 (61.21)	648 753 (64.12)	988 118 (66.80)	1 044 192 (69.54)	589 058 (71.97)	792 998 (73.67)	
Past smoker	32 185 (9.83)	100 858 (9.97)	147 422 (9.97)	147 590 (9.83)	78 736 (9.62)	103 471 (9.61)	
Current smoker	94 794 (28.96)	262 175 (25.91)	343 673 (23.23)	309 869 (20.64)	150 738 (18.42)	179 961 (16.72)	
Alcohol intake, times/week, n (%)							< 0.001
0	218 365 (66.71)	691 599 (68.35)	1 038 819 (70.23)	1 086 635 (72.36)	610 718 (74.61)	834 677 (77.54)	
1–2	65 895 (20.13)	193 714 (19.15)	266 403 (18.01)	252 660 (16.83)	128 411 (15.69)	150 398 (13.97)	
3–4	26 944 (8.23)	80 369 (7.94)	110 497 (7.47)	102 927 (6.85)	50 229 (6.14)	57 538 (5.35)	
≥ 5	16 110 (4.92)	46 104 (4.56)	63 494 (4.29)	59 429 (3.96)	29 174 (3.56)	33 817 (3.14)	
Physical activity, times/week, n (%)							< 0.001
0	175 158 (53.51)	533 537 (52.73)	777 300 (52.55)	784 016 (52.21)	425 757 (52.01)	565 352 (52.52)	
1–2	90 121 (27.53)	275 301 (27.21)	392 575 (26.54)	388 847 (25.89)	207 343 (25.33)	261 610 (24.30)	
3–4	34 441 (10.52)	112 689 (11.14)	170 203 (11.51)	178 480 (11.89)	98 788 (12.07)	128 078 (11.90)	
≥ 5	27 594 (8.43)	90 259 (8.92)	139 135 (9.41)	150 308 (10.01)	86 644 (10.59)	121 390 (11.28)	
Body mass index, kg/m^2, mean (SD)	23.64 (3.15)	23.77 (2.95)	23.87 (2.94)	23.95 (2.94)	24.03 (2.95)	24.11 (3.00)	< 0.001
Total cholesterol, mg/dL, mean (SD)	197.61 (37.45)	197.62 (37.25)	197.73 (37.05)	197.88 (37.54)	198.10 (37.75)	198.55 (37.75)	< 0.001
Systolic blood pressure, mm Hg, mean (SD)	127.56 (17.85)	126.22 (17.24)	125.56 (16.95)	125.21 (16.78)	125.07 (16.69)	125.41 (16.73)	< 0.001
Fasting serum glucose, mg/dL, mean (SD)	98.11 (27.89)	97.65 (26.85)	97.79 (26.94)	98.01 (27.20)	98.16 (27.26)	98.68 (28.09)	< 0.001
Charlson comorbidity index, n (%)							< 0.001
0	264 203 (80.72)	733 283 (72.47)	935 420 (63.24)	810 668 (53.99)	375 947 (45.93)	379 751 (35.28)	
1	40 836 (12.48)	180 462 (17.84)	341 261 (23.07)	409 228 (27.25)	245 984 (30.05)	342 655 (31.83)	
≥ 2	22 275 (6.81)	98 041 (9.69)	202 532 (13.69)	281 755 (18.76)	196 601 (24.02)	354 024 (32.89)	

The *P* values were calculated using a χ^2 test for categorical variables and an analysis of variance for continuous variables. Ordering of variables was not considered on the χ^2 test analysis.
n indicates number of people; and SD, standard deviation.

was 13.0 years. Among 6,214,926 participants, those with ≥ 365 days of antibiotic use were more likely to have a higher BMI, a higher TC, and more comorbidities than the antibiotic non-users. There were significant differences in the distribution of variables (all $P < 0.001$).

