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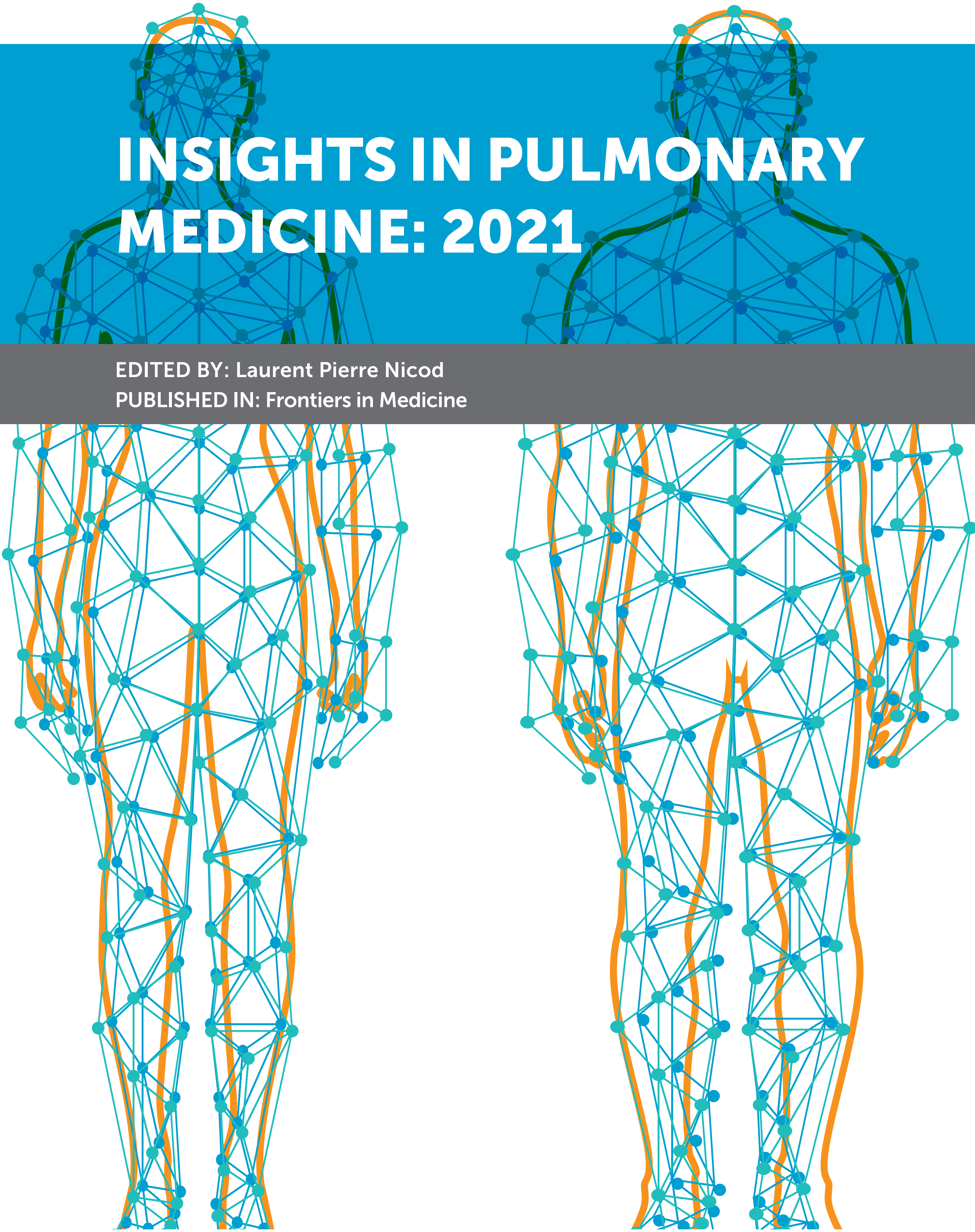

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INSIGHTS IN PULMONARY MEDICINE: 2021

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Lung Cancer Death Attributable to Long-Term Ambient Particulate Matter ($PM_{2.5}$) Exposure in East Asian Countries During 1990–2019

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Background: Ambient particulate matter is a public health concern in East Asia as it contributes to a growing number of all-cause and cancer deaths. This study aimed to estimate lung cancer death attributable to ambient particulate matter (PM) < 2.5 μm ($PM_{2.5}$) in East Asia countries.

Methods: The attributable death rates of lung cancer were estimated based on the calculation of population attributable fraction. We performed joinpoint regression analysis and age-period-cohort (APC) model to estimate temporal trends of the attributable death to $PM_{2.5}$.

Results: In 2019, $PM_{2.5}$ was estimated to have caused 42.2% (nearly 0.13 million) of lung cancer deaths in East Asia men. During 1990–2019, the increase in age-standardized death rates of lung cancer attributable to $PM_{2.5}$ was highest in China, which increased by 3.50% in males and 3.71% in females. The death rate caused by $PM_{2.5}$ also significantly increased in the Democratic People's Republic of Korea (2.16% in males; 3.06% in females). Joinpoint analysis showed that the rates generally increased in younger and older people in both the Democratic People's Republic of Korea and Mongolia, while it only increased in elderly people in other countries'. Age effect from APC analysis demonstrated the risk of lung cancer death attributable to $PM_{2.5}$ generally increased from young to old age. Period effect indicated that from 1994–1998 to 2019–2023 period risk continuously increased by 1.77, 1.68, and 1.72 times in China, the Democratic People's Republic of Korea, and Japan, respectively. The period risk decreased from 1999 to 2009 and subsequently increased from 2009 to 2019 in both the Republic of Korea and Mongolia.

Conclusions: The death rate of lung cancer attributable to $PM_{2.5}$ is increasing in the Democratic People's Republic of Korea, Mongolia, and China. In East Asia, China is facing the highest attributable death rate in recent decades. The period effect suggested a remarkably increased risk of lung cancer death caused by $PM_{2.5}$ in China, the Democratic People's Republic of Korea, and Japan during the long-term period. It is recommended that the governments of these countries should continuously concentrate on particulate matter pollution governance and improvement.

Keywords: death, lung cancer, YLL, $PM_{2.5}$, elder, population attributable fraction (PAF)

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INTRODUCTION

Ambient particulate matter of <2.5 μm ($PM_{2.5}$) pollution is recognized as a major health concern worldwide. $PM_{2.5}$ was associated with an increased risk of disease mortality and morbidities (1–4), and a 10-$\mu g/m^3$ increment in $PM_{2.5}$ was associated with a 4.3% increment in total mortality (5). However, ambient particulate matter pollution continues to be one of the largest increases in risk exposures from 1990 to 2019 worldwide (6).

In 2019, according to the Global Burden of Disease (GBD) 2019 study, among the 87 risk factors identified, long-term exposure to $PM_{2.5}$ caused 118.2 million disability-adjusted life-years (DALYs), representing 4.7% of global DALYs in 204 countries and territories (7). Approximately 4,140,971 all-causes deaths and 307,680 lung cancer deaths in 2019 were attributed to ambient $PM_{2.5}$ globally. However, the ambient $PM_{2.5}$ risk-oriented health problems have mainly occurred in low- and middle-income countries (LMICs) according to previous studies (1, 8). In 2017, ambient particulate matter pollution ranked as the eighth leading risk for death, with a total of 2.94 million deaths globally and 1.05 million deaths in Southeast Asia, East Asia, and Oceania (2). Based on a newly published global comparative risk assessment in 2019, nearly all locations in south Asia, many parts of Southeast Asia, and most provinces in China had 10–15% of DALYs attributable to air pollution (6).

Lung cancer has been the leading cause of cancer deaths for many years worldwide, with the incidence and mortality markedly varying between countries (9, 10). Asia has extremely diverse lung cancer incidences, and it is the leading cause of cancer death in China (10, 11). The major risk factor for the burden of lung cancer was tobacco smoking, followed by air pollution-related age-standardized rates (12). This study aims to focus on lung cancer death caused by particulate matter pollution. Previous studies have evaluated the association of ambient air pollution with lung cancer (13–15). Few studies have been conducted regarding ambient air pollutant-related lung cancer death (16), while no national study has comprehensively evaluated the long-term trend in lung cancer death due to long-term exposure to ambient particulate matter in East Asia countries. Although a recent study reported that the age-standardized death rate attributable to air pollution decreased by 60.6% for China overall between 1990 and 2017 (17), the temporal trend in lung cancer death attributable to air pollution was not clearly analyzed. Therefore, this study aimed to estimate the trend and risk for long-term exposure to $PM_{2.5}$ caused by lung cancer death from 1990 to 2019 among East Asia countries, including China, Mongolia, the Democratic People's Republic of Korea, the Republic of Korea, and Japan.

MATERIALS AND METHODS

Data Sources and Methods for Quantifying the Health Burden

The attributable burden of lung cancer data (1990–2019) for China, the Democratic People's Republic of Korea, the Republic of Korea, Japan, and Mongolia was collected from the most recent GBD project. The datasets analyzed during the current study are published and available in the Global Burden of Disease Database repository. Due to the publicly available nature of data, ethical approval and patient consent to participate were not required in this study.

The GBD 2019 provides a systematic scientific assessment of published, publicly available, and contributed data on incidence, prevalence, mortality, and risk factors for a mutually exclusive and collectively exhaustive list of diseases and injuries. It contains data on 87 behavioral, environmental, occupational, and metabolic risks or clusters of risks, 369 diseases and injuries, and the healthy life expectancy (HALE) for 204 countries and territories (http://ghdx.healthdata.org/).

The original data on lung cancer death were obtained from the local Center for Disease Control and Prevention (CDC), Disease Surveillance Points (DSPs), and the Maternal and Child Surveillance System. Ambient air quality was estimated by extracting the annual average mass concentration data of ambient $PM_{2.5}$ from the multiple-source data, including satellite observation, ground measurement, and chemical migration model simulation, etc., and the grid-level exposure to ambient $PM_{2.5}$ was estimated by data integration model for air quality (DIMAQ) (6, 18). The relative risks (RR) of ambient particulate matter pollution at different exposure levels for different health outcomes were estimated as the Integrated Exposure Response function of exposure based on 81 published systematic reviews, the specific methods are outlined in a previous study (6).

Population attributable fraction (PAF) was defined as if the exposure of a certain risk factor was reduced to the theoretical minimum exposure level in a certain population, the proportion of related diseases or deaths in the population would reduce (2, 19). In this study, the exposure level associated with minimum risk, known as the theoretical minimum risk exposure level, for ambient particulate matter pollution was between 2.4 and 5.9 $\mu g/m^3$. Lung cancer deaths caused by $PM_{2.5}$ were estimated based on defining PAF through combining the distribution of exposure to air pollution with exposure-risk estimates at each level of exposure.

$$PAF = \frac{\sum_i^n p_i(RR_i - 1)}{\sum_i^n p_i(RR_i - 1) + 1} \quad (1)$$

where P_i is the percentage of the population exposed to level i of ambient air exposure, RR_i is the relative risk at exposure level i, and n is the total number of exposure levels.

Attributable death numbers were computed by multiplying PAFs by the relevant outcome quantity for each age-sex-location-year (6, 20). For example, the attributable deaths (AD) were calculated by multiplying the number of the deaths for lung cancer (N) and the PAF:

$$AD = PAF \times N \quad (2)$$

The age-standardized rate of attributable deaths caused by $PM_{2.5}$ was calculated by the world standard population (21).

Statistical Analysis

Joinpoint Regression Analysis

Temporal trends for lung cancer death rates attributable to $PM_{2.5}$ were assessed using the joinpoint regression analysis (Joinpoint regression software, Version 4.5.0.1, available through the Surveillance Research Program of the United States National Cancer Institute). Joinpoint analysis was based on regression with age-standardized mortality rates as the dependent variables and with year as the independent variable. Gender and age group were the by-variables. In the model, logarithmic transformation of the rates was carried out and the standard errors were calculated based on binomial approximation (22). An average annual percentage of change (AAPC), along with the corresponding 95% confidence interval (95%CI), was calculated for each trend. The statistically significant AAPC values indicated a temporal change in mortality trend over the time period. We used the Monte Carlo Permutation method for tests of significance. In this analysis, age groups under 25 years old were excluded due to very low probabilities.

Age-Period-Cohort Analysis

APC analysis could decompose the accumulation of health risks for incidence and death of disease (23). This analysis is developed to reflect the relative risks of disease by estimates on age, period, and cohort effects. Age effect indicates that the risk of death from disease increases with the process of aging. The period effect represents influential factors that simultaneously affect all age groups with advanced periods. The cohort effect represents variations across groups of individuals born in the same year or years. These effects influence morbidity and mortality risk of diseases in specific ways. An intrinsic estimator (IE) method used in this APC analysis could decompose the three effects simultaneously. In this model, the death rates of lung cancer attributed $PM_{2.5}$ were recoded into successive 5-year age groups (25–29, 30–34, ..., 90–94), consecutive 5-year periods (1994–1998, 1999–2003, 2004–2008, 2009–2013, 2014–2018, 2019–2023) and correspondingly consecutive 5-year birth cohort groups (1904–1908, 1909–1913, ..., 1994–1998).

The estimated coefficients of age, period, and cohort effects are plotted in **Figure 2**, and their exponential values [exp(coef.) = $e^{coef.}$] denote the relative risk (RR) of the age, period, or birth cohort effect. Age, period, and cohort effects were analyzed by Stata 12.0 software (StataCorp, College Station, TX, USA). Deviance, Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC), and represent the loss of information caused by using models to represent the process of generating the actual data.

RESULTS

Lung Cancer Attributable Death to $PM_{2.5}$ in East Asia Countries for Males and Females

In 2019, there were 2,042,639 deaths and 2,259,998 new cases of lung cancer worldwide, with a 25.18/100,000 ASDR and 27.66/100,000 ASIR. Importantly, a significant number of these 1,508,993 deaths were attributed to ambient particulate matter pollution that occurred in these East Asia countries, accounting for 36.4% of the global deaths attributed to ambient particulate matter pollution, and 12.3% of the attributed deaths were due to lung cancer. The death rate of lung cancer attributable to $PM_{2.5}$ among men was 2.34 times higher than that in women.

The global number of lung cancer deaths due to ambient particulate matter pollution were 307,680, 60.1% (185,041 deaths) of which were concentrated in East Asia countries, especially in male patients (42.2%). **Table 1** shows lung cancer attributable death to $PM_{2.5}$ in East Asia countries for both male and female patients.

Figure 1 shows the secular trends of the age-standardized rate of lung cancer death attributable to $PM_{2.5}$ in East Asia during 1990–2019. We observed a significant increase in the death rate among patients in China, the Democratic People's Republic of Korea, and Mongolia. We found a continuously decreasing trend in Japan for both male and female patients. In contrast, the death rate from the Republic of Korea peaked in 1999 while a slight rise in female patients has been observed since 2005.

Results of Joinpoint Regression Analysis

We conducted Joinpoint regression analysis to estimate the average annual percent changes in age-standardized and age-specific death rates of lung cancer attributable to $PM_{2.5}$ in East Asia (**Table 2**). Between 1990 and 2019, the increase in the death rate to $PM_{2.5}$ was highest in China [AAPC: 3.50 [95% CI, 3.05–3.94] in male and 3.71 [95% CI, 3.26–4.17] in female patients], and a small decrease was observed in Japan (AAPC: −0.76 [95% CI, −0.92 to −0.60] in male and −0.43 [−0.67 to −0.18] in female patients). As a high SDI country, the Republic of Korea showed no obvious change in the death rate. Similar to China, there was also a substantial increase in the Democratic People's Republic of Korea over the past 30 years (AAPC: 2.16 [95% CI, 2.01–2.32] in male and 3.06 [2.83–3.30] in female patients). We observed a slight increase in Mongolia, and the average percent changes increased by 1.63 (1.19–2.07) annually.

We also estimated changes in the age-specific rates of lung cancer death attributed to $PM_{2.5}$ across age groups (from 25–29 to 90–94 age group) (**Table 2**). We noted that the death rates in all age groups rose significantly in both the Democratic People's Republic of Korea and Mongolia. Other East Asia counties showed declines in the death rate for younger age groups but increases in older age groups. In China, the death rate to $PM_{2.5}$ rose significantly among people aged 65 years and over for male patients, and among people aged 75 years and over for female patients. In Japan, for both sexes, the rate generally declined for most age groups except for 85–89 and 90–94 age groups. In the Republic of Korea, an increase in the rate was also observed in elderly age groups (≥75 years in males and ≥80 years in females). Therefore, lung cancer death due to $PM_{2.5}$ generally increased in the Democratic People's Republic of Korea and Mongolia but decreased for all age groups in Japan. We almost observed a significant increase among elderly people aged 75 years and above in China and the Republic of Korea.

Results of Age-Period-Cohort Analysis

The coefficients of age effect on lung cancer death attributed to $PM_{2.5}$ increased from the 25–29 to 90–94 age group in

TABLE 1 | Lung cancer death and YLL attributable to $PM_{2.5}$ in East Asia in 1990 and 2019.

East Asia	1990				2019			
	Death		YLL*		Death		YLL*	
	Number	Age-standardized Per 100,000	Number	Age-standardized Per 100,000	Number	Age-standardized Per 100,000	Number	Age-standardized Per 100,000
Male								
China	20,576.26	5.42	548,863.25	123.69	120,186.42	13.36	2,704,360.07	271.65
Democratic People's Republic of Korea	243.67	4.09	7,097.63	99.15	899.90	7.04	23,694.74	164.94
Republic of Korea	862.99	7.06	24,070.02	167.94	3,085.32	8.31	60,056.13	147.69
Japan	2,709.53	3.89	57,200.87	76.31	5,513.54	3.21	88,070.16	58.29
Mongolia	28.30	6.55	695.62	144.09	95.61	11.43	2,518.83	240.23
Female								
China	7,060.43	1.69	183,062.82	40.27	51,113.34	4.99	1,107,912.93	105.28
Democratic People's Republic of Korea	85.01	0.89	2,288.95	22.18	367.73	1.94	8,661.43	47.23
Republic of Korea	300.29	1.82	7,878.83	42.89	1,240.90	2.43	21,475.66	44.36
Japan	1,054.48	1.09	21,248.93	22.47	2,516.99	1.02	34,851.02	19.27
Mongolia	6.40	1.17	149.19	26.21	24.35	2.28	577.58	45.55

**YLL, Years of Life Lost.*

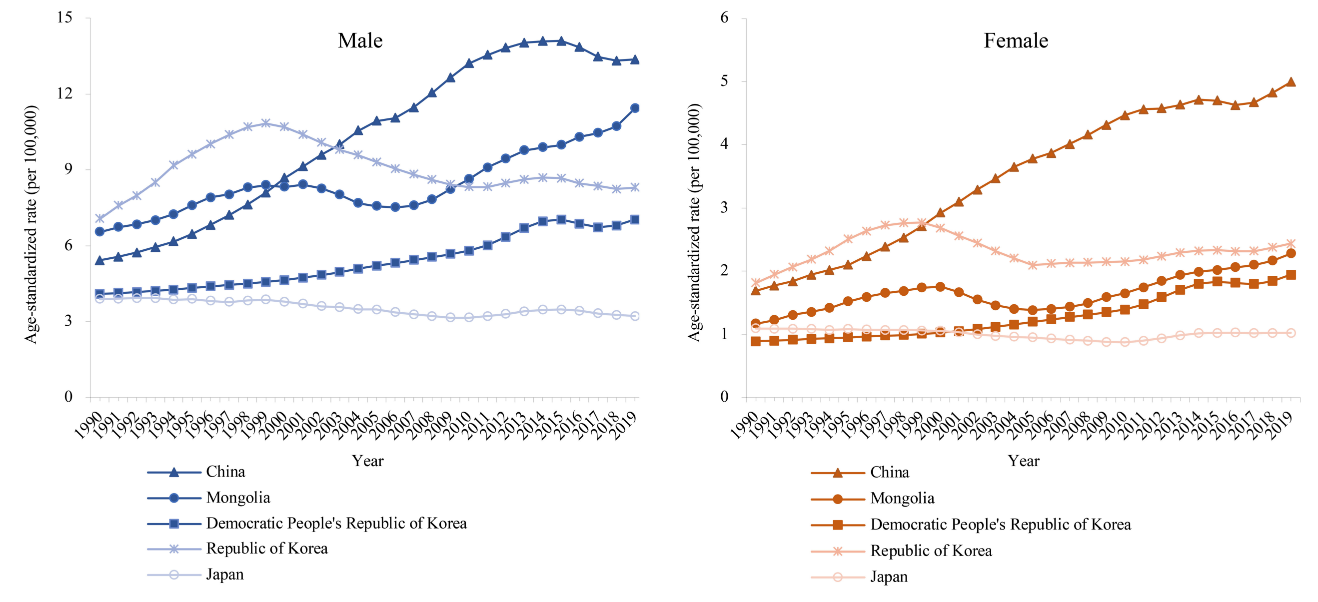

FIGURE 1 | The age-standardized rates of lung cancer attributable death to $PM_{2.5}$ in East Asia for both sexes at all ages, during 1990–2019.

East Asia countries, except for in the Democratic People's Republic of Korea, where the age risk peaked in the 70–74 age group (**Figure 2A**). The age effect showed that in the from 25–29 to 90–94 age group, the risk for lung cancer death attributed to $PM_{2.5}$ increased by 58.99, 36.51, 113.04, 190.68, and 101.86 times in China, the Democratic People's Republic of Korea, Republic of Korea, Japan, and Mongolia, respectively.

The period effect showed different trends in the death rates among East Asia countries (**Figure 2B**). In the from 1994–1998 to 2019–2023 period, the risk increased by 1.77, 1.68, and 1.72 times in China, the Democratic People's Republic of Korea, and Japan, respectively. The period effect in the Republic of Korea and Mongolia showed an N-shape trend, which decreased from 1999 to 2009 and increased from 2009 to 2019.

The cohort effect showed an ongoing decreasing trend from 1904–1908 to 1994–1998 birth cohort in all these countries (**Figure 2C**). From the earliest birth cohort to the most recent cohorts, the risk decreased by around 90% for all East Asia counties.

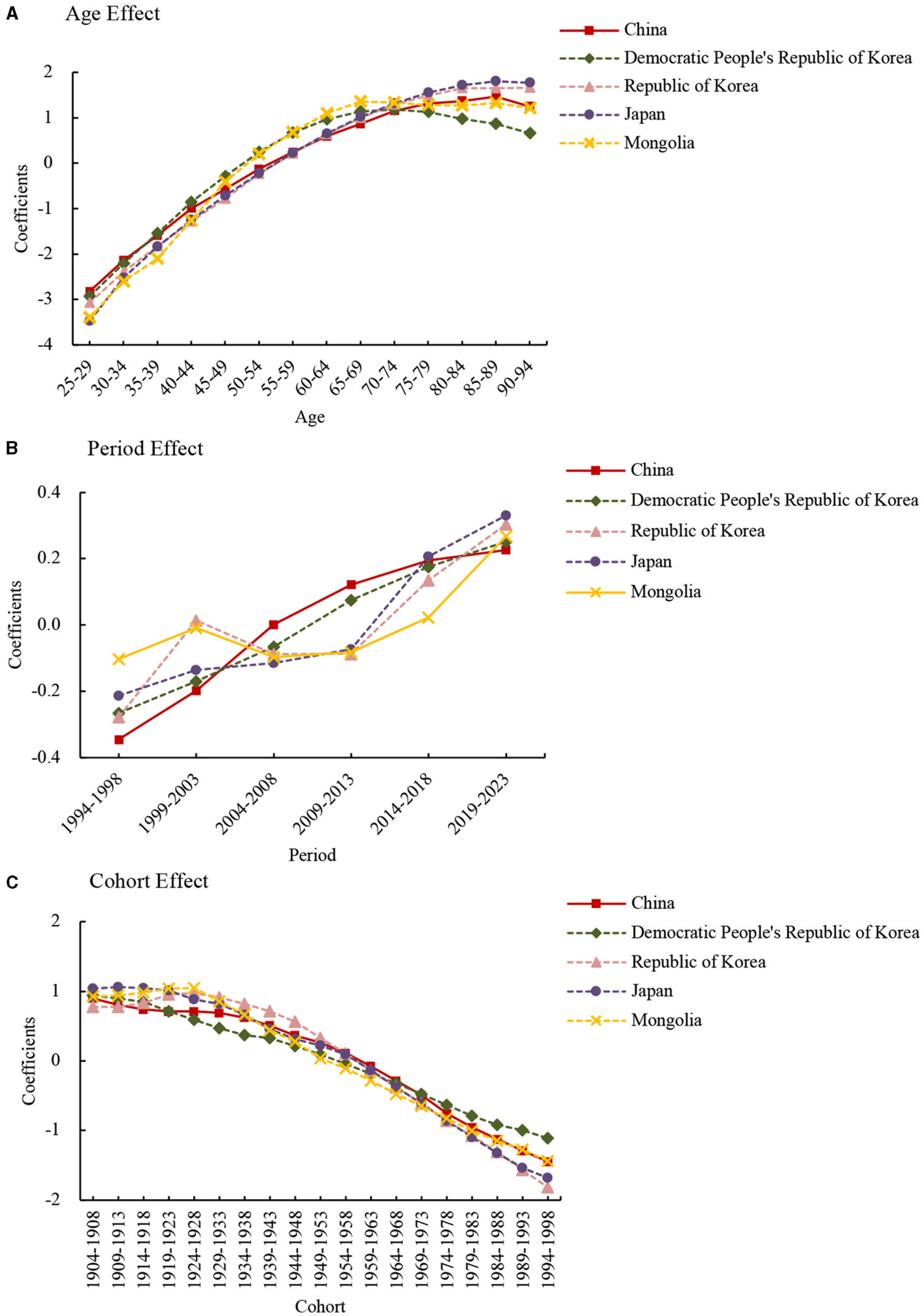

FIGURE 2 | Lung cancer death attributable to $PM_{2.5}$ estimated coefficients for the age **(A)**, period **(B)**, and cohort effects **(C)**.

TABLE 2 | The average annual percent changes (AAPC) in lung cancer deaths attributable to $PM_{2.5}$ in East Asia during 1990–2019 for male and female patients.

ASR* and Age-group (year)	Average annual percent changes (AAPC), male					Average annual percent changes (AAPC), female				
	China (95% CI)	Democratic people's republic of korea (95% CI)	Republic of korea (95% CI)	Japan (95% CI)	Mongolia (95% CI)	China (95% CI)	Democratic people's republic of korea (95% CI)	Republic of korea (95% CI)	Japan (95% CI)	Mongolia (95% CI)
ASR	3.50 (3.05, 3.94)	2.16 (2.01, 2.32)	−0.35 (−0.81, 0.12)	−0.76 (−0.92, −0.60)	1.52 (1.23, 1.81)	3.71 (3.26, 4.17)	3.06 (2.83, 3.30)	−0.07 (−0.52, 0.39)	−0.43 (−0.67, −0.18)	1.63 (1.19, 2.07)
25–29	−1.28 (−1.52, −1.04)	1.63 (1.35, 1.92)	−4.46 (−4.99, −3.94)	−2.07 (−2.26, −1.87)	1.09 (0.56, 1.62)	−2.83 (−3.77, −1.87)	2.84 (2.57, 3.11)	−3.55 (−4.02, −3.08)	−1.64 (−1.87, −1.40)	2.92 (2.68, 3.16)
30–34	−1.25 (−1.49, −1.01)	1.24 (0.95, 1.53)	−3.65 (−4.07, −3.23)	−2.81 (−3.05, −2.57)	2.93 (2.62, 3.23)	−2.73 (−3.43, −2.02)	2.66 (2.42, 2.90)	−2.86 (−3.18, −2.53)	−2.09 (−2.37, −1.80)	0.79 (0.31, 1.28)
35–39	−1.88 (−2.18, −1.58)	0.87 (0.68, 1.06)	−3.47 (−3.83, −3.11)	−2.78 (−2.98, −2.58)	1.97 (1.63, 2.30)	−2.01 (−2.33, −1.70)	2.55 (2.34, 2.76)	−1.84 (−2.04, −1.63)	−2.22 (−2.43, −2.00)	1.11 (0.57, 1.65)
40–44	−1.81 (−2.08, −1.54)	1.01 (0.85, 1.18)	−3.50 (−3.69, −3.30)	−2.66 (−2.93, −2.38)	1.54 (1.21, 1.87)	−1.87 (−2.13, −1.61)	2.52 (2.29, 2.76)	−1.89 (−2.12, −1.66)	−2.27 (−2.59, −1.95)	0.62 (1.12, 2.56)
45–49	−1.07 (−1.33, −0.81)	1.39 (1.22, 1.57)	−3.66 (−3.89, −3.44)	−2.29 (−2.58, −1.99)	1.78 (1.45, 2.11)	−2.01 (−2.47, −1.54)	2.71 (2.49, 2.94)	−1.25 (−1.35, −1.15)	−2.06 (−2.37, −1.74)	1.32 (0.80, 1.85)
50–54	−1.15 (−1.42, −0.88)	1.61 (1.44, 1.77)	−3.56 (−3.75, −3.38)	−1.31 (−1.60, −1.01)	2.00 (1.85, 2.16)	−1.44 (−1.72, −1.15)	2.86 (2.62, 3.10)	−1.32 (−1.60, −1.04)	−1.53 (−1.82, −1.23)	−0.12 (−0.74, 0.51)
55–59	−0.61 (−0.78, −0.43)	1.96 (1.82, 2.10)	−3.11 (−3.39, −2.82)	−0.92 (−1.07, −0.77)	1.63 (1.33, 1.94)	−0.78 (−1.12, −0.44)	2.99 (2.74, 3.24)	−1.44 (−1.65, −1.24)	−0.93 (−1.07, −0.79)	−0.31 (−0.96, 0.35)
60 - 64	0.05 (−0.14, 0.24)	2.03 (1.89, 2.18)	−2.42 (−2.71, −2.12)	−0.84 (−1.13, −0.56)	1.61 (1.16, 2.05)	−0.45 (−0.57, −0.34)	2.96 (2.70, 3.22)	−1.36 (−1.51, −1.20)	−0.37 (−0.55, −0.19)	0.10 (−0.49, 0.69)
65–69	0.26 (0.07, 0.45)	2.29 (2.13, 2.44)	−1.59 (−1.97, −1.21)	−1.05 (−1.39, −0.70)	0.45 (−0.09, 0.99)	−0.25 (−0.44, −0.08)	2.99 (2.73, 3.25)	−1.35 (−1.69, −1.00)	−0.28 (−0.58, 0.02)	0.63 (0.06, 1.21)
70–74	0.57 (0.32, 0.83)	2.44 (2.25, 2.62)	−0.53 (−1.08, 0.02)	−1.29 (−1.61, −0.97)	0.69 (0.19, 1.19)	0.30 (−0.04, 0.65)	3.07 (2.83, 3.31)	−0.82 (−1.36, −0.28)	−0.64 (−0.98, −0.29)	2.03 (1.56, 2.51)
75–79	1.13 (0.81, 1.46)	2.51 (2.35, 2.67)	0.46 (−0.29, 1.22)	−1.32 (−1.47, −1.17)	0.79 (0.35, 1.23)	0.99 (0.64, 1.33)	3.22 (2.98, 3.46)	−0.18 (−0.89, 0.54)	−0.97 (−1.30, −0.65)	1.64 (1.25, 2.04)
80–84	1.77 (1.39, 2.15)	2.46 (2.29, 2.62)	1.62 (0.74, 2.52)	−0.44 (−0.58, −0.29)	2.20 (1.83, 2.58)	1.78 (1.43, 2.13)	3.37 (3.16, 3.57)	0.80 (−0.17, 1.78)	−0.38 (−0.68, −0.07)	2.86 (2.55, 3.17)
85–89	2.57 (2.15, 2.98)	2.37 (2.22, 2.53)	2.88 (2.19, 3.57)	0.46 (0.28, 0.64)	3.87 (3.67, 4.07)	1.65 (1.36, 1.95)	3.59 (3.42, 3.77)	2.50 (1.93, 3.09)	0.31 (0.08, 0.54)	4.28 (4.01, 4.56)
90–94	2.08 (1.66, 2.50)	2.17 (2.06, 2.29)	1.97 (1.36, 2.59)	1.15 (1.04, 1.25)	4.19 (3.89, 4.49)	1.37 (1.17, 1.56)	3.75 (3.56, 3.94)	2.72 (2.18, 3.26)	1.53 (1.38, 1.68)	5.52 (5.08, 5.96)

**ASR, age-standardized rate, which was age-standardized by the GBD 2019 global age-standard population; CI, Confidence interval.*

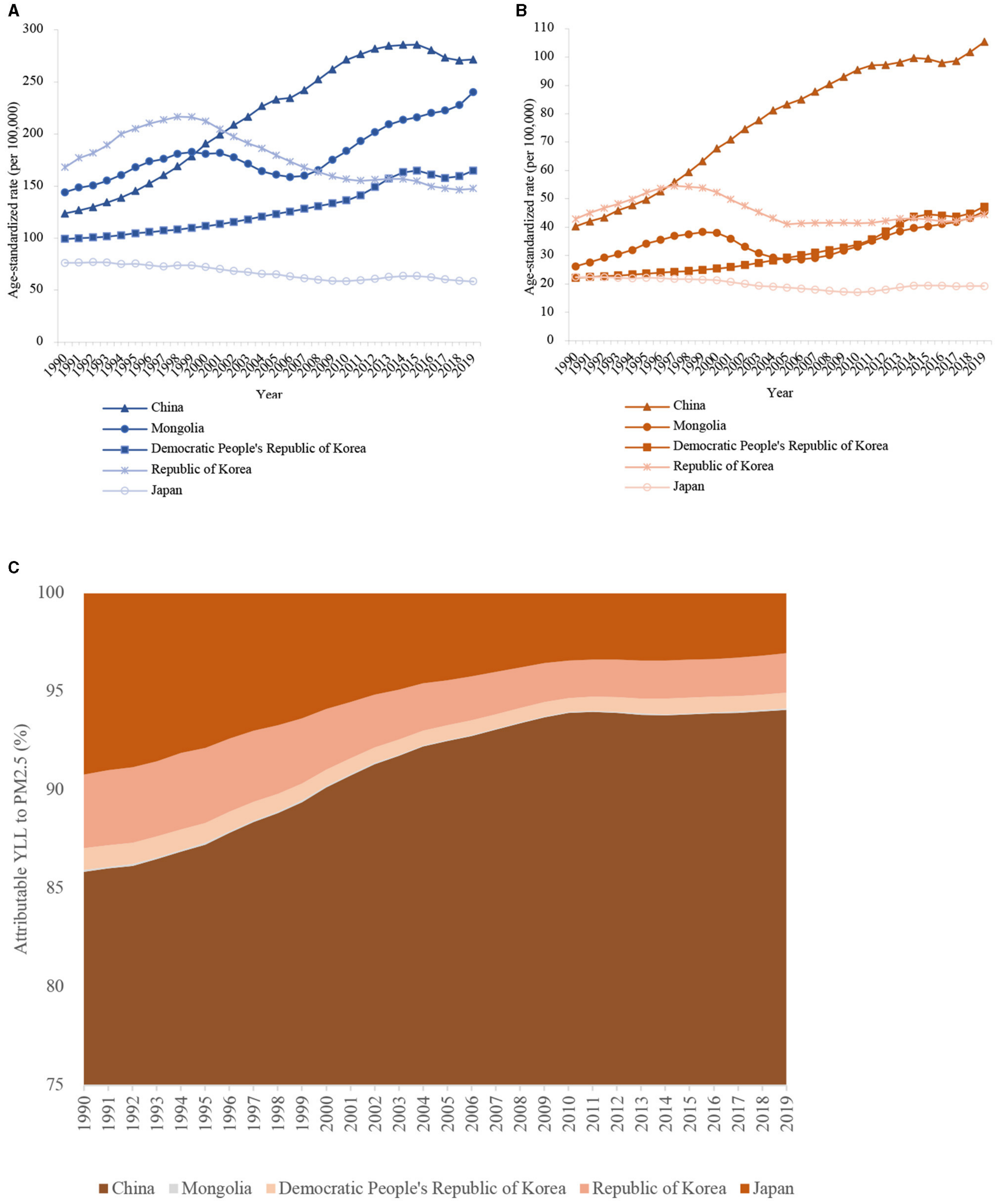

FIGURE 3 | The age-standardized rates of lung cancer attributable YLL to $PM_{2.5}$ in East Asia for both sexes at all ages, during 1990–2019. **(A)** male patients; **(B)** female patients; **(C)** The proportion of lung cancer YLL attributed to ambient $PM_{2.5}$ in East Asia countries from 1990 to 2019.

Lung Cancer Attributable YLL to $PM_{2.5}$ in East Asia Countries

The YLL rate of lung cancer attributable to $PM_{2.5}$ also showed a significant increase for male and female patients in the three regions (**Figure 3**).

In 2019, in male patients, the age-standardized YLL rate of lung cancer attributed to $PM_{2.5}$ was highest in China (271.65/100,000), closely followed by Mongolia (240.23/100,000) (**Table 1**). The YLL rate to $PM_{2.5}$ was relatively lower in the Democratic People's Republic of Korea (164.94/100,000) and the Republic of Korea (147.69/100,000). The YLL rate to $PM_{2.5}$ was lowest in Japan (58.29/100,000). In females, the highest YLL rate was observed in China (105.28/100,000) and the lowest rate in Japan (19.27/100,000). Overall, the YLL rates of lung cancer due to $PM_{2.5}$ were much lower in females than males among all East Asia countries. Compared with 1990, the age-standardized YLL rate of lung cancer attributed to $PM_{2.5}$ increased by 1.20 and 1.61 times in male and female patients in China, respectively (**Table 1**). However, the age-standardized YLL rate of lung cancer attributed to $PM_{2.5}$ decreased in male (24.0%) and female (14.0%) patients in Japan. In the Republic of Korea, the rate decreased by 12.0% in male and increased by 3.0% in female patients.

We also plotted the distribution proportion of lung cancer YLL attributed to ambient $PM_{2.5}$ in East Asia from 1990 to 2019 (**Figure 3C**). The YLLs from East Asia were mainly driven by China. Indeed, 94.08, 0.08, 0.80, 2.01, and 3.03% of YLLs attributable to $PM_{2.5}$ in 2019 were due to lung cancer in China, Mongolia, the Democratic People's Republic of Korea, the Republic of Korea, and Japan, respectively. Overall, apart from death, China also has a substantially increased lung cancer YLL attributed to $PM_{2.5}$ and mainly contributed to the YLLs from East Asia.

Lung Cancer Attributable Death and YLL to $PM_{2.5}$ in East Asia Compared With the Global Level in 2019

Compared with the global level, we plotted the ASDR, ASIR, and death and YLL attributable to the $PM_{2.5}$ of lung cancer in East Asia countries (**Figure 4** and **Supplemental Table 1**). The ASDR and ASIR of lung cancer in these East Asia countries were about 1.0–1.5 times higher than those of the global average in both sexes, while the low ASDR of Japan was 0.84 times that of the global average. In addition, the ASDR and ASIR of lung cancer were higher in male than female patients. In 2019, the death and YLL rates of lung cancer attributable to $PM_{2.5}$ in China were more than 2.0 times higher than those of the world average. In Mongolia, the rates were around 1.5 times that of global levels. It was 1.0–1.3 times more than global levels in the Democratic People's Republic of Korea and the Republic of Korea than the global level, while the level differed in the two countries. There were about 0.4 and 0.5 times higher levels in Japan for lung cancer death and YLL attributable to $PM_{2.5}$. In terms of genders, the death and YLL rates of lung cancer attributed to $PM_{2.5}$ in male patients were higher than that in female patients for most East Asia countries when compared with global levels. The gender difference was exactly different for China (Attributable to death: 231.15% in male patients, and 239.46% in female patients; Attributable to YLL: 217.31% in male patients, 229.92% in female patients). Generally, compared with the global level, China had the highest attributable death and YLL of lung cancer due to $PM_{2.5}$ in East Asia.

DISCUSSION

This study comprehensively estimates on temporal trends in lung cancer death caused by long-term exposure to ambient $PM_{2.5}$ in East Asia. We found that the lung cancer attributable death caused by $PM_{2.5}$ generally increased in younger and older people in both the Democratic People's Republic of Korea and Mongolia by Joinpoint analysis. APC analysis demonstrated that the risk of lung cancer death attributed to $PM_{2.5}$ generally increased from young to old age, and the risk increased continuously in China, the Democratic People's Republic of Korea, and Japan from 1994–1998 to 2019–2023 period. The Republic of Korea and Mongolia showed a different period risk trend. The cohort effect declined to the most recent birth cohorts, which demonstrated a decreasing risk from the old generation to the new generation.