The risk for lung cancer according to the cumulative days of antibiotics prescribed is shown in Table 2. There is a clear duration-dependent relationship between cumulative antibiotic prescription days and lung cancer. A longer duration of antibiotic days was associated with a higher risk of lung cancer in all models (all $P_{trend} < 0.001$). Compared with the non-user group, those with ≥ 365 days of antibiotic use (aHR, 1.21; 95% CI, 1.16–1.26) had a higher risk for lung cancer in a fully adjusted model (model 4). The participants who were prescribed antibiotics for ≥ 365 days (aHR, 1.21; 95% CI, 1.17–1.24) had a higher risk for lung cancer in a fully adjusted model (model 5) which additionally considered infectious diseases, than the 1–14 days user group. The duration-dependent relationship between antibiotic use and lung cancer tended to be preserved regardless of sex (all $P_{trend} < 0.001$) (Supplementary Table S4 and Supplementary Table S5). The crude hazard ratio for lung cancer according to the antibiotic cumulative days is shown in Supplementary Table S6. Competing risk analyses were performed to account for a competing risk caused by overall death when estimating the risk of lung cancer (Supplementary Table S7). This study found an association between antibiotic exposure and lung cancer even considering the competing risk of death. When the competing risk of death was considered, long-term antibiotic exposure was associated with a higher risk of lung cancer (aHR, 1.20; 95% CI, 1.16–1.25), compared to the non-user group.

Sensitivity analyses are presented in Table 3. Participants who were prescribed antibiotics for ≥ 365 days had a higher risk for lung cancer (aHR, 1.22; 95% CI, 1.17–1.28) than the non-user group after excluding those with a cancer diagnosis within the first 5 years of follow-up ($P_{trend} < 0.001$). Participants who were prescribed antibiotics for ≥ 365 days had a higher risk for lung cancer (aHR, 1.23;

Table 2
Risk for lung cancer according to cumulative days antibiotics prescribed.

	Cumulative days of antibiotics prescribed for 5 years before the index date						*P* for trend
	None	1–14 days	15–59 days	60–179 days	180–364 days	≥ 365 days	
Participants, n	327 314	1 011 786	1 479 213	1 501 651	818 532	1 076 430	
Events, n	3 601	10 984	16 916	18 457	10 874	16 940	
Person-years	3 969 209	12 366 792	18 089 446	18 322 202	9 954 079	12 929 944	
aHR (95% CI)[a]							
Model 1	1.00 (ref.)	1.04 (1.01–1.08)	1.10 (1.06–1.14)	1.17 (1.13–1.22)	1.25 (1.20–1.30)	1.40 (1.35–1.45)	< 0.001
Model 2	1.00 (ref.)	1.04 (1.00–1.08)	1.09 (1.05–1.13)	1.15 (1.11–1.20)	1.22 (1.17–1.27)	1.34 (1.29–1.39)	< 0.001
Model 3	1.00 (ref.)	1.03 (0.99–1.07)	1.06 (1.02–1.11)	1.12 (1.08–1.17)	1.18 (1.13–1.23)	1.30 (1.25–1.35)	< 0.001
Model 4	1.00 (ref.)	1.02 (0.98–1.06)	1.05 (1.01–1.09)	1.09 (1.05–1.14)	1.14 (1.09–1.19)	1.21 (1.16–1.26)	< 0.001
aHR (95% CI)[b]							
Model 1		1.00 (ref.)	1.05 (1.03–1.08)	1.12 (1.10–1.15)	1.20 (1.17–1.23)	1.34 (1.31–1.37)	< 0.001
Model 2		1.00 (ref.)	1.05 (1.02–1.07)	1.11 (1.08–1.13)	1.17 (1.14–1.20)	1.29 (1.26–1.32)	< 0.001
Model 3		1.00 (ref.)	1.04 (1.01–1.06)	1.09 (1.07–1.12)	1.15 (1.12–1.19)	1.27 (1.24–1.30)	< 0.001
Model 5		1.00 (ref.)	1.03 (1.01–1.06)	1.08 (1.05–1.11)	1.12 (1.09–1.16)	1.21 (1.17–1.24)	< 0.001