This section discusses the attributable death of lung cancer due to ambient particulate matter. Among East Asia countries, we found that China and Mongolia had high mortality and YLL rates attributable to ambient $PM_{2.5}$. The high population density in China exhibited a huge lung cancer burden attributed to ambient $PM_{2.5}$ and was a major contributor to the death and YLL burden caused by $PM_{2.5}$ in East Asia. This finding was related to the fact that more than a quarter of global deaths caused by ambient particulate matter pollution occurred in China, which is the third risk factor affecting the health of Chinese people (16). A huge proportion of attributable YLL to $PM_{2.5}$ was related to China, which is consistent with studies by the World Bank and the WHO. The Chinese Academy for Environmental Planning on the effect of air pollution on health concluded that between 350,000 and 500,000 people die prematurely each year as a result of outdoor air pollution in China (18, 24). This finding could be contributed to the fact that $PM_{2.5}$ pollution in the winter is worse in China (4), in addition to the increasing high mortality of lung cancer in China (7). Mongolia has a relatively high level of age-standardized attributable death and YLL rates. This is because, during the cold wintertime in Mongolia, a lot of coal is burnt for domestic heating. As reported, ambient particulate levels frequently exceed 100 times the WHO-recommended safety level for sustained periods and account for the majority of personal particulate matter exposure (25). Reducing home heating emissions in traditional housing areas has been the primary focus of air pollution control efforts for Mongolian cities (26). Moreover, we observed that Mongolia still has a much higher level of lung cancer mortality in 2019, which is only second to China, although it has been decreasing over the past decades. Differing from China and Mongolia, Japan's age-standardized attributable death and YLL rates were the lowest from 1990 to 2019. This may be related to the fact that Japan has a declining relatively low level of air pollution, and its mortality rate from lung cancer is relatively low compared with other Asian

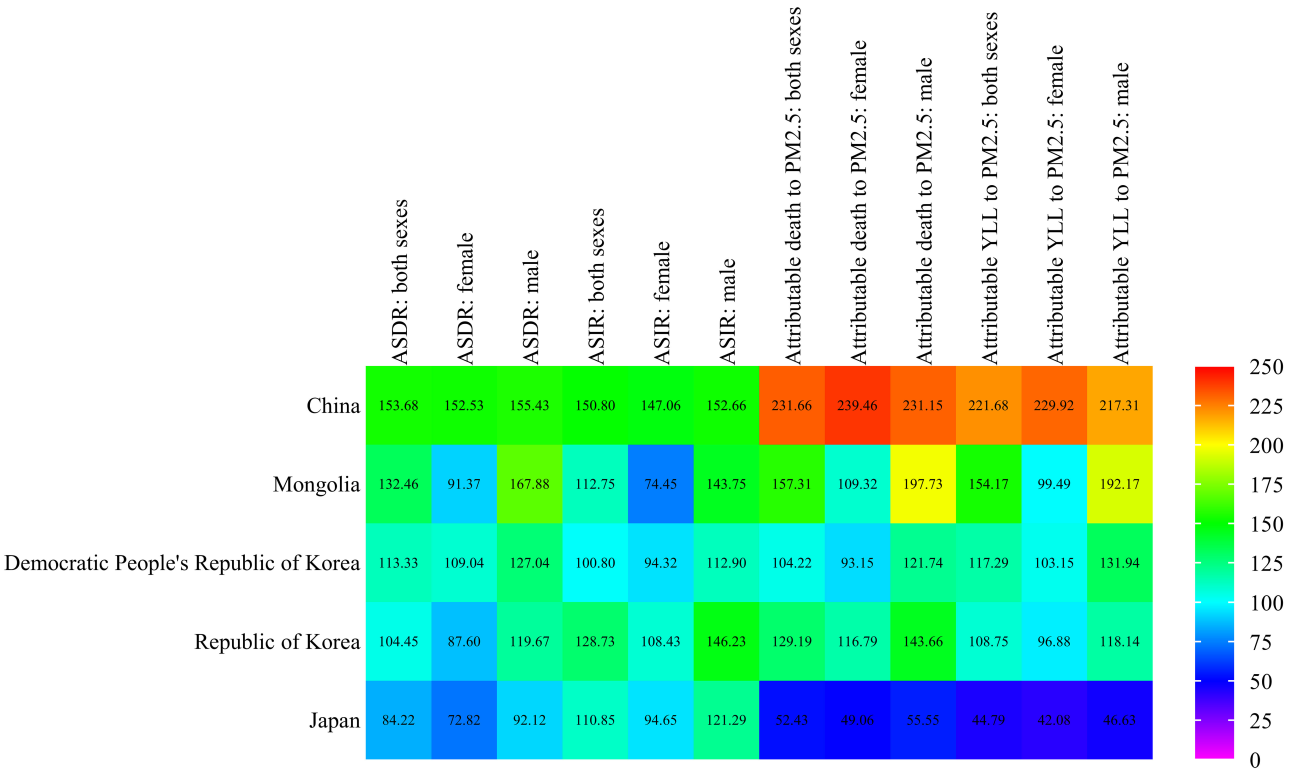

FIGURE 4 | Age-standardized rates of lung cancer attributable death and YLL to $PM_{2.5}$ in East Asia compared with those globally in 2019.

countries (14, 27). A relatively low level of attributable burden was observed in both the Democratic People's Republic of Korea and the Republic of Korea, while the difference was that the death and YLL rate of lung cancer due to $PM_{2.5}$ increased in the former but declined in the latter. Ambient air pollution is recognized as a major environmental health hazard in the Republic of Korea and the average $PM_{2.5}$ exposure concentration for the whole period was estimated to be 30.2 $\mu g/m^3$ during 1990–2013 (28). However, the mortality rate from lung cancer was much lower than in other countries. Therefore, although the Republic of Korea's air pollution is relatively high, the lung cancer attributable burden related to $PM_{2.5}$ is not at a high level. During 1990–2019, lung cancer death and YLL rates to $PM_{2.5}$ have increased in China, the Democratic People's Republic of Korea, and Mongolia, and decreased in Japan and the Republic of Korea. In East Asia countries, these trends were consistent with mean $PM_{2.5}$ concentrations, which showed substantial increases in China, with decreases observed in Japan during 1990–2013 (29).

We observed gender and area-specific distributions of lung cancer attributable burdens due to $PM_{2.5}$. Lung cancer deaths caused by ambient particulate matter were 2.34 times higher in East Asia men than women in 2019. Furthermore, the age-standardized death and YLL rates of lung cancer attributed to $PM_{2.5}$ were much higher in men than women in all countries, which was consistent with the gender difference in trends of lung cancer mortality. A previous study also reported that long-term $PM_{2.5}$ exposure has led to a much higher number of lung cancer premature death in male patients than in female patients (28).

In East Asia, the YLL burden of lung cancer caused by $PM_{2.5}$ was also mainly driven by China, especially in men. This finding was consistent with another previous study, which indicated that the elderly and men had higher health risks than younger people and women. When the $PM_{2.5}$ concentrations meet the WHO air quality guidelines of 10 $\mu g/m^3$, 84% of the premature deaths would be avoided (30). Compared to 1990, the age-standardized death rate of lung cancer attributed to $PM_{2.5}$ increased in 2019, while the increase rate was smaller in males than females in the Democratic People's Republic of Korea, Republic of Korea, and Mongolia. Outdoor air pollution occurs predominantly in developing countries, particularly in Asia (1, 31). In China, industrial and residential sources were the two leading sources of mortality due to exposure to ambient $PM_{2.5}$ (32), and annual concentrations of ambient $PM_{2.5}$ are more than 5 times higher than the WHO guideline values in many populous cities. In Mongolia, sources concentrated on increased coal consumption in the cold season (33). Whether females were exposed to a higher risk of death in these countries, need to be further explored in relation to the different sources of mortality caused by ambient $PM_{2.5}$. In Japan, we observed that lung cancer death attributable to $PM_{2.5}$ decreased in both sexes.

Increasing automobile traffic has caused considerable increases in concentrations of particulate matter, and concentrations have gradually decreased since control measures based on the automobile PM law were enforced in 2001 (34).

It is well-known that the major risk factor for lung cancer is tobacco smoking (12). Reactive oxygen or nitrogen species (ROS, RNS) and oxidative stress in the respiratory system could initiate or promote mechanisms of carcinogenesis, and the lungs are likely exposed to oxidants generated exogenously (air pollutants, cigarette smoke, etc.) daily (35). As reported, inhalable quartz, metal powders, mineral asbestos fibers, ozone, soot from gasoline and diesel engines, tobacco smoke, and ambient PM are involved in various oxidative stress mechanisms (35–38). It seems to be highly possible that $PM_{2.5}$ not only adds to lung cancer risk but also smoking habits over the years might be related to some of the differences of lung cancer, especially for men and women. Because smoking significantly increases the risk of lung cancer, which has been reported to be the main cause of lung cancers in 90% of male and 79% of female patients (39, 40). China is still facing heavy tobacco use, and lung cancer is the leading cause of cancer death and morbidity. A series of policies to control tobacco consumption and prevent lung cancer have been carried out. However, the burden of lung cancer is still serious, and the smoking rate in men is still very high (41).

In terms of age difference, the attributable death to $PM_{2.5}$ of lung cancer generally increased among elderly people (age $\geq$75 years) in both China and the Republic of Korea. This finding was also observed in other studies, which showed that long-term exposure to $PM_{2.5}$ was associated with an increased risk of disease death among people aged 65 years and older (42, 43). However, it is noteworthy that lung cancer death attributable to $PM_{2.5}$ increased for all age groups in the Democratic People's Republic of Korea and Mongolia. We also found that the percentage change of ASR of lung cancer death caused by $PM_{2.5}$ for China was significantly higher than that in any age groups in **Table 2**. This result was related to the age-standardized rate, which eliminates the influence of different age components of the population, and ensures the comparability of the lung cancer death rate.

Days of heavy pollution regularly occur in Asian megacities (23). China and the Democratic People's Republic of Korea had increasing attributable deaths rates of lung cancer caused by $PM_{2.5}$, and the period risk has also increased over the past few decades. According to the most up-to-date risk factor assessment, global exposure to harmful environmental risks has been declining, with the notable exception of ambient particulate matter pollution (6). In China, a large proportional increase in $PM_{2.5}$ was observed between 1990 and 2013 (29). While the early air pollution policies were ineffective at reducing emissions since the 1980s, and air pollution problems dominated by $PM_{2.5}$ have emerged and worsened since 2005 (44). As $PM_{2.5}$ was not been included in the National Air Reporting System until 2013, we collected data on the national annual mean $PM_{2.5}$ concentrations in China and found that it declined with a range of 72–36 $\mu g/m^3$ from 2013 to 2019 (**Supplemental Figure 1**). Another previous study also reported that the average annual population-weighted $PM_{2.5}$ exposure in China was 52.7 $\mu g/m^3$ in 2017, which is 9% lower than in 1990 (17). This decline is because of effective air pollution control policies after the winter-long $PM_{2.5}$ episode in China (44). However, the annual average $PM_{2.5}$ concentration in China was 36 $\mu g/m^3$ in 2019 and the planned reductions in annual average $PM_{2.5}$ concentration from the current level to 10 $\mu g/m^3$ still have not been achieved. As early as 2013, 87% of the global population lived in areas exceeding the WHO air quality guideline of 10 $\mu g/m^3$ $PM_{2.5}$ (annual average) (29). Thus, the changes derived from the policy evolution have implications for future studies, as well as further reforming the management of health risk and air quality control. More interventions are required to achieve the guideline levels of $PM_{2.5}$ as the Chinese population is still facing a high exposure level. The lung cancer death attributable to $PM_{2.5}$ declined in Japan but the period risk in this country still be increasing. This is possibly related to long-term exposure to particulate matter causing health problems, such as normal lung function not being restored even after the improvement of air pollution in Japan (45). It is therefore essential for countries in East Asia to prevent air pollution.

This study has limitations. Although GBD 2019 collected missing data and improved the quality of data and its comparability by modifying and adjusting data sources and collection and evaluation methods, there might be uncertainty about exposure estimates, as there was no measurement in some areas or the data was not available in the GBD 2019 study. Therefore, the results should be carefully interpreted for these countries. Second, the majority of air quality monitoring stations were located in urban areas where air pollution mean concentrations are expected to be high. Third, the APC analysis only takes the effects of age, period, and cohort into account and does not further analyze other risk factors underlying lung cancer death.

CONCLUSIONS

Our study showed that ambient air pollution could impose a substantial burden in terms of lung cancer death in East Asia, where China is facing the highest attributable death rate for lung cancer caused by $PM_{2.5}$. An increasing trend of lung cancer death attributed to $PM_{2.5}$ was observed in East Asia from 1990 to 2019, except for Japan and the Republic of Korea. However, the period effect nevertheless suggests a remarkably increasing risk in China, the Democratic People's Republic of Korea, and Japan in the long-term, and overall reduction of air pollution would have significant benefits for the health of the populations in these countries.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

CY conceived of the study and participated in its design and coordination. XL led data collection and analysis, wrote the original draft, and oversaw editing of the final manuscript. All authors contributed to the drafting and revision of the article, read, and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.742076/full#supplementary-material

Supplemental Figure 1 | National exposure in China to $PM_{2.5}$ for 12 months during 2013-2019.

Supplemental Table 1 | The age standardized rates of death and incidence lung cancer and its attributable burden to $PM_{2.5}$ in East Asia countries compared with the global's in 2019 (%).

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Proximity to Heavy Traffic Roads and Patient Characteristics of Late of Onset Asthma in an Urban Asthma Center

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Background: Traffic-related pollution is associated with the onset of asthma and the development of different phenotypes of asthma. Few studies have investigated the association between traffic proximity and late-onset of asthma (LOA) and early-onset asthma (EOA). This study was conducted to investigate the associations of LOA phenotypes with a function of the distance between residence and heavy traffic roads (HTRs).

Methods: The study group consisted of 280 patients who were (LOA: 78.4%) recruited consecutively from a pay-for-performance asthma program to clarify the patient characteristics and proximity to HTRs within 1,000 m from their residences between EOA and LOA in three urban centers in Taiwan. The subsequent analysis focused on patients with LOA ($n = 210$) linking phenotypes and distance to HTRs.

Results: Subjects with LOA tended to be older than those with EOA and had shorter asthma duration, poorer lung function, lower atopy, and less exposure to fumes or dust at home. Patients with LOA were more likely than those with EOA to live within 900 m of two or more HTRs (14.3 vs. 3.4%, $p = 0.02$). Among patients with LOA, minimum distance to an HTR was negatively associated with numbers of specific IgE as well as positively associated with the age of onset and body weight significantly. A higher proportion of patients with atopy (26.3 vs. 20.6%, $p = 0.001$. odds ratio [OR]: 2.82) and anxiety/depression (21.0 vs. 18.1%, $p = 0.047$. OR: 1.81) and a trend of lower proportion of patients with obese (5.7 vs. 12.4%, $p = 0.075$) were found to be living within 900 m from HTRs.

Conclusions: Late-onset of asthma (LOA) tended to live in areas of higher HTR density compared to EOAs. Among patients with LOA living close to HTRs, the interaction between traffic-related pollution, allergy sensitization, and mood status were the factors associated with asthma onset early. Obesity may be the factor for later onset who live far from HTRs.

Keywords: late onset asthma, traffic proximity, urban environment, asthma phenotype, traffic density

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BACKGROUND

After an initial appearance in childhood, asthma may remain inactive for an extended period or reappear later in life. A number of recent studies have also described the onset of asthma during adulthood (1). Thus, early-onset asthma (EOA) and late-onset asthma (LOA) can be viewed as two distinct phenotypes, based on the categorization of disease entities according to underlying mechanisms or endotypes, such as risk factors, remission rates, co-morbidities, and gene expression profiles (2–4). The age at diagnosis determining the early vs. late-onset asthma varies from 12 or 18 years (4–6) of age to 40 (7) years of age. However, emerging evidence indicates that environmental factors also play a critical role in the development of asthma. The risk factors of EOA include single nucleotide polymorphisms on chromosome 17q21, atopic status, rhinovirus infection, and exposure to traffic-related air pollution (TRAP) (8–12). TRAP is one of the major environmental impacts of urbanization and previous research has shown the long-term effects on asthma onset in children (5, 13). Among children, proximity to traffic has been linked to an elevated risk of asthma (12, 14–16).

Despite the non-negligible incidence of adult-onset asthma, the causes have not been extensively investigated. Identifying the risk factors of LOA is crucial to understanding the underlying mechanisms and LOA was identified as the most significant independent risk factor for non-remission in patients with asthma (17). It should be noted however that the risk factors for LOA are more complex than those for EOA. Specific characteristics have been mentioned for their different influence on EOA and LOA. A population-based study in Finland showed the incidence of allergic asthma decreases with advancing age and after the age of 40, new asthma cases are almost non-atopic (18). The other study in Finland discovered the influence of the family history of asthma is higher on EOA than LOA (risk ratio (RR): 4.10 vs. 1.44) (9). A European survey discovered patients with chronic rhinosinusitis reported less EOA (RR: 0.45) but more LOA (RR: 3.09) (19). In the cluster study of asthma phenotypes, obese female is one of the major cluster of LOA (20). One large-scale genome-wide association study (GWAS) suggested that non-genetic risk factors play a more important role in LOA than in EOA. This is a clear indication that environmental factors are worthy of further consideration (21). The important role of TRAP in adult-onset asthma has also been emphasized (22). The association between traffic proximity and LOA has been identified by a certain amount of traffic volume and patients in risk (23–25). One study of our adult asthma cohort reported that higher IL-17A expression in the epithelium of patients among those living within 1,000 m from heavy traffic roads (HTRs) than among those living more than 1,000 m from HTRs (26).

In the current study, we were interested in whether the traffic proximity was different in EOA and LOA. We sought to determine whether proximity or density of HTRs is associated with the LOA in the asthma cohort of an urban medical center. We also examined patient characteristics in order to identify factors significantly associated with traffic proximity in LOA.

MATERIALS AND METHODS

Study Design and Subject Recruitment

This was a cross-sectional study of asthma patients recruited consecutively in a pay-for-performance program at Chang Gung Memorial Hospital, Linkou branch, which has been implemented by the National Health Insurance Administration (NHIA) in Taiwan since 2001 (27). Certified physicians and case managers provide in-person training pertaining to asthma control, asthma care planning, and proper inhaler usage. The outcomes are regularly monitored by the NHIA (https://www.nhi.gov.tw/Content_List.aspx?=nEBDEAEDEC639490C&topn=5FE8C9FEAE863B46). Inclusion criteria included the diagnosis of asthma by a pulmonologist in accordance with ICD-10 code J45 at least twice within 90 days. Note that diagnoses were based on episodic respiratory symptoms (wheezing, breathlessness, chest tightness, and cough), variable or persistent obstructive pulmonary function, and response to asthma therapy. All patients provided written informed consent. The study protocol was approved by the Chang Gung Medical Foundation Institutional Review Board (No. 201900211B0).

Patient Data Collection

At the initial recruitment, we recorded the characteristics of the subjects based on questionnaires or medical records, including age at the time of asthma diagnosis by a physician, asthma control test (ACT) results, family history of asthma, the use of asthma medication, co-morbidities, childhood history of dyspnea, frequency of bronchitis, exposure to fumes or dust at home or work, smoking status, and current residence (in the last 6 months). Pulmonary function and allergy-related biomarkers, including eosinophils, eosinophil cation protein (ECP), immunoglobulin E (IgE), and specific IgE (ImmunoCAP, Phadia, Sweden) were recorded. Patients with any positive specific IgE to allergens (>0.35 KU/L,) were considered atopic. The tests above were done at physicians' discretion in real-world practice. For example, according to the regulation of Taiwan healthcare insurance, the reimbursement of specific IgE would not be offered unless the total IgE > 25 KU/L. Therefore, a test of specific IgE was not mandatory and the numbers of sIgE to check were based on the physicians' discretion.

Outcome Measurement

The total cohort of 283 patients was divided into two groups according to age at the time of asthma onset. Patients who were $\geq$ 18 years old at the time of asthma onset without a childhood history of dyspnea and frequency of bronchitis were defined as LOA. Otherwise, they were considered as EOA. It was determined that 94.7% of the total cohort were living in major urban centers: EOA ($n = 58$) and LOA ($n = 210$). This group of urban patients was subjected to further analysis to determine the proximity to HTRs within 1,000 m from their residences (26, 28) and whether distance to HTRs affected the age of asthma onset (analysis plan was illustrated in **Supplementary Figure S1**).

Definition of Heavy Traffic Roads (HTRs)

Heavy traffic roads (HTRs) were identified using open-data daily PCU (Passenger Car Unit) statistics from the Directorate

General of Highways of Taiwan (https://www.thb.gov.tw/sites/en/). Based on the geographic distribution of patients, we selected the ten busiest traffic monitoring sites, each of which had a daily mean bidirectional PCU exceeding 36,329 in 2018 (**Supplementary Figure S2**).

Model of Geometric Analysis

Geometric data were extracted from maps obtained from the open-data service – Open Street Map (https://www.openstreetmap.org/export). Data covered the region between latitudes from 24.74 to 25.33 N and longitudes from 120.86 to 122.04 E. All map data other than roads and streets were excluded. Criteria for the selection of routing vectors were based on the Top-10 PCU routes. We sought to mitigate sphere projection bias by re-projecting the coronadite system of geometrics (e.g., routes or patient locations) from EPSG:4326 (WGS84 – World Geodetic System 1984, used in GPS) to EPSG:32651 (WGS 84 – UTM Zone 51N). This converted the spherical representation in radian units into a 2D flat surface presentation in meters, which is a convenient format for subsequent calculation. Geodata was processed using a custom script written in JavaScript under the NodeJS (version 12.9.1. OpenJS Foundation. San Francisco, California. Joyent, Inc.) runtime environment, and all exchangeable data formats were standardized according to GeoJSON format. The graphical representation of input variables and calculation results was handled using QGIS software (version 3.10.1. QGIS.org, 2021. QGIS Geographic Information System. QGIS Association. https://www.qgis.org).

Linear algebra (i.e., point to vector distance) was used to calculate the minimum distance between the domicile of each patient and the nearest HTR based on geometric data (**Supplementary Figure S3**). We also calculated the overall density of traffic in the areas surrounding the domicile of each patient by counting the number of HTRs within circles of various sizes, starting at 100 m and extending to a maximum distance of 1,000 m (26, 28).

Statistical Analysis

All data were expressed as mean ± *SD* or percentage. The Student's *t*-test was used to compare the means of continuous variables and normally distributed data; otherwise, the Mann-Whitney test was used. Categorical variables including patients with EOA and LOA living in ≥ 1 or 2 heavy-traffic roads within indicated distance were tested using the Chi-square test or Fisher exact test. For tests done by physicians' discretion, the numbers are tested were smaller than the total cohort. The analysis was done on the patients who were checked and the number of participants who provided information and the number of participants with positive results were specified. Unadjusted odds ratio (OR) and 95% CI were calculated for selective variables in geometric analysis during the Chi-square test. The association between the minimum distance to an HTR and patient factors was tested using the Spearman rank correlation because the distance was not normally distributed. All analysis was performed using IBM SPSS Statistics version 19. Armonk, NY: IBM Corp. Statistically significant results were defined as $p \leq 0.05$.

TABLE 1 | The characteristics of patients with early and late-onset asthma.

Variables	EOA (*n* = 61)	LOA (*n* = 222)	*p* value
Age, years, mean (SD)	46.6 (19.2)	60.9 (15.5)	<0.001
Male, *n* (%)	29 (47.5)	108 (48.6)	0.9
Body mass index, kg/m^2	25.7 (4.9)	25.8 (5.0)	0.8
Never smoker	48 (81.4)	142 (64.3)	0.006
ACT score, mean (SD)	19.7 (5.1)	21.0 (4.3)	0.1
Age of asthma onset, years, mean (SD)	8.6 (5.4)	52.2 (17.9)	<0.001
Asthma duration, years, mean (SD)	38.0 (21.1)	9.0 (12.9)	<0.001
Family history of asthma, *n* (%)	26 (42.6)	48 (21.6)	0.02
Home exposure to fumes or dust, *n* (%)	28 (45.9)	71 (32.0)	0.049
Occupational exposure to fumes/dust, *n* (%)	25 (41.0)	76 (34.2)	0.4
Comorbidities, *n* (%)			
Gastroesophageal reflux	29 (47.5)	110 (49.5)	0.9
Allergic rhinitis	42 (68.9)	137 (61.7)	0.3
Rhinosinusitis with or without polyp	14 (23.0)	53 (23.9)	0.9
Aspirin sensitivity	5 (8.2)	15 (6.8)	0.7
Anxiety or depression	23 (37.7)	87 (39.2)	0.8
Obstructive sleep apnea	9 (14.8)	51 (23.0)	0.2
Pulmonary function and allergic status			
FVC, Liter	2.74 (1.12)	2.17 (0.87)	<0.001
FVC, % of pred.	82.5 (18.9)	74.2 (20.2)	0.005
FEV1, Liter	2.12 (0.98)	1.63 (0.71)	<0.001
FEV1, % of pred.	75.3 (22.0)	68.3 (21.2)	0.03
IgE level, KU/L, median (range)	209.0 (6–2,075)	159.5 (2–283)	0.04
ECP level, μg/L, mean (SD)	16.1 (13.4)	15.0 (25.7)	0.8
ECP level ≥ normal range (18μg/L)	40.4 (21/52)	17.9 (29/162)	0.02
Eosinophil counts, cells/μL, mean (SD)	204.9 (182.2)	215.8 (218.8)	0.8
Atopy, % (n/N)	69.1 (38/55)	47.3 (87/184)	0.05

Data are presented as number and percentage or mean and SD, unless otherwise indicated.
N, number of participants who provided information; n, number of participants with positive results.
EOA, early-onset asthma; LOA, late-onset asthma; ACT, asthma control test; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; BD; pred., prediction. IgE, immunoglobulin E; ECP, eosinophil cationic protein.

RESULTS

Characteristics of Patients With LOA Differed From Those of Patients With EOA

Between July 2019 and June 2020, a total of 283 asthma patients were consecutively enrolled in the pay-for-performance program. Among these asthmatics, 222 subjects (78.4%) were LOA. **Table 1** shows the characteristics of patients with EOA and LOA. **Supplementary Figure S4** presents the distribution of patient ages and ages of onset. The mean age of onset of EOA and LOA was 8.6 years and 52.2 years, respectively. Compared to the EOA group, participants with LOA were older, had a shorter duration of asthma onset, less association of asthma family history, less exposure to fumes or dust at home, and a higher proportion of smoking habits. There was no difference in gender, co-morbidities, weight status, exacerbation history in

FIGURE 1 | Minimum distance from the residence of asthmatic patients to the nearest heavy traffic road in New Taipei City, Taoyuan, and Hsinchu, Taiwan. EOA residences are indicated by X and LOA residences are indicated by circles. The heavy traffic roads in this study included three national highways (Highway 1, 2, and 3) and five expressways (Expressway 1, 2, 3, 64, and 66).

the last year, and ACT score between the two groups. The pre-bronchodilator forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were lower in patients with LOA than in patients with EOA. As for allergic biomarkers, IgE levels were significantly higher in the EOA group than in the LOA group. The proportion of patients with ECP levels exceeding the normal range (18 μg/L) was higher in the EOA group than in the LOA group. The two groups were comparable in terms of eosinophil count. Patients with EOA tended to be more atopic and were also more susceptible to home dust mite (HDM), cat dander, and dog dander than patients with LOA (**Supplementary Table S1**).

The Distribution of Patients With EOA and LOA Living in Areas of High Traffic Density

As shown in **Figures 1**, **2**, we observed no significant differences between the LOA and EOA groups in terms of minimum distance to the nearest HTR (EOA vs. LOA: 1,124 ± 787 m vs. 1,412 ± 175 m; $p = 0.07$). We further analyzed the number of HTRs within regions that were measured from patient residences at set distances of up to 1,000 m. When patients resided with a distance of 900 m in the high-traffic road, more patients with LOA were living in multiple HTRs areas compared to patients with EOA (≥ 2 HTRs; 14.3 vs. 3.4%, $p = 0.023$. **Table 2**). Subgroup analysis was performed to identify patient characteristics associated with multiple HTRs within 900 m. Briefly, this involved pooling 30 patients with LOA with two patients with EOA for analysis. Compared to patients living in fewer than two HTRs within 900 m, those living in more than two HTRs had higher IgE levels (**Table 3**, mean ± *SD*, 426.9.1 ± 798.2 vs. 252.9 ± 360.8 KU/L, $p = 0.05$), more positive atopic status (75 vs. 49.8%, $p = 0.02$), and a higher sensitivity to HDM (64.3 vs. 43.2%, $p = 0.04$).

The Associations of Patients With LOA With the Minimum Distance to HTRs

To clarify the influence of high traffic density on patients with LOA, we conducted further analysis on the correlation between the distance to HTRs and patient characteristics in the LOA group (**Figure 3** and **Supplementary Table S2**). The minimum distance to an HTR was positively correlated with age of onset (**Figure 3A**, Spearman's rho 0.151, $p = 0.025$), BMI (**Figure 3B**, Spearman's rho 0.157, $p = 0.023$) as well as negatively correlated with the numbers of positive specific IgEs (**Figure 3C**, Spearman's rho −0.213, $p = 0.005$).

The Characteristics of Patients With LOA According to the Distance Away From HTRs

For the binary variables and confirming the results of the correlation test, we compared the atopy, mood status, and obesity (BMI ≥ 30) of patients with LOA living within or beyond 900 m of HTRs (**Figure 4**). A higher proportion of patients with LOA with atopic status (26.3 vs. 20.5%, $p = 0.001$, unadjusted OR: 2.82, 95%CI: 1.519–5.235. **Figure 4A**)

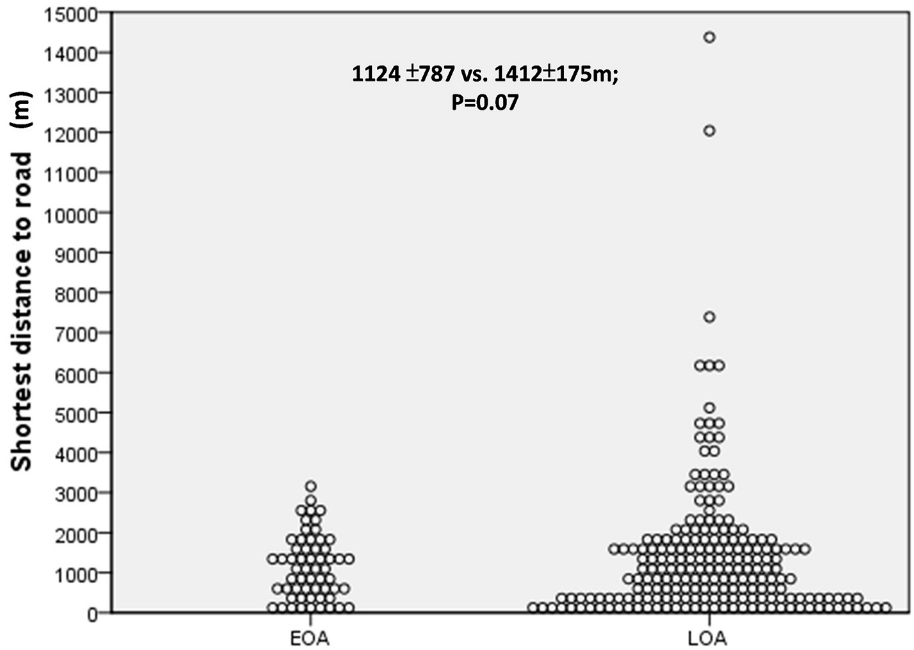

FIGURE 2 | The minimum distance to the nearest heavy traffic road (HTR) between early-onset asthma (EOA) vs. late-onset asthma (LOA). No significant difference was observed between patients with EOA and LOA in terms of minimum distance to HTR.

TABLE 2 | The density of heavy traffic roads proximal to residences of patients with EOA or LOA*.

	EOA (*n* = 58)	LOA (*n* = 210)	*p*-value
Road density within 900 m			
Heavy traffic roads ≥1	27 (46.6)	100 (47.6)	0.86
Heavy traffic roads ≥2	2 (3.4)	30 (14.3)	0.02
Road density within 600 m			
Heavy traffic roads ≥1	16 (27.6)	77 (36.7)	0.19
Heavy traffic roads ≥2	1 (1.7)	20 (9.5)	0.05
Road density within 300 m			
Heavy traffic roads ≥1	9 (15.5)	47 (22.4)	0.249
Heavy traffic roads ≥2	0	5 (2.4)	0.234
Road density within 100 m			
Heavy traffic roads ≥1	5 (8.6)	17 (8.1)	0.905
Heavy traffic roads ≥2	0	1 (0.5)	0.598

**Patients living in New Taipei City, Taoyuan, and Hsinchu, Taiwan.*
Data are presented as patient numbers and percentages.

and depression or anxiety were found to be living within 900 m from HTRs (21.0 vs. 18.1%, $p = 0.047$, unadjusted OR: 1.81, 95%CI: 1.031–3.165. **Figure 4B**). In contrast, there was a trend of a higher proportion of obese patients were living beyond 900 m from HTRs (12.4 vs. 5.7%, $p = 0.075$, unadjusted OR: 1.974, 95%CI: 0.936–4.165. **Figure 4C**). Interestingly, the proportion of obese patients living beyond 1,000 m from HTRs was statistically higher than those living within 1,000 m to HTRs (data not shown).

TABLE 3 | Asthma-associated inflammatory markers in asthmatic patients living in areas with or without high-density traffic as indicated by at least two heavy traffic roads within 900 meters.

	Residence with ≥ 2 major roads (*n* = 32)	Residence with ≤ 1 major road (*n* = 236)	*p* value
IgE level, KU/L, median (range)	144.0 (6–3,548)	105.0 (0–2,075)	0.05
ECP level, μg/L	16.1 (13.4)	15.0 (25.7)	0.7
Eosinophil counts, cells/μL	170.6 (95.4)	217.0 (226.8)	0.3
Atopy, % (n/N)	75.0 (21/28)	49.8 (100/201)	0.02
Positive of specific IgE			
Home dust mite, % (n/N)	64.3 (18/28)	43.2 (83/192)	0.04
Cockroach, % (n/N)	32.1 (9/28)	19.8 (38/192)	0.1
Cat dander, % (n/N)	10.7 (3/28)	13.0 (25/192)	1.0
Dog dander, % (n/N)	17.9 (5/28)	14.1 (27/192)	0.6
Blomia tropicalis, % (n/N)	450.0 (13/26)	40.7 (72/177)	0.4
Penicillium natatum, % (n/N)	17.6 (3/17)	5.6 (9/161)	0.09
Cladosporium herbarum, % (n/N)	11.8 (2/17)	1.9 (3/161)	0.07

Data are presented as number and percentage, mean and standard deviation (SD), or percentage and positive proportion.
N, number of participants who provided information; n, number of participants with positive results.
EOA, early-onset asthma; LOA, late-onset asthma; IgE, immunoglobulin E; ECP, eosinophil cationic protein.

DISCUSSION

Our analysis revealed that patients with LOA tended to be older than patients with EOA, to have had asthma for a shorter

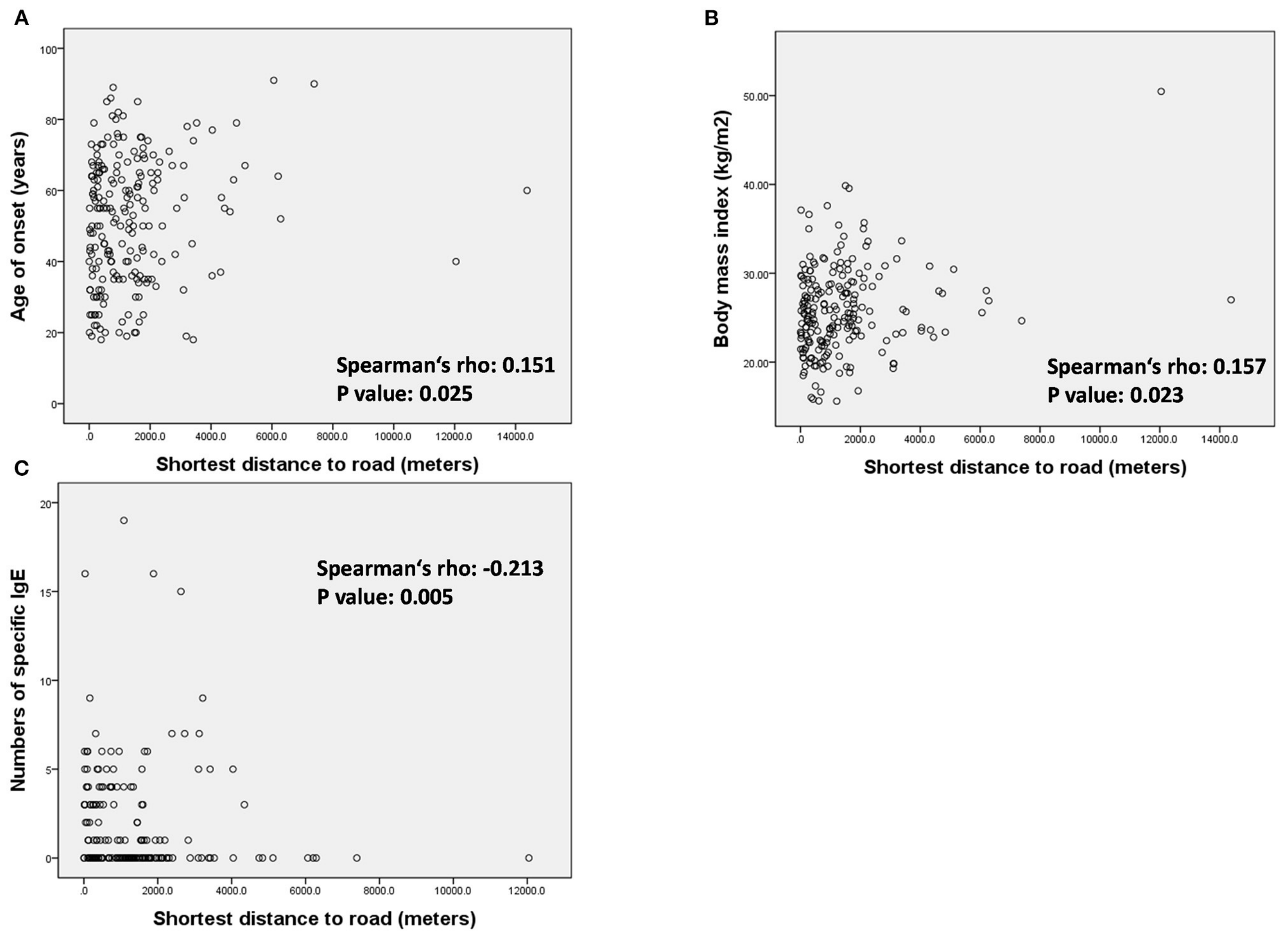

FIGURE 3 | Association between minimum distance to heavy traffic road and **(A)** age of onset, **(B)** body mass index, and **(C)** number of specific IgE in the patients with LOA.

duration, were less likely to have a family history of asthma, were less exposed to fumes or dust in the home, were less likely to be atopic, were less sensitive to common inhaled allergens, and were more likely to have poor lung function. In urban areas, more patients with LOA had multiple HTRs (≥ 2 HTRs) within a distance of 900 m. Patients living within 900 m from multiple HTRs had a higher total IgE level, a more atopic status, and a higher sensitivity to HDM, compared to patients with ≥ 1 HTRs at a comparable distance. Among patients with LOA, the minimum distance to an HTR was positively associated with the age of onset and BMI and was negatively associated with atopy and mood status. Among patients with LOA, a higher proportion of atopic patients were living within 900 m from HTRs, a higher proportion of patients with anxiety or depression were living within 900 m from HTRs and a trend of a higher proportion of obese patients were living beyond 900 m from HTRs. To the best of our knowledge, this is the first study demonstrating the different traffic density between EOA and LOA and different phenotypes in LOA by geolocation, which showed the novel relation between asthma phenotypes and the urban environment.