The aHRs were calculated by Cox proportional hazards regression after adjustments for multivariate variables. Model 1 was adjusted for age, sex, household income, smoking status, alcohol intake, and physical activity. Model 2 was adjusted for the variables in Model 1 plus body mass index, total cholesterol, systolic blood pressure, fasting serum glucose, and Charlson comorbidity index. Model 3 was adjusted for the variables in Model 2 plus acid suppressants use, statin use, NSAIDs use, and aspirin use. Model 4 was adjusted for the variables in Model 3 plus COPD, tuberculosis, and asthma. Model 5 was adjusted for the variables in Model 4 plus infectious diseases (respiratory diseases, urinary tract infections, skin, soft tissue, bone, and joint infections, intra-abdominal infections, intestinal infectious diseases, and others).
[a]Antibiotics non-user group was set as a reference group.
[b]Antibiotics 1–14 days user group was set as a reference group.
n indicates number of people; aHR, adjusted hazard ratio; CI, confidence interval; and ref, reference.

Table 3
Sensitivity analysis of risk for lung cancer according to cumulative days of antibiotics prescribed.

	Cumulative days of antibiotics prescribed for 5 years before the index date								*P* for trend
	Total	Events	None	1–14 days	15–59 days	60–179 days	180–364 days	≥ 365 days	
Wash-out period									
Model 1									
1-year wash-out	6 200 130	75 749	1.00 (ref.)	1.02 (0.98–1.06)	1.06 (1.01–1.10)	1.10 (1.05–1.14)	1.14 (1.09–1.19)	1.22 (1.17–1.27)	< 0.001
3-year wash-out	6 152 934	68 930	1.00 (ref.)	1.02 (0.98–1.06)	1.06 (1.02–1.10)	1.10 (1.06–1.15)	1.14 (1.09–1.20)	1.23 (1.17–1.28)	< 0.001
5-year wash-out	6 089 261	60 499	1.00 (ref.)	1.00 (0.96–1.05)	1.05 (1.00–1.10)	1.10 (1.06–1.15)	1.14 (1.09–1.20)	1.22 (1.17–1.28)	< 0.001
Model 2									
1-year wash-out	5 873 630	72 248		1.00 (ref.)	1.04 (1.01–1.06)	1.08 (1.05–1.11)	1.13 (1.09–1.16)	1.21 (1.17–1.24)	< 0.001
3-year wash-out	5 828 815	65 748		1.00 (ref.)	1.04 (1.01–1.07)	1.09 (1.06–1.11)	1.13 (1.10–1.17)	1.22 (1.18–1.26)	< 0.001
5-year wash-out	5 768 252	57 696		1.00 (ref.)	1.05 (1.02–1.08)	1.10 (1.07–1.13)	1.14 (1.11–1.18)	1.23 (1.19–1.27)	< 0.001
Exclusion									
Diagnosis of COPD, tuberculosis, and asthma before the index date									
Model 1	5 399 107	61 312	1.00 (ref.)	1.02 (0.98–1.06)	1.05 (1.00–1.09)	1.10 (1.05–1.14)	1.14 (1.09–1.19)	1.20 (1.15–1.25)	< 0.001
Model 2	5 080 992	57 912		1.00 (ref.)	1.03 (1.00–1.06)	1.08 (1.06–1.11)	1.13 (1.09–1.16)	1.19 (1.16–1.23)	< 0.001
Variation of the exposure period and follow-up period									
7-year exposure period									
Model 1	6 803 654	72 122	1.00 (ref.)	1.02 (0.97–1.08)	1.06 (1.01–1.12)	1.13 (1.07–1.19)	1.16 (1.09–1.22)	1.25 (1.18–1.32)	< 0.001
Model 2	6 610 373	70 378		1.00 (ref.)	1.04 (1.01–1.07)	1.11 (1.08–1.14)	1.14 (1.10–1.18)	1.24 (1.20–1.28)	< 0.001