Correlation between TRAP and asthma is usually assessed in terms of pollutant concentration, such as nitrogen oxide and particulate matter (PM), or the distance to HTRs. Numerous cohort studies have demonstrated a positive association between exposure to TRAP and the risk of asthma (11, 23, 29, 30); however, studies on the link between proximity to HTRs and the risk of asthma have been inconsistent. A birth cohort study in southwestern British Columbia failed to observe a significant correlation between proximity to highways and major roads (<150 m) and development of childhood asthma (15). A birth cohort study in New York City reported a significant correlation between proximity to heavy traffic (<250 m) and childhood asthma among patients without a history of moving prior to the age of 5 (16). In a cohort study of children with asthma attending elementary school in an urban area of the northeastern United States, the incidence of asthma symptoms was shown to increase inversely with the distance to major roads (12). One cohort study conducted in three cities in Sweden reported a positive association between adult-onset asthma and proximity (<50 m) to major roads (≥8,000 vehicles/day)

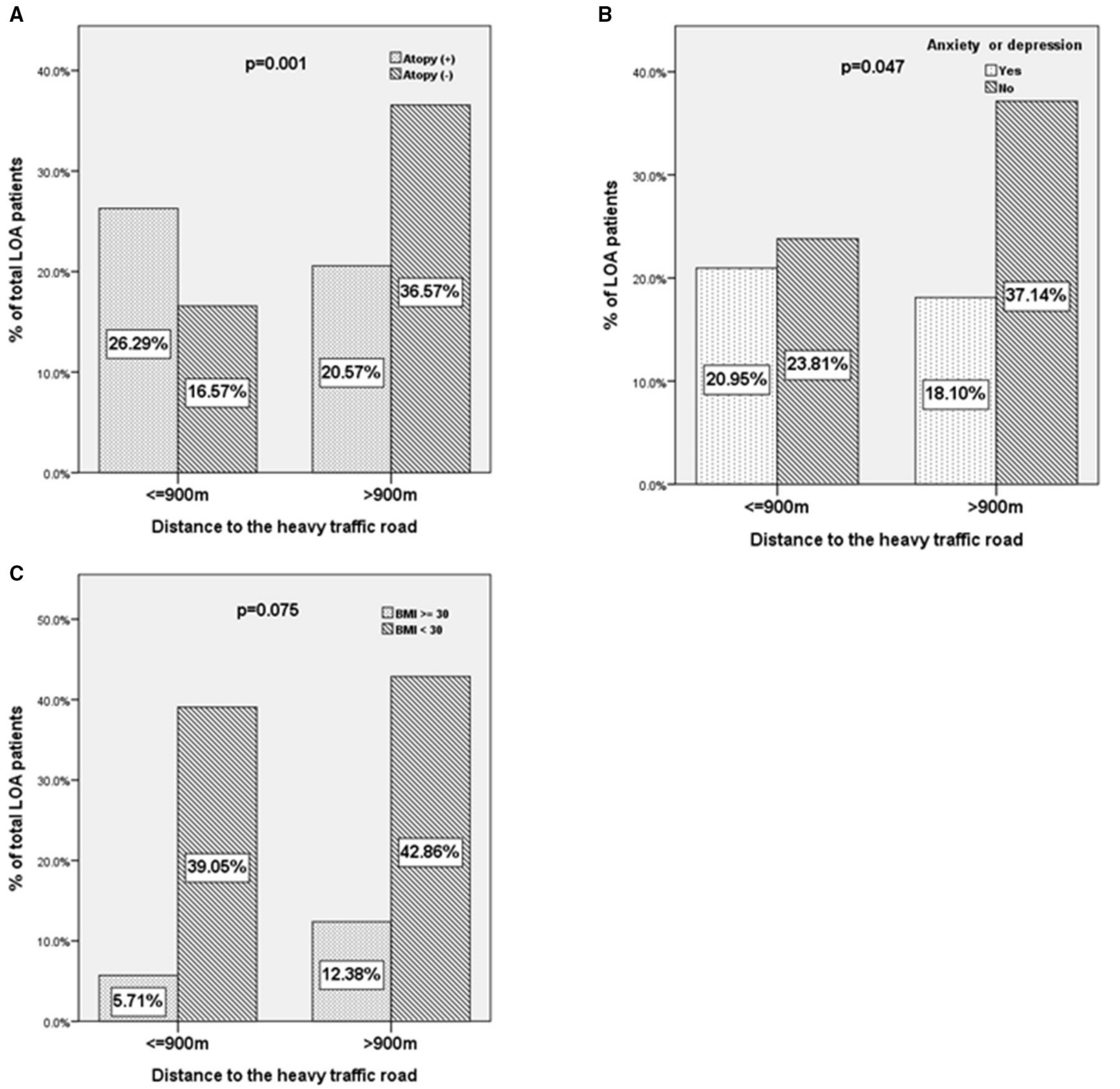

FIGURE 4 | The characteristics of patients with LOA according to the distance away from heavy traffic roads. **(A)** Atopy status, **(B)** anxiety or depression, and **(C)** body mass index ≥30 of patients with LOA as a function of the distance between residence and heavy traffic roads (≤900 vs. >900 m).

(23). Two cross-sectional studies examined the links between subgroups of asthma and proximity to major roads. One study on adults in southern Sweden discovered that proximity (>100 m) to a major road (>10 cars/min) was associated with allergic asthma but not with non-allergic asthma (24). One study on adults in Tasmania, Australia, (i.e., an area with low air pollution levels) reported that proximity (<200 m) to a major road was associated with an elevated risk of asthma; however, this was only among carriers of glutathione S-transferase theta-1 (25). The different distances to HTR for asthma risk in the studies above may result from the different traffic volumes, the population in risks, and the local environment of individual cities. In the current study, HTRs were defined by daily mean PCU exceeding 36,329, which was much higher than that of other studies (23, 24). The range of 1,000 m to HTR was selected initially because the relevant reports included one study from our asthma patients (26, 28). By defining HTRs with the top-ten high traffic volume from the official database, we determined that living within 900 m of multiple HTRs was associated with an elevated risk of LOA compared to EOA. The current study provides new evidence of the greater impact of heavy traffic exposure for LOA. In addition to the direct distance to HTRs, exposure to the density of HTRs is also an important determinant for asthma control.

The patient profiles revealed by the subgroup analysis of patients living with multiple HTRs provided one of the possible mechanisms of asthma inception of LOA (**Table 3**). Patients living within 900 m from multiple HTRs presented higher total IgE levels, were more likely to be atopic and were more likely to present sensitivity to HDM, compared to patients with fewer than two HTRs at a comparable distance. We also found that asthma onset was earlier among patients with LOA living near HTRs. This suggests that an interaction between TRAP and allergic sensitization may be the force driving asthma inception at an early age among LOA living near **Figures 3**, **4**. Similar to our results, a cross-sectional study revealed associations between exposure to high road density and the prevalence of allergic sensitization and small airway function in subjects with a family history of asthma (31). Previous animal studies on TRAP exposure and allergic sensitization support our results. In adult and neonatal mice, co-exposure to diesel exhaust particles (DEPs) and house dust mites was shown to promote the persistence of TH2/TH17 effector/memory cells in the lungs (32). In studies in an adult mouse model, DEP and HDM co-exposure has also been shown to enhance airway hyper-responsiveness and generate a mixed TH2 and TH17 response or the number of type 2 innate lymphoid cells (33, 34). Co-exposure to HDM and benzo(a)pyrene has also been shown to enhance IL-33 and TSLP production in an asthma mouse model (35). Environmental factors (e.g., ambient air polyaromatic hydrocarbons, PM, and DEP) have been linked to epigenetic changes that modify the gene expression of T regulatory cells or innate response, further promoting the Th2 response (36).

Anxiety and depression contribute to asthma symptoms and stressful life events have been shown as the risk factor of onset of asthma (37, 38). Some studies have shown psychiatric stress enhances allergic inflammation (39, 40). We found the risk of anxiety or depression increased in patients living within 900 m to HTRs (**Figure 4B**). This finding further demonstrated the complex interactions between the psychiatric status, traffic exposure in patients with LOA, and further studies for better analysis are required.

The incidence of obesity in patients with LOA was higher among those living >900 m from HTRs than among those living <900 m from HTRs (**Figure 4C**). This conflicts with a number of previous studies suggesting that air pollution plays a role in the incidence of asthma among the obese (41). By contrast, the incidence of atopic asthma in patients with LOA was lower among those living far from HTRs (**Figures 3**, **4**). We surmise that exposure to TRAP may play a more important role in the pathogenesis of atopic asthmatics than it does in obese asthmatics. Future studies on obesity-related LOA and TRAP in urban areas are required.

The study has several limitations. First, the cohort had a smaller patient number of EOA compared to LOA. This is because we recruited adult patients consecutively without selection in the pay-for-performance program to prevent selection bias. The higher ratio of LOA to EOA in our cohort is possible due to the remission rate of EOA being much higher than LOA and LOA is suggested to be more severe than EOA (2, 42). Therefore, similar to other cohorts (43), patients with LOA were referred from local clinics to our center more frequently than patients with EOA. The present results from a single center will have to be confirmed further in subsequent longitudinal studies in a larger population. Second, self-reports pertaining to the age of the patient at the time of diagnosis by a physician were subject to recall bias. Note that we were unable to obtain documented medical records related to asthma diagnosis; however, the patient characteristics in the current study (e.g., family history of lung function) were comparable with those obtained in large-cohort studies and major review articles related to LOA (2, 3, 6, 9, 18, 44). Third, covariates of exposure to fume or dust or comorbidities were defined by questionnaires or medical records, not by real inspection of patients' environments or strict medical diagnostic criteria. The results of a negative association between exposure and comorbidities and HTR proximity in the current study are required further studies to confirm. Fourth, we were unable to obtain information related to TRAP concentrations; therefore, our findings are not necessarily generalizable to all HTRs. Our findings could have been affected by local climatic conditions, occupational exposure, and indoor air pollution in the individual urban environment.

In conclusion, the characteristics of patients with LOA were distinct from those of patients with EOA. By geolocation in urban centers, we discovered the correlation between asthma phenotypes and the urban environment in LOA. patients with LOA tended to live in areas of higher HTR density, which was associated with an elevated incidence of atopic symptoms, sensitivity to HDM, and mood disorder. Proximity to HTRs and obesity may be factors contributing to uncontrolled asthma in cases of LOA. The novel evidence of patient-environment interaction provides further explanation for asthma persistence in the modern world.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Chang Gung Medical Foundation Institutional Review Board (No. 201900211B0). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

T-YL: conceptualization, data curation, investigation, formal analysis, and writing of the original draft. Y-SL: methodology, software, formal analysis, and visualization. H-CL: data curation, resources, and formal analysis. S-ML: data curation, resources supervision, formal analysis, and writing with review and editing. Y-LL, C-HW, P-JC, and C-YL: data curation and resources. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.783720/full#supplementary-material

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Characteristics and Prognostic Factors of Pulmonary Fibrosis After COVID-19 Pneumonia

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Background: Few is known about the long-term pulmonary sequelae after COVID-19 infection. Hence, the aim of this study is to characterize patients with persisting pulmonary sequelae at follow-up after hospitalization. We also aimed to explore clinical and radiological predictors of pulmonary fibrosis following COVID-19.

Methods: Two hundred and 20 consecutive patients were evaluated at 3–6 months after discharge with high-resolution computed tomography (HRCT) and categorized as recovered (REC) or not recovered (NOT-REC). Both HRCTs at hospitalization ($HRCT_0$), when available, and $HRCT_1$ during follow-up were analyzed semiquantitatively as follows: ground-glass opacities (alveolar score, AS), consolidations (CONS), and reticulations (interstitial score, IS).

Results: A total of 175/220 (80%) patients showed disease resolution at their initial radiological evaluation following discharge. NOT-REC patients (45/220; 20%) were mostly older men [66 (35–85) years vs. 56 (19–87); $p = 0.03$] with a longer in-hospital stay [16 (0–75) vs. 8 (1–52) days; $p < 0.0001$], and lower P/F at admission [233 (40–424) vs. 318 (33–543); $p = 0.04$]. Moreover, NOT-REC patients presented, at hospital admission, higher ALV [14 (0.0–62.0) vs. 4.4 (0.0–44.0); $p = 0.0005$], CONS [1.9 (0.0–26.0) vs. 0.4 (0.0–18.0); $p = 0.0064$], and IS [11.5 (0.0– 29.0) vs. 0.0 (0.0–22.0); $p < 0.0001$] compared to REC patients. On multivariate analysis, the presence of CONS and IS at $HRCT_0$ was independent predictors of radiological sequelae at follow-up [OR 14.87 (95% CI: 1.25–175.8; $p = 0.03$) and 28.9 (95% CI: 2.17–386.6; $p = 0.01$, respectively)].

Conclusions: In our population, only twenty percent of patients showed persistent lung abnormalities at 6 months after hospitalization for COVID-19 pneumonia. These patients are predominantly older men with longer hospital stay. The presence of reticulations and consolidation on HRCT at hospital admission predicts the persistence of radiological abnormalities during follow-up.

Keywords: SARS-CoV-2, coronavirus disease 2019, pulmonary fibrosis, high-resolution computed tomography, pulmonary sequelae

BACKGROUND

Coronavirus disease 2019 (COVID-19), which is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has infected more than 130 million people worldwide. COVID-19 leads to respiratory manifestations that can range from mild flu-like symptoms such as fever, cough, and fatigue to severe respiratory failure requiring intensive care (1, 2).

Data from previous pandemics caused by coronaviruses suggested that there may be pulmonary sequelae in one-third of patients at 12 weeks after discharge (3, 4).

Some recent studies tried to characterize radiological sequelae after COVID-19 pneumonia (5, 6). This condition, which is referred to as "post-COVID syndrome," still lacks a universally agreed definition (7). On May 2020, a document of the British Thoracic Society (BTS) proposed an algorithm on post-discharge management of patients with COVID-19 and distinguished two groups of interest: patients with severe pneumonia and patients with mild-to-moderate pneumonia (8). Following up on this document, George and colleagues suggested a structured respiratory follow-up for patients with clinico-radiological confirmation of COVID-19 pneumonia (9). Importantly, they proposed patients with severe pneumonia undergo a full clinical assessment at 12 weeks with a chest X-ray whereas patients with persisting radiological abnormalities should undergo a high-resolution computed tomography (HRCT) scan. In this regard, the role of chest X-ray and HRCT in disease management both during hospitalization and follow-up is well established (10, 11). Han and coauthors recently reported that fibrotic-like changes on CT performed at 6 months during follow-up persist in approximately one-third of patients with COVID-19 (12), but the data on long-term pulmonary sequelae in this patient population remain scarce. The aim of this study is to characterize, among patients hospitalized for COVID-19 pneumonia, those presenting persisting pulmonary sequelae during follow-up, and to define which clinical and radiological features are predictive of persistent radiological abnormalities.

MATERIALS AND METHODS

Study Population and Study Design

We prospectively collected patients evaluated at the post-COVID clinic of the University Hospital of Padova between June and December 2020. The patients evaluated at the post-COVID clinic were initially admitted to the Division of Infectious and Tropical Diseases of the University Hospital of Padova between February and September 2020 for SARS-CoV-2 infection confirmed by the real-time polymerase chain reaction (RT-PCR) at nasopharyngeal swab.

Among all patients evaluated, we specifically followed up every 3 months those presenting a COVID-19-related severe disease according to the WHO criteria ($n = 220$) (13). Demographics and clinical data at hospital admission [symptoms, gas exchange values (paO_2/FiO_2)] and during hospitalization [days of hospital stay, maximal FiO_2 (FiO_2 max) needed, level of care, treatment] were collected. Comorbidities were categorized as cardiovascular diseases (CVDs), respiratory diseases, metabolic diseases (including diabetes mellitus, obesity, and dyslipidemia), autoimmune diseases, and oncologic diseases (including lung, prostate, pancreatic, breast, and colon cancer). Based on patient's clinical conditions during hospitalization, we distinguished those requiring a low- (LIMC) and high-intensity medical care (HIMC), as previously described (14).

Radiological Evaluation

At follow-up, HRCT was available for the entire study population ($HRCT_1$) whereas at hospital admission, it was available in only a subgroup of patients ($HRCT_0$) ($n = 79$, 36%). The HRCTs were performed by a 64 slice Siemens Somatom Sensation (Siemens Healthcare, Erlangen, Germany) applying a slice thickness $\leq$0.5 mm.

According to the presence or absence of radiological abnormalities on $HCRT_1$, the study population was categorized as recovered patients (REC, $n = 175$) or not recovered patients (NOT-REC, $n = 45$).

Two expert thoracic radiologists (CG and AG), who were blinded to clinical data and timing of HRCTs, scored the images independently using a composite semiquantitative scale. This represented a modification of the previously reported scoring systems standardized by our group (13). Specifically, ground-glass opacities (GGO) (alveolar score, AS), consolidations (CONS), and reticulations (interstitial score, IS) were analyzed. For each lung lobe, the two radiologists assessed the extent of AS, CONS, and IS using a scale from 0 to 100 and estimated extent to the nearest 2%. The result was expressed as the mean value of the five lobes in AS, CONS, and IS. The level of interobserver agreement was obtained for each patient as a mean of 5 lobes and for each radiological abnormality (AS, CONS, and IS) and expressed as Cohen's k value. Disagreement between radiologists was resolved by consensus.

Statistical Analysis

Categorical variables were described as absolute (n) and relative values (%), whereas continuous variables were described as median and range. To compare demographic and clinical data between REC and NOT-REC patients, chi-square test and Fisher's exact test ($n < 5$) for categorical variables and Mann–Whitney U tests for continuous variables were used, as appropriate.

To compare radiological scores at $HRCT_1$ in NOT-REC patients, Mann–Whitney U test for continuous variables was used, whereas Wilcoxon signed-rank test was used to compare radiological scores between $HRCT_0$ and $HRCT_1$. A univariate logistic regression analysis, followed by a regression model adjusted for gender, pack-years, paO_2/FiO_2 at admission, degree of medical care (high or low), and FiO_2 max, was performed to detect the predictive factors of radiologic sequelae (NOT-REC)

Abbreviations: IPF, idiopathic pulmonary fibrosis; FVC, forced vital capacity; HRCT, high-resolution computed tomography; REC, recovered; NOT-REC, not recovered; AS, alveolar score; CONS, consolidations; IS, interstitial score; COVID-19, Coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, real- time polymerase chain reaction; LIMC, low-intensity medical care; HIMC, high-intensity medical care; BMI, body mass index; CVDs, cardiovascular diseases; TLC, total lung capacity; DLCO, diffusing capacity of the lung for carbon monoxide.

TABLE 1 | Baseline demographics and clinical features of the overall population evaluated at post-COVID clinic, and of the two subgroups categorized according to the presence of radiological recovery during the follow-up period.

	Overall population (n = 220)	**REC (n = 175; 80%)**	**NOT−REC (n = 45; 20%)**	***P* value**
Male—*n (%)*	115 (52)	86 (49)	29 (64)	0.06
Age at admission—*years*	59 (19–87)	56 (19–87)	66 (35–85)	<0.0001
Smoking history—*pack-years*	0 (0–67)	0 (0–67)	0 (0–60)	0.07
Current—*n (%)*	15 (7)	10 (6)	5 (11)	0.20
Former—*n (%)*	70 (32)	54 (31)	16 (36)	0.54
Nonsmokers—*n (%)*	135 (61)	111 (63)	24 (53)	0.21
BMI—(kg/m^2)	26 (18–39)	27 (18–39)	26 (21–35)	0.35
Cardiovascular diseases—*n* (%)	98 (45)	72 (41)	26 (58)	0.04
Respiratory diseases—*n* (%)	39 (18)	30 (17)	9 (20)	0.65
Autoimmune diseases—*n* (%)	36 (16)	25 (14)	11 (24)	0.10
Metabolic diseases—*n* (%)	102 (4)	78 (45)	24 (53)	0.29
Oncologic diseases—*n* (%)	25 (11)	17 (8)	8 (18)	0.12
PaO_2 / FiO_2 at admission	314 (33–543)	318 (33–543)	233 (40–424)	0.04
FiO_2max during hospitalization—%	28 (21–100)	27 (21–100)	45 (21–100)	<0.0001
Hospitalization—days	9 (0–75)	8 (1–52)	16 (0–75)	<0.0001
Low degree of care—*n* (%)	163 (74)	138 (79)	25 (56)	0.002
High degree of care—*n* (%)	57 (26)	37 (21)	20 (44)	

Values are expressed as numbers and (%) or median and range, as appropriate. To compare demographics between recovery (REC) and not recovery (NOT-REC), chi-square test and Fisher's t-test (n < 5) for categorical variables and Mann–Whitney t-test for continuous variables were used.

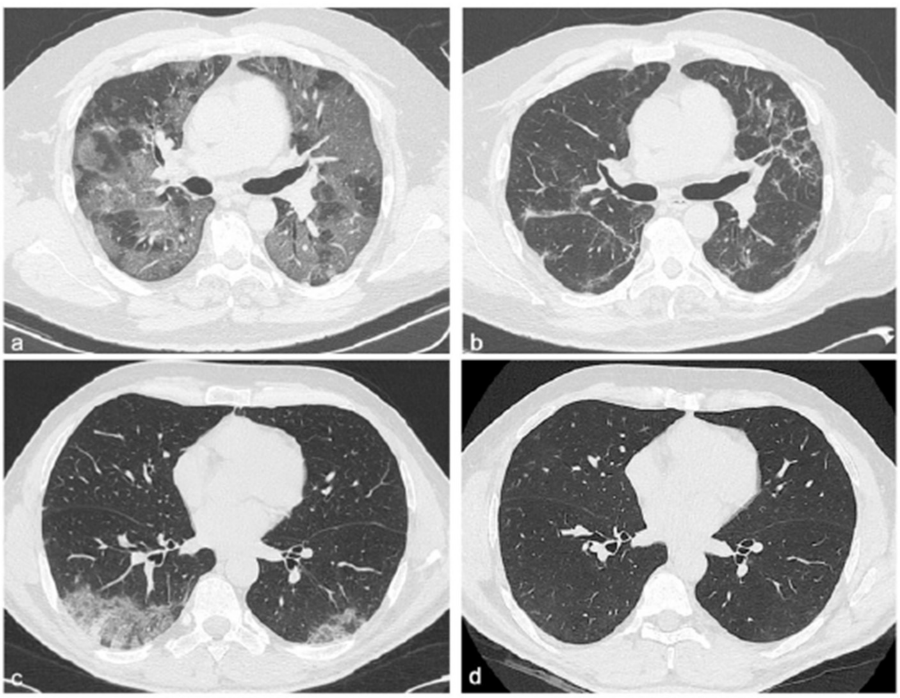

FIGURE 1 | Chest CT features of two patients with COVID-19 pneumonia at different time points: hospitalization and 6 months after discharge. Chest CT images of a 58-year-old male patient with COVID-19, not recovery patient **(a,b)**. The first CT performed at admission shows bilateral areas of ground-glass opacities in a peripheral distribution **(a)**, and after 6 months from discharge, CT shows persistent of interlobular septal thickening with peripheral distribution **(b)**. Chest CT images of a 51-year-old male patient with COVID-19, recovery patient **(c,d)**. The first CT shows, at admission, a small consolidation at the right lower lobe accompanied by ground-glass opacities in both lower lobes **(c)**, and after 6 months from discharge, no residual abnormalities were observed **(d)**.

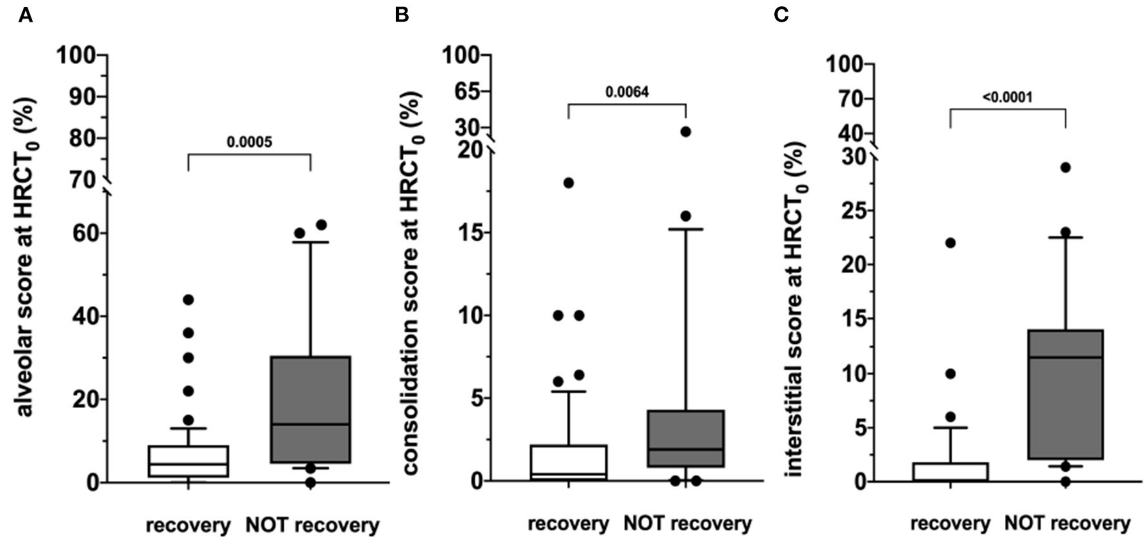

FIGURE 2 | HRCT scores during hospitalization (HRCT0) of the two subgroups categorized according to the presence of radiological recovery [recovery (REC) or NOT-recovery (NOT-REC)] at follow-up period. Horizontal bars represent median values; bottom and top of each box plot 25th and 75th; brackets show 10th and 90th percentiles; and circles represent outliers. White boxes indicate values for recovery group and gray boxes not recovery group. **(A)** ALV [14.0 (0.0–62.0) vs. 4.4 (0.0–44.0); $p = 0.0005$]; **(B)** CONS [1.9 (0.0–26.0 vs. 0.4 (0.0–18.0); $p = 0.0064$]; **(C)** INT [11.5 (0.0–29.0) vs. 0.0 (0.0–22.0); $p < 0.0001$].

TABLE 2 | HRCT scores during hospitalization ($HRCT_0$) of the overall population evaluated at post-COVID clinic and of the two subgroups categorized according to the presence of radiological recovery during the follow-up period.

	Overall population (*n* = 220)	REC (*n* = 175; 80%)	NOT—REC (*n* = 45; 20%)	*p*-value
Alveolar score—%	5.0 (0.0–62)	4.4 (0.0–44.0)	14.0 (0.0–62.0)	0.0005
Consolidations—%	0.8 (0.0–26.0)	0.4 (0.0–18.0)	1.9 (0.0–26.0)	0.006
Interstitial score—%	0.8 (0.0–29.0)	0.0 (0.0–22.0)	11.5 (0.0–29.0)	<0.0001

Values are expressed as median and range, as appropriate. To compare HRCT scores at hospitalization ($HRCT_0$) between recovery (REC) and not recovery (NOT-REC), Mann–Whitney t-test for continuous variables was used.

at follow-up. All data were analyzed using SPSS Software version 25.0 (US: IBM Corp., New York, NY, USA). *p*-Values < 0.05 were considered statistically significant. The graphs were obtained using the statistical package GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Ethics Statement

The study protocol complies with the ethical guidelines of the 1975 Declaration of Helsinki, and in agreement with national regulation on observational studies, it was notified and approved by the local ethics committee (number: 46430/03.08.2020) and the need for patient's informed consent was waived.

RESULTS

Clinical Evaluation at Hospital Admission and During Hospitalization

Two hundred and 20 patients with COVID-19 pneumonia evaluated at the post-COVID clinic were included in the study (**Table 1**). A total of 115 patients (52%) were men, with a median age of 59 years (range 19–84) and body mass index (BMI) 26 (18–39). The most prevalent comorbidities were CVDs ($n = 98$, 45%), followed by the chronic respiratory diseases (18%). Based on the presence of radiological sequelae on HRCT performed at follow-up ($HRCT_1$), 175 (80%) patients were categorized as REC and 45 (20%) as NOT-REC (**Figure 1**). Baseline demographic and clinical data of REC and NOT-REC patients are summarized in **Table 1**.

No differences in sex, smoking history, or BMI were observed between the two groups, with a prevalence of men in NOT-REC compared to REC (64 vs. 49%, respectively). NOT-REC patients were significantly older compared to REC [66 (35–85) vs. 56 (19–87) years; $p < 0.0001$]. CVDs were significantly more frequent in NOT-REC compared to REC [26 (58%) vs. 72 (41%); $p = 0.04$] whereas autoimmune, metabolic, and oncologic diseases did not differ between the two groups. Symptoms before hospital admission were also similar, except for a higher proportion of patients presenting with dyspnea in NOT-REC compared to REC group [33 (73%) vs. 64 (37%); $p < 0.0001$] (**Supplementary Table 1**).

At hospital admission, NOT-REC had a worse gas exchange with a lower PiO_2/FiO_2 ratio than REC [233 (40–424) vs. 318 (33543); $p = 0.04$]. In addition, compared to REC, during hospitalization, NOT-REC required more frequently

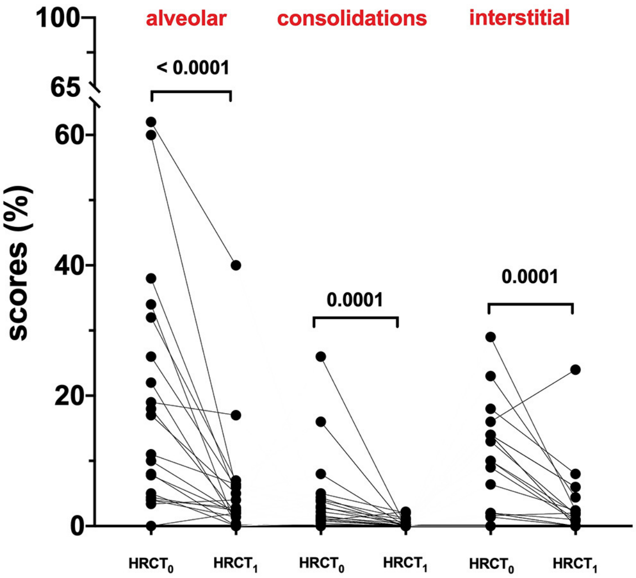

FIGURE 3 | HRCT scores of the not recovery population (NOT-REC) from HRCT0 to HRCT1: ALV. [from 14 (0.0–62.0) to 2.6 (0.0–40.0); p < 0.0001], CONS [from 1.9 (0.0–26.0) to 0.0 (0.0–2.2); p = 0.0001] and INT [1.5 (0.0–29.0) to 1.4 (0.0–24.0)].

high-intensity medical care (HIMC) (20, 44 vs. 37, 21%; p = 0.002), higher FiO_2 max [45 (21–100) vs. 27 (21–100); $p <$ 0.0001], and longer in-hospital stay [16 (0–75) vs. 8 (1–52) days; $p <$ 0.0001].

The majority of patients were admitted during the first SARS-CoV-2 wave when no standardized protocols existed for treatment of hospitalized patients. NOT-REC patients were more frequently treated with hydroxychloroquine (n = 37, 82 vs. 111, 63%; p = 0.01), antibiotics other than ceftriaxone and azithromycin (n = 25, 56 vs. 44, 25%; p < 0.0001), remdesevir (n = 7, 16 vs. 10, 6%, p = 0.02), tocilizumab (n = 8, 18 vs. 12, 7%; p = 0.02), and steroids (n = 27, 60 vs. 74, 42%; p = 0.03) compared to REC. Conversely, the two groups did not differ with regard to the use of ceftriaxone, azithromycin, lopinovir/ritonavir, and hyperimmune plasma (**Supplementary Table 2**). At discharge, a similar proportion of patients in both groups were prescribed steroids.

Clinical, Functional, and Radiologic Evaluation at Follow-Up

Patients were evaluated at post-COVID clinic at regular 3-month intervals after discharge. At first evaluation, NOT-REC patients presented more frequently a modified Medical Research Council (mMRC) scores of 1 and 2 compared to REC [15 (33%) vs. 22 (13%), p = 0.0009 and 7 (16%) vs. 3 (2%), p < 0.0001, respectively]. In the overall population, pulmonary function tests (PFTs) revealed a median forced vital capacity (FVC) of 3.40 liters (L) (range 1.40–7.96), 96%pred. and a median total lung capacity (TLC) of 5.36 L (3.63–8.09), 89% pred. within the normal range. Likewise, NOT-REC patients showed preserved lung volumes within normal range (**Supplementary Table 3**). A number of 32 patients out of 220 (14.5%) had an abnormal diffusing capacity of the lung for carbon monoxide (DLco) at the 6-month follow-up, which occurred in those with persistent interstitial lung abnormalities (NOT-REC patients). At follow-up CT ($HRCT_1$), NOT-REC patients presented higher ALV [2.8 (0.0–40.0)] compared to CONS [0.0 (0.0–2.0); p < 0.0001] and IS [0.6 (0.0–24.0); p < 0.0001] (**Supplementary Figure 1**). Overall, the interobserver agreement between the two radiologists with regard to change in AS, CONS, and IS was good (Cohen's kappa = 0.79 for AS, k = 0.88 for CONS, and k = 0.81 for IS).

Longitudinal Evaluation of Radiologic Manifestation: From Hospitalization to Follow-Up

At hospital admission, HRCT ($HRCT_0$) was available for 79/220 (36%) patients. ALV [5.0 (0.0–62.0)] was significantly more prevalent compared to CONS [0.8 (0.0–26.0); p < 0.0001] and IS [0.8 (0.0–29.0); p < 0.0001]. When this patient subgroup was stratified in NOT-REC and REC, NOT-REC patients (n = 20) had at hospital admission higher ALV [14.0 (0.0–62.0) vs. 4.4 (0.0–44.0); p = 0.0005] (**Figure 2A**), CONS [1.9 (0.0–26.0 vs. 0.4 (0.0–18.0); p = 0.0064] (**Figure 2B**), and IS [11.5 (0.0–29.0) vs. 0.0 (0.0–22.0); p < 0.0001] (**Figure 2C**) compared to REC patients (n = 59) (**Table 2**). Finally, when comparing $HRCT_0$ with $HRCT_1$, we observed that in NOT-REC patients, ALV [from 14 (0.0–62.0) to 2.6 (0.0–40.0); p < 0.0001], CONS [from 1.9 (0.0–26.0) to 0.0 (0.0–2.2); p = 0.0001], and IS [1.5 (0.0–29.0) to 1.4 (0.0–24.0)] decreased significantly (**Figure 3**).

Prognostic Factors for Radiological Sequelae at Follow-Up

Univariate analysis showed that older age, a prolonged in-hospital stay, a lower $PiO2/FiO_2$ at hospital admission, cardiovascular comorbidities, a higher degree of medical care, a higher FiO_2 max, and higher ALV, CONS, and INT scores at $HRCT_0$, not use of hydroxychloroquine, antibiotics other than azithromycin and ceftriaxone, tocilizumab, remdesevir, and systemic steroids are associated with persistent radiological abnormalities at follow-up. Multivariate analysis revealed that CONS [OR: 20.6 (95%CI: 1. −301.2); p = 0.02] and IS score [23.0 (1.4–377.2); p = 0.02] are independent predictors of radiological sequelae at follow-up (**Table 3**).

Finally, on multivariate analysis adjusted for gender, pack-years, PiO_2/FiO_2 ratio at admission, degree of care (high or low), and FiO_2 max, both CONS and IS at $HRCT_0$ are independent predictors of radiological sequelae at follow-up with an OR of 14.87 (95% CI: 1.25–175.8; p = 0.03) and 28.9 (95% CI: 2.17–386.6; p = 0.01), respectively (**Table 4**).

DISCUSSION

In our study, we demonstrated that only a significant minority of patients hospitalized for COVID-19 pneumonia has persistent radiological abnormalities at follow-up. Patients who did not recover are mainly elder men, with a more severe gas exchange

TABLE 3 | Predictive factors of radiological sequelae at follow-up in patients hospitalized for SARS-COV-2-related pneumonia.

	Univariate analysis		Multivariate analysis	
	OR (95% IC)	***p***	**OR (95% IC)**	***p***
Sex				
Female	Ref.	-	-	-
Male	1.87 (0.95—3.69)	0.07	-	-
Age—years				
<59	Ref.	-	Ref.	-
≥59	2.99 (1.47–6.08)	0.002	0.81 (0.10–6.39)	0.84
BMI—(kg/m^2)				
<26	Ref.	-	-	-
≥26	0.80 (0.41–1.58)	0.52	-	-
Smoking history–*pack–years*				
= 0	Ref.	-	-	-
>0	1.56 (0.79–3.10)	0.19	-	-
Hospitalization—*days*				
<9	Ref.	-	Ref.	-
≥9	4.77 (2.15–10.5)	<0.0001	12.77 (0.65–248.8)	0.09
PiO$_2$/FiO$_2$ at admission				
<314	Ref.	-	Ref.	-
≥314	0.33 (0.13–0.80)	0.01	1.24 (0.13–11.46)	0.84
CVD				
No	Ref.	-	Ref.	-
Yes	1.95 (1.00–3.80)	0.04	1.40 (0.15–12.48)	0.76
Respiratory diseases				
No	Ref.	-	-	-
Yes	1.20 (0.52–2.77)	0.65	-	-
Autoimmune diseases				
No	Ref.	-	-	-
Yes	1.94 (0.87–4.32)	0.11	-	-
Metabolic diseases				
No	Ref.	-	-	-
Yes	1.42 (0.73–2.74)	0.29	-	-
Oncologic diseases				
No	Ref.	-	-	-
Yes	2.01 (0.80–5.01)	0.13	-	-
Degree of care				
Low	Ref.	-	Ref.	-
High	2.98 (1.49–5.95)	0.002	1.35 (0.13–13.12)	0.79
FiO$_2$ max—%				
<28	Ref.	-	Ref.	-
≥28	3.25 (1.54–6.80)	0.002	1.01 (0.07–16.2)	0.99
Alveolar score HRCT0—%				
<7	Ref.	-	Ref.	-
≥7	4.0 (1.33–11.98)	0.01	0.74 (0.09–5.99)	0.78
Consolidations HRCT0—%				
<0.8	Ref.	-	Ref.	-
≥0.8	6.29 (1.66–23.87)	0.007	20.6 (1.40–301.2)	0.02
Interstitial score HRCT0—%				
<1.4	Ref.	-	Ref.	-
≥1.4	41.2 (5.1–331.8)	<0.0001	23.0 (1.40–377.2)	0.02
Hidroxicloroquina				
Yes	Ref 2.66 (1.17–6.07)	0.02	Ref 1.26 (0.18–8.82)	0.80
No				
Azithromycin				
Yes	Ref.	-	-	-
No	0.76 (0.39–1.47)	0.41	-	-
Ceftriaxone				
Yes	Ref.	-	-	-
No	1.74 (0.89–3.40)	0.10	-	-
Other antibiotics				
Yes	Ref.	-	Ref.	-
No	3.72 (1.88–7.34)	<0.0001	4.87 (0.52–45.7)	0.16
Lopinovir/Ritonavir				
Yes	Ref.	-	-	-
No	1.49 (0.75–2.94)	0.24	-	-
Remdesevir				
Yes	Ref.	-	Ref.	-
No	3.03 (1.08–8.49)	0.03	12.5 (0.41–3.85)	0.14
Glutathione				
Yes	Ref.	-	-	-
No	0.22 (0.09–1.75)	0.15	-	-
Tocilizumab				
Yes	Ref.	-	Ref. 0.6 (0.03–11.1)	-
No	2.93 (1.12–7.69)	0.02		0.73
Plasma				
Yes	Ref.	-	-	-
No	1.49 (0.37–5.86)	0.56	-	-
Steroids during hospitalization				
Yes	Ref.	-	Ref. 1.04 (0.09	-
No	2.04 (1.05–3.99)	0.03	– 11.6)	0.97

(Continued)

TABLE 3 | Continued

Values are expressed as OR (95%CI). Logistic regression analysis was used to determine the relationship of clinical data with radiological sequelae at follow-up.

impairment at hospital admission and a more severe clinical course during hospitalization. Interestingly, the presence of reticulation and consolidation at admission was predictive of persistent interstitial changes at follow-up.

To date, few studies have reported on the follow-up of patients hospitalized for COVID-19 pneumonia (5, 6). Different approaches based on disease severity have been proposed with the aim to standardize patients' follow-up. Specifically, the British Thoracic Society guidelines for management of post-COVID-19 syndrome distinguished patients with severe pneumonia requiring intensive care from patients with mild-to-moderate

TABLE 4 | Multivariate analysis for factors independently associated with radiological sequelae at follow-up in patients hospitalized for SARS-COV-2-related pneumonia.

	Multivariate analysis*	
	OR (95% IC)	***p***
Alveolar score HRCT0—%		
<7	Ref. 1.80 (0.39-–8.20)	-
≥7		–0.44
Consolidations HRCT0—%		
<0.8	Ref. 14.87 (1.25-–175.8)	-
≥0.8		–0.03
Interstitial score HRCT0—%		
<1.4	Ref. 28.9 (2.17-–386.6)	-
≥1.4		-**0.01**

*Values are expressed as OR (95%CI). Univariate and multivariate-adjusted odds ratio for radiological NOT recovery according to radiological patterns during hospitalization ($HRCT_0$). *Adjusted for gender, pack-years, PiO_2/FiO_2 ratio at admission, degree of care (high or low), FiO_2 max.*

pneumonia treated in a medical ward or at home (4). However, it is becoming increasingly clear that radiological changes following COVID-19 pneumonia do not resolve completely in a large minority of patients (5, 15). Some studies have started to use CT to assess the presence of long-term lung abnormalities. A recent work from the Chongqing University Three Gorges Hospital evaluated 41 patients and showed that in most patients, the chest CT lesions were no longer present at 7 months after discharge, whereas older patients with severe comorbidities were more prone to develop fibrosis. (16). From the Wuhan cohort, Han and colleagues investigated 114 patients with severe pneumonia according to the WHO criteria (12) and observed fibrotic changes in one-third of them at the 6-month follow-up. Of note, on multivariate analysis, they found that a higher baseline/initial CT lung involvement score (>18 in a score of 25) was independently associated with fibrotic-like changes in the lung (12). Huang and colleagues conducted a cohort study that included 353 patients who were enrolled between January and May 2020 who underwent HRCT at follow-up after discharge. They found that more than 50% of the patients had residual lung abnormalities. Moreover, they found that disease severity in the acute phase was independently associated with the percentage change of CT score in a multivariable analysis (17).

In our hospital, the first patients with COVID-19 pneumonia were admitted in February 2020 and were evaluated in the post-COVID clinic in June 2020. We enrolled prospectively patients diagnosed with COVID-19 pneumonia according to the WHO criteria. Two hundred and 20 patients were evaluated at 3 months after discharge and every 3 months thereafter, according to the current guidelines (8). We found that as many as 20% of our entire patient population had radiological pulmonary sequelae at follow-up. This percentage is lower than that observed in previous studies (12, 17), but our patients' population has been followed up for a longer period of time, thus allowing non-fibrotic pulmonary abnormalities to clear. Patients who did not recover (NOT-REC) were older, mostly men and with worse disease impairment both at admission and during hospitalization compared to patients without radiological sequelae at follow-up. Specifically, NOT-REC patients had a lower PiO_2/FiO_2 ratio at admission and a more severe clinical course. Moreover, NOT-REC patients who required higher maximal FiO_2 during hospital stay were more often treated in a high-intensive care setting and required a longer in-hospital stay, consistent with the findings from the Wuhan cohort (17). Furthermore, we have shown that, in NOT-REC patients, the HRCT performed at hospital admission is more likely to display ground-glass opacities, consolidations, and reticulation. These data suggest that the risk of pulmonary sequelae may be related to the severity of the acute illness and to the intensity of care needed. This is in line with the hypothesis that a cytokine storm might contribute to the pathogenesis of COVID-19 whereas its severity is associated with poor outcomes (18). However, mechanical ventilation and ventilator-induced lung injury, and high-flow oxygen therapy might also have contributed to the development of fibrotic-like changes (19, 20).