The aHRs were calculated by Cox proportional hazards regression after adjustments for multivariate variables. Model 1 was adjusted for age, sex, household income, smoking status, alcohol intake, physical activity, body mass index, total cholesterol, systolic blood pressure, fasting serum glucose, Charlson comorbidity index, acid suppressants use, statin use, NSAIDs use, aspirin use, COPD, tuberculosis, and asthma. Model 2 was adjusted for the variables in Model 1 plus infectious diseases (respiratory diseases, urinary tract infections, skin, soft tissue, bone, and joint infections, intra-abdominal infections, intestinal infectious diseases, and others).
aHR indicates adjusted hazard ratio; CI, confidence interval; and ref, reference.

95% CI, 1.19–1.27) than the 1–14 days user group after excluding those with cancer diagnosis within the first 5 years of follow-up ($P_{trend} < 0.001$). After excluding patients with prevalent lung diseases, including COPD, tuberculosis, and asthma before the index date, similar patterns to the main results were observed in the analyses. The association between lung cancer and antibiotic use was found to be significant in the ≥ 365 days user group (aHR, 1.20; 95% CI, 1.15–1.25) compared to the non-user group. Finally, when the antibiotic exposure period was lengthened to 7 years instead of 5 years, participants who were prescribed antibiotics for ≥ 365 days had a greater risk for lung cancer (aHR, 1.25; 95% CI, 1.18–1.32) than the non-users.

The results from the stratified analyses are shown in Table 4. We did not observe significant interactions of the cumulative days of antibiotics with CCI, COPD, tuberculosis, and asthma on lung cancer ($P_{interaction} > 0.05$). Association of antibiotic use with lung cancer appeared to be stronger in participants aged ≥ 50 years ($P_{interaction} < 0.001$). The interaction of antibiotic use with smoking status on lung cancer ($P_{interaction} < 0.001$) was observed, but a longer duration of antibiotic use was associated with a higher risk of lung cancer regardless of smoking status ($P_{trend} < 0.001$).

The risk for lung cancer according to the number of antibiotic classes prescribed is shown in Table 5. Compared to the non-user group, participants who used five or more antibiotic classes had a higher risk of lung cancer (aHR, 1.15; 95% CI, 1.10–1.21; $P_{trend} < 0.001$).

The restricted cubic spline curve showed that the lung cancer risk increased with the long-term cumulative antibiotic subscription days, similar to the categorical analyses (Supplementary Fig. S1).

Discussion

In this retrospective cohort study, we revealed that lung cancer incidence was associated with cumulative days of antibiotic prescription and the increasing number of antibiotic classes in a duration-dependent relationship. Furthermore, this association was significantly consistent in various analyses after adjustments for potential confounding factors, including cigarette smoking, chronic lung diseases, and infectious diseases. To the best of our knowledge, this nationally representative study is the first in Asia to provide real-world evidence of antibiotic exposure as a risk-enhancing factor for lung cancer.

Lungs were formerly regarded as sterile for a long time. However, rapid strides in microbial research confirmed the presence of lung microbiota [30]. The relationship between lung microbiota and lung cancer has been proposed, providing plausible mechanisms for the lung microbiome leading to lung tumorigenesis. Dysregulated lung microbiome is manifested by decreased symbiotic bacteria and increased pathogenic bacteria [31–33]. Thus, dysbiotic lung microbiota may propagate a chronic inflammatory environment, and chronic inflammation is known to result in carcinogenesis by inducing apoptosis, cellular proliferation, and mutation [9].