The primary aim of our study was to identify predictors of radiological sequelae following COVID-19 pneumonia. Whereas on univariate analysis age, prolonged in-hospital stay, lower $PiO2/FiO_2$ at hospital admission, cardiovascular comorbidities, higher intensity of medical care, and higher FiO_2 max, not using hydroxychloroquine, antibiotics other than azithromycin and ceftriaxone, tocilizumab, remdesevir, or systemic steroids were significantly associated with the presence of interstitial changes during follow-up, we found that higher CONS [OR: 20.6 (95%CI: 1.4–301.2); $p = 0.02$] and IS [23.0 (1.4–377.2); $p = 0.02$] at hospitalization were the only variables independently associated with the persistence of fibrotic changes at follow-up in multivariate analysis. In particular, this latter observation is consistent with that of Han and colleagues who found that a more extensive baseline or initial CT lung involvement was independently associated with permanent fibrotic-like changes in the lung (12). Additionally, the higher amount of consolidation and reticulation at admission remained significantly associated with persistent radiological abnormalities when adjusted for gender, pack-years of smoking, and PiO_2/FiO_2 ratio. However, it remains uncertain whether the fibrotic-like changes we observed represent irreversible pulmonary fibrosis, and further monitoring is warranted to answer this question.

The findings of our study should be interpreted in light of some limitations. First, this is a single-center study; however, it is among the first to analyze HRCT changes over time in a large population of patients hospitalized for COVID-19 pneumonia. In addition, we included a large proportion of patients with severe COVID-19, who are at higher risk of developing persistent lung disease. Second, the CT scan at hospital admission was available for only a subset of patients; however, the aim of our study was to characterize the radiological changes occurring over time as previously done in idiopathic pulmonary fibrosis (21) and to identify predictors of persistent radiological abnormalities.

In conclusion, in our study, about 20% of patients with COVID-19 pneumonia had radiological sequelae at follow-up. Patients who did not fully recover showed a more severe impairment at hospital admission and during hospitalization. Moreover, the presence of reticulation and consolidation on the initial chest CT is predictive of persistent radiological interstitial changes at follow-up.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the University Hospital of Padova, *via* Niccolò Giustiniani n.2, 35128 Padova (nr.: 46430/03.08.2020). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EB, EC, and PS contributed in conceptualization, writing, reviewing and editing, and supervision. EB and EC performed writing original draft—preparation, visualization, and investigation. EC, NB, CG, GC, and AG provided resources and conducted investigation. SP, GC, DL, and AF performed data curation. EB, PS, AC, and MS contributed in resources, visualization, and supervision. All authors have written, read, and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.823600/full#supplementary-material

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Beyond the Lung: Geriatric Conditions Afflict Community-Dwelling Older Adults With Self-Reported Chronic Obstructive Pulmonary Disease

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Rationale: Chronic obstructive pulmonary disease (COPD) predominantly affects older adults. However, the co-morbid occurrence of geriatric conditions has been understudied.

Objective: Characterize the prevalence of geriatric conditions among community-dwelling U.S. older adults with self-reported COPD.

Methods: We conducted a nationally representative, cross-sectional study of 3,005 U.S. community-dwelling older adults (ages 57–85 years) from the National Social Life, Health, and Aging Project (NSHAP). We evaluated the prevalence of select geriatric conditions (multimorbidity, functional disability, impaired physical function, low physical activity, modified frailty assessment, falls, polypharmacy, and urinary incontinence) and psychosocial measures (frequency of socializing, sexual activity in the last year, loneliness, cognitive impairment, and depressive symptoms) among individuals with self-reported COPD as compared to those without. Using multivariate logistic and linear regressions, we investigated the relationships between COPD and these geriatric physical and psychosocial conditions.

Main Results: Self-reported COPD prevalence was 10.7%, similar to previous epidemiological studies. Individuals with COPD had more multimorbidity [modified Charlson score 2.6 (SD 1.9) vs. 1.6 (SD 1.6)], more functional disability (58.1 vs. 29.6%; adjusted OR 3.1, 95% CI 2.3, 4.3), falls in the last year (28.4 vs. 20.8%; adjusted OR 1.4, 95% CI 1.01, 2.0), impaired physical function (75.8 vs. 56.6%; adjusted OR 2.1, 95% CI 1.1, 3.7), more frequently reported extreme low physical activity (18.7 vs. 8.1%; adjusted OR 2.3, 95% CI 1.5, 3.5) and higher frailty prevalence (16.0 vs. 2.7%; adjusted OR 6.3, 95% CI 3.0,13.0) than those without COPD. They experienced more severe polypharmacy (≥10 medications, 37.5 vs. 16.1%; adjusted OR 2.9, 95% CI 2.0, 4.2). They more frequently reported extreme social disengagement and were lonelier, but the association with social measures was eliminated when relationship status was accounted for, as those with COPD were less frequently partnered. They more frequently endorsed depressive symptoms (32.0 vs. 18.9%, adjusted OR 1.9, 95% CI 1.4, 2.7). There was no noted difference in cognitive impairment between the two populations.

Conclusions: Geriatric conditions are common among community-dwelling older adults with self-reported COPD. A "beyond the lung" approach to COPD care should center on active management of geriatric conditions, potentially leading to improved COPD management, and quality of life.

Keywords: geriatrics, functional impairment, COPD–chronic obstructive pulmonary disease, polypharmacy (source: MeSH, frailty), loneliness, cognitive impairment

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the United States and fifth cause of disability in the world (1–3). COPD predominately affects older adults (4). In a 2006 study, the median global COPD pooled prevalence in people 65 years old and greater was 15%, whereas the prevalence among those 40–64 years old was 8% (5). Further, 12% of Medicare beneficiaries have COPD (6).

As people age, the development of geriatric conditions can complicate management of chronic diseases like COPD. Geriatric conditions are multifactorial disease states that transcend discrete diagnosis categories, and confer additional risk for quality of life impairment, hospitalizations, medication non-adherence and death (7–9). Frailty, a syndrome of multisystem impairment defined and assessed variably, is perhaps the best studied geriatric condition in COPD (10). Its presence has been associated with increased risk of hospitalizations and death. The prevalence of frailty in those with COPD has been estimated at almost 60%, and has been demonstrated to predict mortality better than forced expiratory volume in 1 s (11, 12). Eisner et al. found that in those with COPD, developing "non-respiratory impairment" (e.g., loss of lower extremity muscle strength) and functional limitations were associated with increased risk of disability (13). The prevalence and impact of other geriatric conditions such as multimorbidity, activities of daily living disability, physical function impairment, falls, polypharmacy, urinary incontinence, and social frailty among those with COPD have been largely understudied.

In recent years, an evolving understanding of geriatric conditions has helped paint a richer picture of the complexity of health of older people with chronic diseases. For example, research from The Health and Retirement Study, a nationally representative study of older adults, has demonstrated high rates of urinary incontinence and falls in individuals with congestive heart failure, coronary artery disease, and diabetes (14). In tandem with physical disease, social context is critical when considering health in older adults. Social frailty is an emerging concept identifying risk of losing (or loss) of valuable social resources (15). Social frailty, including social disengagement and loneliness, is more common with advancing age, increases vulnerability to catastrophic health events beyond what can be predicted by medical comorbidities alone (16), and is associated with all-cause mortality (17). In several small studies, loneliness is prevalent in those with COPD, and independently associated with more emergency room visits and reduced health perception (18–20).

The primary objective of this study is to report the prevalence of geriatric physical and psychosocial conditions among community-dwelling older adults with COPD using data from the National Social Life, Health, and Aging Project (NSHAP), a nationally representative sample. NSHAP, as compared to other longitudinal studies of older adults, is unique in its robust assessments of social health, along with other physical geriatric conditions (21). Our secondary objective was to determine whether the presence of COPD was associated with having a geriatric condition in the entire sample. We hypothesized that older adults with COPD experience accelerated physiologic aging, manifested by a much higher prevalence of geriatric conditions compared to older adults without COPD even after adjustment for demographics. Our study provides new insights into the high national rates of geriatric conditions among community dwelling people with COPD.

METHODS

Study Population

We conducted a cross-sectional study of respondents enrolled in the first of three rounds of data collected in NSHAP. NSHAP is the first longitudinal, nationally-representative study to assess simultaneously social relationships, physical and mental health, function, and cognition in older adults (aged 57–85 at first interview) in the United States (22). This de-identified analysis was approved by the Institutional Review Board at the University of Chicago and data usage approved by the NSHAP Data Usage Agreement. All respondents provided informed consent.

Data Collection

Round 1 was collected in 2005–2006, and enrolled 3,005 adults (1,551 women and 1,454 men) of 4,017 eligible persons, born from 1920 to 1947 (aged 57–85 at time of interview) who resided in the community (none resided in assisted living or skilled nursing facilities) (23). The unweighted response rate was 74.8% and weighted response rate was 75.5%. Data collection was comprised of three components: (1) an in-home questionnaire; (2) biomeasure collection; and (3) a self-administered leave-behind questionnaire. Potential participants were excluded in round one if they were deemed too cognitively impaired to give formal consent and/or complete the interview as determined by the field interviewer (no formal criteria).

Professional interviewers from NORC (previously known as the National Opinion Research Center) at the University of Chicago conducted the in-home assessments. Further details are available elsewhere (22, 24–28).

Chronic Obstructive Pulmonary Disease Diagnosis

Respondents were asked the question "Has a medical doctor ever told you that you have any of the following conditions: Emphysema, chronic bronchitis, or chronic obstructive lung disease?" Responses to this question (yes/no) were used to divide the sample into comparator groups.

Demographics

Age was calculated using date of birth and survey date. Gender (male or female), race/ethnic group (White/Caucasian, Black/African American, Hispanic/non-black, and other), smoking history, education, and relationship status were self-reported. Smoking status was categorized as "never smoker," "former smoker," and "current smoker," and determined by asking respondents, "do you smoke cigarettes?" and "have you ever smoked cigarettes regularly?" Education levels were categorized as "less than high school," "high school equivalent," "vocational certificate," and "bachelor's degree." Current relationship status (currently married or in a romantic relationship) was reported as "yes" or "no".

Geriatric Conditions

Additional methodologic details for the geriatric conditions can be found in the **Supplementary Material**. Select geriatric conditions were assessed: multimorbidity (modified Charlson index score, with COPD excluded from the morbidity calculation, scale ranging from 0 to 25.5 where a 0 score indicates no co-morbid conditions and 25.5 indicates all co-morbid conditions included) (29, 30), activities of daily living (ADL) disability (see **Supplementary Material**), impaired physical function (timed up and go, TUG, performance time $\geq$ 10 s), extreme low physical activity (<once a month of moderate to vigorous activity on average), modified frailty (an adapted and abbreviated scale including exhaustion, low activity and slow TUG performance time; frailty was defined as a score of 3) (21), any fall in the last 12 months, polypharmacy (<4, 4–10, or $\geq$10 medications), and any urinary incontinence in last 12 months. Psychosocial measures assessed were social frailty measures: extreme social disengagement (socializing one time in the last year or less with friends or relatives), moderate social engagement (socializing several times in the last year or less with friends or relatives), and loneliness [NSHAP Felt Loneliness Measure (NFLM) $\geq$ 1] (28). Cognitive impairment was evaluated [moderate cognitive impairment was defined as a score of <6 on the Short Portable Mental Status Questionnaire (SPMSQ)] (31, 32). Significant depressive symptoms were assessed using the NSHAP Depressive Symptoms Measure (NDSM), with a score $\geq$9 demonstrating significant depressive symptoms (28). Additionally, high-risk medication usage was summed for each respondent [anti-histamines, anticholinergics, benzodiazepines, anti-psychotics, anxiolytics/sedatives, tricyclic antidepressants, muscle relaxants, anti-arrhythmic agents, cyclooxygenase (COX)-2 inhibitors, and narcotics] (33) using a medication log (34). Moderate polypharmacy was defined as taking $\geq$4 medications and severe polypharmacy was defined as $\geq$10 medications (see **Supplementary Material**).

Statistical Analysis

Sample characteristics were compared among respondents with and without a self-reported doctor diagnosis of COPD. Continuous variables are presented as means with standard deviations (SD). Categorical variables are presented as percentages. *T*-tests and chi-square tests, respectively, detected significant differences between the groups.

Multivariate logistic regression models assessed the association between self-reported COPD diagnosis and each geriatric condition, adjusted for age, gender, race/ethnicity, and education. Multivariate linear regression was used to assess the association between self-reported COPD diagnosis and the modified Charlson index score. Social measures included adjustment for these demographics as well as adjustment for partner status. Odds ratios or linear regression coefficients with 95% confidence intervals (CI) are reported for all variables. $P \leq 0.05$ were considered statistically significant. No adjustment for multiple comparisons was made. All analyses were survey weighted, accounting for the survey design, therefore reported estimates reflect the U.S. community-dwelling older adult population in 2005. Analyses were conducted in Stata 15.1 (StataCorp LLC, College Station, Texas, USA).

RESULTS

Demographics

Of the 3,005 adults in Round 1, 322 respondents (10.7%) endorsed having COPD or emphysema (**Table 1**). Those with COPD were older (mean 69.6 years, SD 7.4 vs. 67.8 years, SD 7.7; $p = 0.01$) and more often self-identified as being white/Caucasian individuals (87.6%) as compared to the non-COPD group (79.8%). Individuals with COPD reported lower education levels (completed bachelor's degree: 19.1 vs. 25.2%), had a lower prevalence of being partnered (65.8 vs. 75.6%) and were more commonly current or former smokers (77.8 vs. 57.0%).

Geriatric Conditions

Older adults with self-reported COPD had more multimorbidity than those without COPD (**Table 2**); the average modified Charlson co-morbidity score was significantly higher (2.6, SD 1.9) as compared to the non-COPD group (1.6, SD 1.6) ($p < 0.0001$). The relationship between the modified Charlson

TABLE 1 | Demographic characteristics of US older adults with and without COPD by self-report.

	COPD ($n = 322$) Weighted % or mean (SD)	Non-COPD ($n = 2,683$) Weighted % or mean (SD)	p-value
TOTAL prevalence	10.7	89.3	
Age, mean years (SD)	69.6 (7.4)	67.8 (7.7)	0.01
Gender, women	55.7	51.0	0.2
Race/Ethnicity	($n = 320$)	($n = 2,673$)	0.02
White/Caucasian	87.6	79.8	
Black/African American	6.4	10.5	
Hispanic, non-black	4.2	7.2	
Other	1.8	2.6%	
Education			0.02
Less high school	23.6	17.9	
High school equivalent	27.8	26.8	
Vocational certificate	29.5	30.1	
Bachelor	19.1	25.2	
Relationship status			
Partnered	65.8	75.6	0.1
Smoking status	($n = 322$)	($n = 2,681$)	<0.0001
Current smoker	27.1	13.7	
Former smoker	50.7	43.3	
Never smoker	22.2	43.0	

TABLE 2 | Prevalence of multimorbidity among US older adults with and without COPD by self-report.

	COPD ($n = 322$)	Non-COPD ($n = 2,683$)	
Modified Charlson*, mean (SD)	2.6 (1.9)	1.6 (1.6)	<0.0001
Select conditions			
Asthma	34.6%	7.1%	<0.0001
Arthritis	68.4%	49.5%	<0.0001
History of stroke	14.7%	7.3%	<0.0001
Heart failure	15.4%	7.4%	0.0009
History of MI	19.5%	10.7%	0.003
Diabetes	22.7%	19.4%	0.3
Cancer (ever had)	12.5%	11.4%	0.6

COPD, chronic obstructive pulmonary disease; MI, myocardial infarction.
**Modified Charlson co-morbidity index: as previously described in the NSHAP data set based on the original index of 19 weighted conditions; co-morbidities were added with varying weights as follows: 1 point assigned to history of myocardial infarction, gastric ulcer disease, congestive heart failure, peripheral vascular disease, arthritis, dementia, asthma, and stroke; 1.5 points assigned to diabetes, 2 points assigned to liver disease, leukemia, lymphoma, renal disease, and cancer history; and 6 points assigned to metastatic cancer. COPD was removed from the score. Possible score ranged from 0 to 25.5 where a 0 score indicates no co-morbid conditions and 25.5 indicates all co-morbid conditions included.*

co-morbidity score and COPD persisted after adjustment for age, race/ethnic group, gender, and education (coefficient 0.89, 95% CI 0.51, 1.27; $p < 0.0001$). They also had more asthma (34.6 vs. 7.1%), heart failure (15.4 vs. 7.4%), history of myocardial infarction (19.5 vs. 10.7%), history of cerebral vascular events/stroke (14.7 vs. 7.3%), and arthritis (68.4 vs. 49.5%).

Older adults with self-reported COPD had higher rates of at least one ADL disability (58.1 vs. 29.6%, adjusted model OR 3.1, 95% CI 2.3, 4.3; $p < 0.0001$; **Table 3**). They reported more difficulty performing every reported ADL (walking a block, walking across a room, dressing, bathing, eating, bed mobility, and toileting) (**Figure 1**). The most profound impairment was difficulty walking a block compared to those without COPD (OR 3.4, 95% CI 2.5, 4.6; $p < 0.0001$).

Older adults with self-reported COPD had more frequently impaired physical function as measured by a slow TUG test ($\geq$10 s): 75.8 vs. 56.6%, adjusted OR 2.1, 95% CI 1.1, 3.7; $p < 0.02$; **Table 3**). They also reported more extreme physical inactivity (18.7 vs. 8.1%, adjusted OR 2.3, 95% CI 1.5, 3.5; $p < 0.0001$). Modified physical frailty (as identified by presence of 3 criteria using an adapted and modified 3-point scale) was more common: 16.0 vs. 2.7% (adjusted OR 6.3, 95% CI 3.0, 13.0; $p < 0.0001$). They reported falling in the last year more frequently than those without COPD (28.4 vs. 20.5%, adjusted OR 1.4, 95% CI 1.01, 2.0; $p = 0.04$; **Table 3**). Urinary incontinence was highly prevalent in older adults with COPD (53.9 vs. 39.6%, adjusted OR 1.7, 95% CI 1.3, 2.1; $p < 0.0001$; **Table 3**).

Older adults with self-reported COPD had significantly more moderate polypharmacy ($\geq$4 medications) (80.6 vs. 58.4%, adjusted OR 2.7, 95% CI 2.0, 3.8; $p < 0.0001$) and severe polypharmacy ($\geq$10 medications) (37.5 vs. 16.1%, adjusted OR 2.9, 95% CI 2.0, 4.2; $p < 0.0001$; **Table 4**). Respondents in the COPD group were found to be taking many more high-risk medications, such as anti-histamines, benzodiazepines, and narcotics (**Table 4**).

Community-dwelling older U.S. adults with self-reported COPD had more extreme social disengagement, as assessed by higher frequency of socializing less than once a year with family and friends (4.5 vs. 2.1%, unadjusted OR 2.2, 95% CI 1.2, 4.0; $p = 0.01$, adjusted OR 0.7, 95% CI 0.1, 4.8, $p = 0.7$). Moderate social disengagement was not significantly different between the COPD and non-COPD groups (23.1 vs. 22.7%, unadjusted OR 1.0, 95% CI 0.8, 1.4, $p = 0.18$, adjusted OR 0.8, 95% CI 0.5, 1.5; $p = 0.5$). They also had higher rates of sexual inactivity in the last year (60.9 vs. 42.8%, unadjusted OR 2.1, 95% CI 1.5, 2.8; $p < 0.0001$, adjusted OR 1.5, 95% CI 0.7, 2.9, $p = 0.3$). They were also lonelier (57.7 vs. 42.1%, unadjusted OR 1.9, 95% CI 1.4, 2.5; $p < 0.0001$, adjusted OR 1.2, 95% CI 0.7, 2.2; $p = 0.5$; **Table 3**). These differences were largely due to partnership status, as the significance of these associations diminished in the models which adjusted for relationship status.