Advances in the understanding of gut and lung microbiota have enabled the explanation of mechanisms. The fact that the gastrointestinal and respiratory tracts have the same embryonic origin and structural similarities supports the discovery that the gut and lung interact through microbial and immune pathways [9]. The gut microbiota-lung axis is a key pathway that directly links lung cancer and gut dysbiosis due to antibiotic use. Gut dysbiosis triggers the production of inflammatory cytokines and cytotoxic substances [9]. Moreover, bioactive molecules originating from microbiomes can induce cancer initiation by causing the production of toxic substances in the lung [9]. More specifically, aberrant forms of intestinal bacteria produce secondary bile acids, like deoxycholic acid and lithocholic acid, from bile acids, which can lead to DNA damage and gradually initiate lung carcinogenesis [9]. Another possible mechanism is genotoxicity due to changes in microbiome composition [10]. Toxins and free radical formation, DNA lesions, and cell cycle arrest occur as processes of genotoxicity, taking part in carcinogenesis in the lung [9].

There are additional possible explanations for our findings. First, antibiotics may be carcinogenic [34]. In the context that cancer is an environmental disease, antibiotics could have the ability to act as extrinsic environmental chemical carcinogenic factors themselves and to alter the normal microbiota toward carcinogenic [35]. Specific antibiotic classes, such as beta-lactams, cephalosporins, and fluoroquinolones, were associated with a higher risk of cancer [36]. However, further studies about the mechanism are needed. Second, several diseases by gut and lung dysbiosis in accordance with long-term antibiotic use may have increased the risk of lung cancer. Gut dysbiosis is implicated in the etiology of inflammation and other diseases such as COPD and asthma [37–40]. Though we excluded lung cancer-related pulmonary diseases and adjusted for infectious diseases, there may still be risk factors during the observation period.

Several limitations must be addressed when interpreting the findings of our research. First, we could not consider the clinical information, such as the stage and the histopathologic type, because of the lack of information in the database. Second, even though we adjusted for numerous potential confounding variables related to lung cancer, the possibility of residual confounders may not have been eliminated. We attempted to minimize the possibility of bias by considering major risk factors such as CCI, COPD, tuberculosis, asthma, and a variety of infectious diseases in multivariable Cox regression analysis. However, due to the study's retrospective nature, we could not completely rule out the possibility of indication bias. Further prospective studies that minimize the likelihood of indication bias are required to verify our results. Third, the prescription records for antibiotics may not accurately represent the actual intake. Fourth, the result might not be generalized to other ethnicities and countries. Finally, even though there was an association between antibiotic use and the risk of lung cancer, it may be unclear whether our findings represent a causal effect because of the retrospective cohort study design.

Research on antibiotic exposure and lung cancer is limited, and as such, little is known about their association. Nevertheless, our study made advances in assessing the association between antibiotic exposure and lung cancer risk. This study is currently the largest in scale and may represent the general population. We also used the operational definition of lung cancer diagnosis based on the ICD-10 codes and the critical condition codes for cancer simultaneously to minimize any misclassification bias. In addition, the extensive list of confounders comprises sociodemographic characteristics, health behavior and status, medication use, major lung diseases, and infectious diseases, strengthening the credibility of our results. Finally, the results of the sensitivity analyses with the 5-year latent period minimized potential reverse causality, thereby sustaining the robustness of our hypotheses. Furthermore, the tendency of the study results was maintained even when the antibiotic exposure period varied. Based on diverse pharmacoepidemiological methodologies, including competing risk analysis, this nationwide longitudinal study considering multiple risk factors suggests the possibility and evidence of a hypothesis for the association between antibiotic exposure and lung cancer risk.

This nationwide cohort study discovered a significant duration-dependent relationship between antibiotic prescription and lung cancer risk. Therefore, a judicious antibiotic prescription with thorough consideration of the potentially detrimental effects of long-term antibiotic prescription is required. In other words, antibiotics should not be prescribed or taken indiscriminately. Physicians should use caution when prescribing antibiotics and patients should only take the dosage suggested by their physician. Future prospective and experimental studies that explore the association between antibiotic use and lung cancer incidence, possibly mediated

Table 4
Stratified analysis of risk for lung cancer according to cumulative days antibiotics prescribed.

	Total	Events	Cumulative days of antibiotics prescribed for 5 years before the index date						*P* for trend	*P* for interaction
			None	1–14 days	15–59 days	60–179 days	180–364 days	≥ 365 days		
Age										< 0.001
40–49 years old	2 632 646	8 998	1.00 (ref.)	1.00 (0.90–1.11)	1.04 (0.93–1.15)	1.07 (0.96–1.19)	1.09 (0.96–1.22)	1.09 (0.97–1.23)	0.918	
50–59 years old	1 859 148	21 028	1.00 (ref.)	1.04 (0.97–1.12)	1.06 (0.99–1.14)	1.10 (1.02–1.18)	1.13 (1.04–1.22)	1.25 (1.16–1.35)	< 0.001	
≥ 60 years old	1 723 132	47 746	1.00 (ref.)	0.97 (0.92–1.02)	0.98 (0.93–1.03)	1.01 (0.96–1.07)	1.06 (1.003–1.12)	1.13 (1.07–1.19)	< 0.001	
Smoking status										< 0.001
Never smoker	4 263 454	36 596	1.00 (ref.)	1.05 (0.98–1.11)	1.09 (1.02–1.15)	1.12 (1.06–1.19)	1.16 (1.09–1.24)	1.21 (1.13–1.28)	< 0.001	
Past smoker	610 262	8 583	1.00 (ref.)	1.01 (0.89–1.14)	1.04 (0.91–1.17)	1.05 (0.93–1.19)	1.12 (0.98–1.27)	1.20 (1.05–1.37)	< 0.001	
Current smoker	1 341 210	32 593	1.00 (ref.)	1.002 (0.95–1.06)	1.02 (0.96–1.08)	1.07 (1.01–1.14)	1.11 (1.04–1.18)	1.22 (1.14–1.29)	< 0.001	
Charlson comorbidity index										0.614
0	3 499 272	34 764	1.00 (ref.)	1.00 (0.95–1.05)	1.04 (0.99–1.09)	1.09 (1.03–1.14)	1.11 (1.05–1.18)	1.20 (1.14–1.27)	< 0.001	
1	1 560 426	21 639	1.00 (ref.)	1.08 (0.98–1.18)	1.07 (0.98–1.17)	1.12 (1.02–1.22)	1.17 (1.06–1.28)	1.22 (1.11–1.33)	< 0.001	
≥ 2	1 155 228	21 369	1.00 (ref.)	1.06 (0.95–1.19)	1.06 (0.95–1.18)	1.10 (0.99–1.23)	1.16 (1.03–1.29)	1.24 (1.11–138)	< 0.001	
COPD										0.172
No	6 046 008	71 380	1.00 (ref.)	1.02 (0.98–1.06)	1.05 (1.01–1.09)	1.09 (1.05–1.14)	1.14 (1.09–1.19)	1.22 (1.17–1.27)	< 0.001	
Yes	168 918	6 392	1.00 (ref.)	0.91 (0.72–1.16)	0.92 (0.73–1.16)	0.95 (0.76–1.20)	0.98 (0.78–1.24)	1.03 (0.82–1.29)	0.015	
Tuberculosis										0.764
No	6 133 685	75 795	1.00 (ref.)	1.02 (0.98–1.06)	1.05 (1.01–1.09)	1.09 (1.05–1.14)	1.14 (1.09–1.19)	1.21 (1.16–1.26)	< 0.001	
Yes	81 241	1 977	1.00 (ref.)	1.05 (0.74–1.50)	1.14 (0.81–1.61)	1.18 (0.84–1.65)	1.22 (0.86–1.73)	1.31 (0.93–1.84)	< 0.001	
Asthma										0.188
No	5 556 373	65 885	1.00 (ref.)	1.02 (0.98–1.06)	1.05 (1.01–1.09)	1.10 (1.06–1.15)	1.14 (1.09–1.19)	1.20 (1.15–1.25)	< 0.001	
Yes	658 553	11 887	1.00 (ref.)	1.03 (0.84–1.27)	1.05 (0.86–1.28)	1.03 (0.85–1.26)	1.11 (0.91–1.36)	1.24 (1.02–1.51)	< 0.001	

The aHRs were calculated by Cox proportional hazards regression after adjustments for age, sex, household income, smoking status, alcohol intake, physical activity, body mass index, total cholesterol, systolic blood pressure, fasting serum glucose, Charlson comorbidity index, acid suppressants use, statin use, NSAIDs use, aspirin use, COPD, tuberculosis, and asthma.
aHR indicates adjusted hazard ratio; CI, confidence interval; and ref, reference.

Table 5
Risk for lung cancer according to the number of antibiotic classes prescribed.

	Number of antibiotic classes prescribed during 5 years before the index date						*P* for trend
	None	1	2	3	4	5 or more	
Participants, n	327 314	804 016	1 385 047	1 759 358	1 414 106	525 085	
Events, n	3 601	9 335	16 703	22 419	18 799	6 915	
Person-years	3 969 209	9 780 850	16 871 976	21 420 973	17 190 479	6 398 185	
Model 1							
aHR (95% CI)	1.00 (ref.)	1.03 (0.99–1.07)	1.06 (1.01–1.10)	1.10 (1.06–1.14)	1.11 (1.07–1.16)	1.15 (1.10–1.21)	< 0.001
Model 2							
aHR (95% CI)		1.00 (ref.)	1.02 (1.00–1.05)	1.07 (1.04–1.09)	1.08 (1.05–1.11)	1.13 (1.09–1.17)	< 0.001

The aHRs were calculated by Cox proportional hazards regression after adjustments for multivariate variables. Model 1 was adjusted for age, sex, household income, smoking status, alcohol intake, physical activity, body mass index, total cholesterol, systolic blood pressure, fasting serum glucose, Charlson comorbidity index, acid suppressants use, statin use, NSAIDs use, aspirin use, COPD, tuberculosis, and asthma. Model 2 was adjusted for the variables in Model 1 plus infectious diseases (respiratory diseases, urinary tract infections, skin, soft tissue, bone, and joint infections, intra-abdominal infections, intestinal infectious diseases, and others). Antibiotics were divided into eleven classes consisting of penicillin, cephalosporin, macrolide, fluoroquinolone, sulfonamides, tetracyclines, and lincosamides or others. n indicates number of people; aHR, adjusted hazard ratio; CI, confidence interval; and ref, reference.

by alterations of the lung and gut microbiota, are necessary to verify the results presented herein.

Ethical approval

This study was approved by the Seoul National University Hospital Institutional Review Board (IRB number: E-2204–023–1312) and performed in accordance with the Declaration of Helsinki. The requirement for informed consent from the patients was waived because the database was anonymized according to strict confidentiality guidelines.

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CRediT authorship contribution statement

Minseo Kim: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft**. Sun Jae Park:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft**. Seulggie Choi:** Conceptualization, Methodology, Writing – review & editing**. Seogsong Jeong:** Conceptualization, Methodology, Writing – review & editing**. Jooyoung Chang:** Conceptualization, Methodology, Writing – review & editing**. Young Jun Park:** Methodology, Writing – review & editing**. Joung Sik Son:** Methodology, Writing – review & editing**. Ji Soo Kim:** Methodology, Writing – review & editing**. Yoosun Cho:** Methodology, Writing – review & editing**. Yun Hwan Oh:** Methodology, Writing – review & editing**. Ahryoung Ko:** Methodology, Writing – review & editing**. Sang Min Park:** Conceptualization, Methodology, Validation, Writing – review & editing**,** All authors approved the manuscript submitted for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2023.05.006.

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