The SPMSQ cognitive assessment did not uncover significant differences in cognitive impairment in those with self-reported COPD compared to those without (12.9 vs. 17.6%, adjusted OR 0.6, 95% CI 0.2, 1.9, $p = 0.4$). Those with COPD more frequently reported depressive symptoms by the NSHAP Depressive Symptoms Measure (32.0 vs. 18.9%, adjusted OR 1.9, 95% CI 1.4, 2.7; $p < 0.0001$).

TABLE 3 | Unadjusted and adjusted multivariate logistic regression models comparing the prevalence of geriatric conditions among US older adults with and without COPD by self-report.

	COPD %	Non-COPD %	Unadjusted model OR (95% CI)	Adjusted model OR (95% CI)
Physical measures				
At least 1 ADL limitation	58.1	29.6	3.3 (2.4, 4.5)	3.1 (2.3, 4.3)
Slow gait (TUG) speed (≥10 s)	75.8	56.6	2.4 (1.4, 4.1)	2.1 (1.1, 3.7)
Extreme low physical activity (<once a month)	18.7	8.1	2.6 (1.8, 3.7)	2.3 (1.5, 3.5)
Frail (abbreviated scale)	16.0	2.7	6.8 (3.5, 13.2)	6.3 (3.0, 13.0)
Fall (in last 12 months)	28.4	20.8	1.5 (1.1, 2.1)	1.4 (1.0, 2.0)
Urinary incontinence (in last 12 months)	53.9	39.6	1.8 (1.4, 2.3)	1.7 (1.3, 2.1)
Psychosocial measures				
Extreme social disengagement* (once a year or less)	4.5	2.1	2.2 (1.2, 4.0)	0.7 (0.1, 4.8)
Moderate social disengagement* (several times a year or less)	23.1	22.7	1.0 (0.8, 1.4)	0.8 (0.5, 1.5)
No sex (in last year)*	60.9	42.8	2.1 (1.5, 2.8)	1.5 (0.7, 2.9)
Loneliness* (NFLM ≥ 1)	57.7	42.1	1.9 (1.4, 2.5)	1.2 (0.7, 2.2)
Moderate cognitive impairment (SPMSQ < 6)	12.9	17.6	0.7 (0.3, 2.1)	0.6 (0.2, 1.9)
Frequent depressive symptoms (NDSM ≥ 9)	32.0	18.9	2.0 (1.5, 2.8)	1.9 (1.4, 2.7)

OR, odds ratio; CI, confidence interval; ADL, activity of daily living; TUG, timed up-and-go; NFLM, NSHAP felt loneliness measure; NDSM, NSHAP depressive symptoms measure.
Adjusted model: adjusted for age, gender, race/ethnic group, and education.
**Adjusted model also included relationship status.*

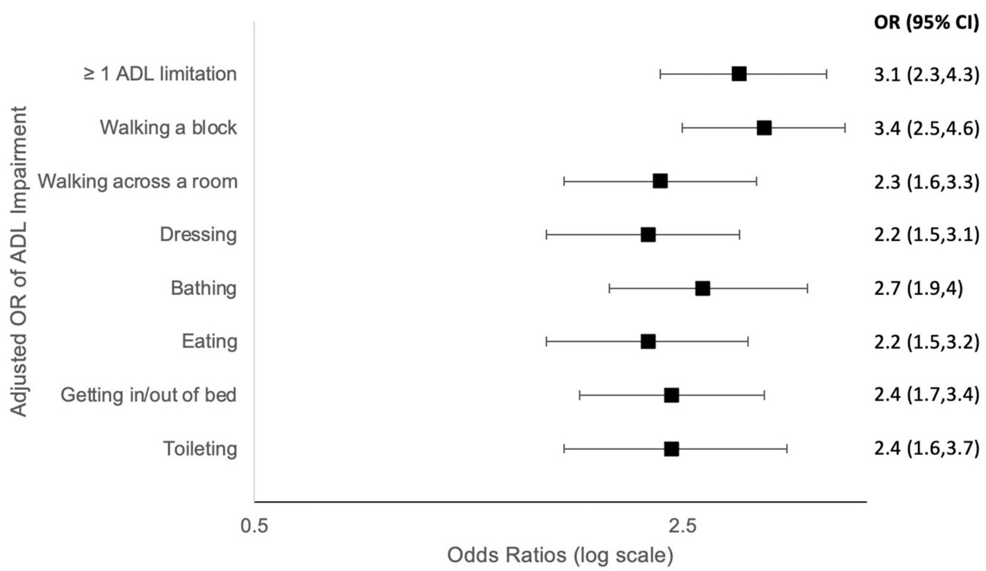

FIGURE 1 | Forest plots (odds ratios with 95% confidence intervals) based on Multivariate Logistic regression models comparing activities of daily living (ADL) impairment among US older adults with vs. without COPD by self-report. OR, odds ratio; ADL, activities of daily living; Adjusted model: adjusted for age, gender, race/ethnic group, and education.

DISCUSSION

Our study establishes that COPD is frequently co-prevalent with multiple, non-respiratory domains of age-related vulnerability requiring complex and coordinated interdisciplinary care and specialized geriatrics training and knowledge (35). Our findings also demonstrate compelling evidence of social frailty among older U.S. adults with COPD living at home, likely related to the significant difference in partnership status between the groups.

In the United States, community-dwelling older adults with COPD are disproportionately afflicted with geriatric conditions that reflect worse global physical and social health. Previous work has demonstrated a high rate of disability and social disengagement among community dwelling older adults with COPD (36). Our findings confirm this and expand upon other

TABLE 4 | Polypharmacy and high-risk medications among US older adults with and without COPD by self-report.

	COPD %	Non-COPD %	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Polypharmacy				
Moderate (≥4 medications)	80.6	58.4	3.0 (2.1, 4.1)	2.7 (2.0, 3.8)
Severe (≥10 medications)	37.5	16.1	3.1 (2.2, 4.5)	2.9 (2.0, 4.2)
High-Risk meds				
Anti-histamines	16.8	6.0	3.2 (2.2, 4.6)	3.4 (2.2, 5.1)
Anticholinergic/ anti-spasmodic	4.0	1.3	3.2 (1.4, 7.5)	2.7 (1.2, 6.4)
Benzodiazepines	11.1	5.4	2.2 (1.4, 3.5)	1.9 (1.2, 3.0)
Anti-psychotics	2.9	1.3	2.2 (1.1, 4.5)	1.9 (0.99, 3.7)
Anxiolytic/Sedatives	15.3	8.0	2.1 (1.4, 3.1)	1.9 (1.3, 2.8)
Tricyclic anti-depressant	4.8	2.0	2.6 (1.2, 5.7)	2.4 (1.1, 5.2)
Muscle relaxants	4.9	1.6	3.1 (1.3, 7.1)	3.4 (1.4, 8.0)
Anti-arrhythmics	3.3	1.2	2.9 (1.4, 6.0)	2.6 (1.2, 5.7)
COX-2 inhibitors	4.1	2.0	2.0 (1.1, 4.0)	1.9 (0.98, 3.9)
Narcotics	9.3	4.5	2.2 (1.1, 4.1)	2.0 (1.1, 3.8)

COPD, chronic obstructive pulmonary disease; OR, odds ratio; CI, confidence interval; COX-2, cyclooxygenase-2.
Adjusted model: adjusted for age, gender, race/ethnic group, and education.

physical and social burdens of COPD, with new information about the high burden of multimorbidity, functional disability, impaired physical function (by slow TUG performance time), low physical activity, falls, polypharmacy, urinary incontinence, depressive symptoms, and both physical and social frailty in a nationally representative community dwelling population with COPD. These findings make clear the larger ecological burden of COPD on older Americans.

The U.S. health system siloes disease management by organ system and subspecialty. This has led to traditional clinical assessments of COPD severity that miss the mark and focus narrowly on COPD-specific issues such as exacerbations, lung function and dyspnea. Our data show that clinicians caring for people with COPD need to consider larger issues of social health and ecology in the care of these patients.

Social health is one critical pillar of wellbeing that often is not captured by traditional organ-centric medical models of health (16). Our findings of social disengagement are of clear importance in the broader care of patients with COPD. As one example, we found that these patients are lonelier, have more extreme social disengagement, and pursue less frequent sexual activity. Interestingly, these effects appears to be primarily related to not having a partner as the significant effect was eliminated once the analyses were adjusted for relationship status. This finding highlights that the social history may be useful to understand the wider burden of COPD in this population and the common lack of a strong social infrastructure to assist with disease management. This finding has clinical relevance because loneliness has been demonstrated to be associated with more emergency room visits and reduced health perception in people with COPD (20). Compared to previous studies on the prevalence of loneliness in which estimates ranged from 25 to 29%, both the COPD and non-COPD populations were lonelier (37). Further, sexual relationships and dysfunction have been demonstrated previously to be common among those with COPD and have an underappreciated impact on quality of life (38–40). Identifying loneliness and social disengagement in patients with COPD may allow clinicians and other caregivers to develop strategies to improve engagement, aided by recommendations from interprofessional team members such as social workers and physical therapists.

A high prevalence of depressive symptoms was demonstrated in the self-reported COPD population, which has been reported previously (41). Depressive symptoms in COPD has been linked to increased acute exacerbations and mortality (42, 43). Frequent assessments for depression with in-office tools such as the PHQ-2 and PHQ-9 are critical, and mental health support and referrals should be pursued by primary care providers and specialty teams caring for patients with COPD and depressive symptoms.

Among older U.S. adults with COPD, there were high rates of ADL disability and physical function impairment along with physical frailty by a modified index. These individuals also were less physically active and suffered more falls. Disability and impaired physical function lead to a decline in independent living, sometimes in catastrophic situations (e.g., following hip fracture), and people who maintain mobility have higher late-life function and quality of life (44–46). Those with COPD are particularly vulnerable due to breathlessness and loss of muscle mass (sarcopenia) (47–49). We propose incorporating simple geriatric assessments into the routine care of people with COPD. Such assessments are likely to uncover unmet need for assistive devices (e.g., walkers and canes, durable medical equipment (e.g., shower chairs), strength training or consideration for additional care (e.g., disability parking placards, in-home caregiving) (50).

Polypharmacy increases mortality in the general older adult population (51). We found significant polypharmacy in patients with COPD as well as increased use of potentially inappropriate and high-risk medications. Measures to identify and limit polypharmacy are especially important in older adults with COPD to limit potentially harmful side effects. Several medications in the high-risk categories for these patients include narcotics and benzodiazepines that may depress respiration. Polypharmacy may be related to their higher rates of multimorbidity which often leads to increased clinical encounters, including subspecialty visits and hospitalizations, and subsequent medication prescribing, as has been demonstrated in other contexts (52, 53). Previous work has demonstrated limited understanding of such geriatric issues in subspecialty and general medical trainees (54) which we hypothesize carries forward to long-term practice patterns that result (in part) in polypharmacy. Pulmonary specialty training should include of geriatrics education, in which geriatric conditions, polypharmacy and high-risk medications are learned, as such knowledge may equip specialists with tools to manage COPD more optimally. The impact of this training will require further study.

Urinary incontinence is a highly prevalent geriatric comorbidity that impairs quality of life and leads to falls (55, 56). We found that urinary incontinence was common in both groups, but older adults with COPD had significantly more urinary incontinence in the prior year as compared to those without COPD. The urinary incontinence definition used in NSHAP was very inclusive as it captured any related symptoms regardless of frequency in the last year. This definition may have included those with rare symptoms. We hypothesize that contributors to urinary incontinence in COPD include frequent coughing, medication side effects, generalized sarcopenia that includes pelvic floor muscles, and decreased ability to ambulate to the bathroom and thus functional incontinence. Screening of and treatment for urine incontinence, including non-pharmacologic options (e.g., pessaries, pelvic floor physical therapy), should be offered to patients with COPD when identified and can greatly improve quality of life.

Surprisingly, higher rates of cognitive impairment were not seen in the NSHAP COPD population, which differs from many previous studies (57, 58). A possible cause for this finding is the low-sensitivity of the SPMSQ cognitive assessment tool used in Round 1 of NSHAP data collection, which is unable to detect early, more subtle cognitive changes. This assessment tool was replaced by a survey-adapted Montreal Cognitive Assessment (MoCA-SA) in subsequent rounds. Future studies will need to assess the burden of cognitive impairment among NSHAP's self-reported COPD population using this more sensitive screening tool. Another possible cause of this finding is that potential participants were excluded in Round 1 if they were too cognitively impaired to give formal consent, which likely excluded participants with more severe cognitive impairment.

A strength of our study is the generalizability of our findings which are based on a nationally representative study of older U.S. adults, with robust assessments of social function and context and simultaneous measures of physical health. Our study is limited by the lack of spirometric data in NSHAP to verify obstructive lung disease diagnosis or stratify outcomes by COPD severity. Additionally, we suspect there may be overlap with other airway disease in some individuals who self-reported asthma but not COPD; this is a diagnostic challenge in the field more generally. Because COPD is a clinical diagnosis that must include assessment of symptoms and exposures along with spirometry, we caution that using spirometry alone to determine case definition of COPD would also have challenges. For example, age-related lung function changes may cause an obstructive pattern and could lead to inclusion of participants without COPD. We note that the prevalence of COPD by self-report in the NSHAP population is consistent with previously epidemiologic reports based on rigorous criteria (1). Self-reported disease data may also have affected the accuracy of the modified Charlson comorbidity index (for example the high reported co-prevalence of asthma and COPD suggest that participants may have mischaracterized their lung disease in reporting). However, this method of reporting is common, as the US Centers for Disease Control assesses COPD prevalence via self-report via the Behavioral Risk Factor Surveillance System telephone survey (59).

Another potential limitation of our study is the significant age difference between the COPD and non-COPD participant groups; those with COPD were almost 2 years older than the non-COPD group. While our analyses were adjusted for age, there may be unaccounted for age effects that influenced the findings of increased geriatric conditions in this group. Our frailty assessment was adapted and not validated, given absence of weight loss and hand grip data in Round 1, so this should be interpreted with caution. Our frailty prevalence was lower than expected compared to national rates in the National Health and Aging Trends Study, which used validated scales and found a prevalence of 15% (95% CI: 14, 16%) in the older non-nursing home population (60). Finally, NSHAP lacks COPD-specific quality of life questions to assess for cough and breathlessness which is another limitation. This information is now used to classify severity of COPD and may be linked to deteriorating physical function and social disengagement (61).

Our findings suggest that a geriatric-focused approach to COPD care could reap significant benefits for affected individuals. Unfortunately, geriatricians are in short-supply and cannot practically care for all patients that could benefit. In 2018, there were about two pulmonologists to every geriatrician in the U.S. (14,899 vs. 7,290), so it is imperative that health systems innovate in order to extend age-friendly care to those that need it (62, 63). The field of geriatric oncology has been a pioneer in geriatric-subspecialty care and have endorsed comprehensive geriatric assessments (CGAs) in older patients with cancer (64). In practice, execution of these geriatric evaluations range from sponsoring embedded consulting geriatricians to perform CGAs for high-risk patients, training interprofessional team members to deliver simple screening assessments, or empowering subspecialists to become dually trained in geriatrics and their intended subspecialty (65). All of these models are possible in ambulatory pulmonary care.

When social or physical frailty are identified, management recommendations should include referrals to interprofessional and multidisciplinary team members, which is a core tenet of age-friendly care. For example, social workers can offer support, counsel, and referrals to social engagement and caregiving resources, physical therapists can help address sarcopenia and frailty, behavioral health specialists can provide counseling and treatment for depressive symptoms, and medical assistants, nursing staff, respiratory therapists and pharmacists can ensure medication lists are up to date and patients are trained in correct inhaler device use. Well-informed providers and clinics can and should assess for unmet medical equipment needs to reduce the mismatch between an individual's environment and their physical capabilities (e.g., shower chairs, raised toilet seats, grab bars, canes, walkers, and disability parking placards) (50). Finally, pulmonary specialty training should include geriatrics education, and providers should enter independent practice armed with specialization in age-friendly COPD care (65, 66). This multi-pronged, "beyond the lung" approach is likely to lead to improved COPD management and quality of life for this population.

CONCLUSION

Geriatric conditions disproportionately afflict community-dwelling older adults with COPD. The presence of multiple domains of vulnerability directly impact COPD management, therefore COPD care requires a geriatric lens. A "beyond the lung" approach to COPD care should be prioritized by the siloed U.S health system, health care organizations and individual providers, which will potentially lead to improved quality of life and COPD management for affected individuals.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. Round 1 data is available through the National Archive of Computerized Data on Aging (NACDA): https://www.icpsr.umich.edu/web/pages/NACDA/nshap.html.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board at the University of Chicago. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LW, KW, JP, EW, MM, WD, SW, VP, and MH-S made substantial contributions to the conception and design of the work. LW and MH-S wrote the first draft of the manuscript. All authors listed above agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved and substantial contributions to the acquisition, analysis, or interpretation of data for the work. All authors contributed to the article and approved the submitted version.

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Diagnostic Significance of Metagenomic Next-Generation Sequencing for Community-Acquired Pneumonia in Southern China

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Background: The morbidity and mortality of community-acquired pneumonia are relatively high, but many pneumonia pathogens cannot be identified accurately. As a new pathogen detection technology, metagenomic next-generation sequencing (mNGS) has been applied more and more clinically. We aimed to evaluate the diagnostic significance of mNGS for community-acquired pneumonia (CAP) in the south of China.

Methods: Our study selected CAP patients who visited the 3rd Xiangya Hospital from May 2019 to April 2021. Pathogens in bronchoalveolar lavage fluid (BALF) specimens were detected using mNGS and traditional microbiological culture. mNGS group: detected by both mNGS and BALF culture; control group: detected only by BALF or sputum culture. The diagnostic performance of pathogens and the antibiotic adjustments were compared within mNGS group.

Results: The incidence of acute respiratory distress syndrome (ARDS) was 28.3% in the mNGS group and 17.3% in the control group. Within the mNGS group, the positive rate of pathogens detected by mNGS was 64%, thus by BALF culture was only 28%. Pathogens detected by mNGS were consisted of bacteria (55%), fungi (18%), special pathogens (18%), and viruses (9%). The most detected pathogen by mNGS was *Chlamydia psittaci*. Among the pathogen-positive cases, 26% was not pathogen-covered by empirical antibiotics, so most of which were made an antibiotic adjustment.

Conclusions: mNGS can detect pathogens in a more timely and accurate manner and assist clinicians to adjust antibiotics in time. Therefore, we recommend mNGS as the complementary diagnosis of severe pneumonia or complicated infections.

Keywords: mNGS, pneumonia, diagnostic significance, pathogen, BALF

INTRODUCTION

Community-acquired pneumonia (CAP) is a common disease with high mortality (1). According to the clinical phenotype, the pathogens for up to 60% of infectious diseases were still unknown (2, 3), and the mortality of CAP in need of emergency treatment exceeds 40% (4). Pathogens that cause pneumonia include common bacteria (such as *Streptococcus pneumoniae*), fungi, viruses, and some atypical pathogens such as *Mycoplasma pneumoniae*, *Chlamydia,* and *Legionella* (1). In a study of 329 clinical samples, it was found that the main pathogens of patients with different immune status were diverse. Among patients with normal immunity, the pathogens are mainly *S. pneumoniae*, *rhinovirus*, and *influenza*. The main pathogens in immunocompromised patients are *Pneumocystis*, *Klebsiella pneumonia*, *S. pneumoniae*, *Haemophilus influenza,* and *Pseudomonas aeruginosa* (5). In recent years, rare and atypical pathogens have been continuously detected, such as *Mycobacterium abscessus*, *Mycobacterium kansas*, etc. These pathogens may cause pneumonia, multiple-organ disorders and even acute respiratory distress syndrome (ARDS). Due to the limitations of current traditional pathogen detection methods in terms of sensitivity, detection speed and detection spectrum, rapid and accurate diagnosis of pneumonia pathogens are a big challenge (6, 7). Therefore, early and effective identification of the pathogens are essential for the precise treatment of pneumonia patients.

mNGS is the second-generation sequencing technology of metagenomics, which can identify bacteria, fungi, parasites, and viruses without much guidance from clinical experience. mNGS can identify the pathogens deeply and rapidly without culturing, and even have higher sensitivity than traditional cultivation methods (8). Another advantage of mNGS is the diversity of samples which could detect almost all pathogens in clinical samples (9) such as bronchoalveolar lavage fluid (BALF), tissue, sputum, pleural effusion, cerebrospinal fluid, pus, bone marrow, and nasal swabs (10–12), etc. Since the sensitivity and specificity of mNGS are less perturbative by the antibiotic treatment presently (13). mNGS may become a routine diagnostic test, partially replacing the traditional sputum culture method (14). However, the interpretation of the mNGS reports, especially the identification of pathogenic bacteria, colonizing bacteria, and the mixture of normal oral microbiota in respiratory tract samples in pneumonia patients need further study (15). The pathogenicity of microorganisms in different regions is generally different. There are few large-scale analysis research with respiratory samples studying the correlation between the detection efficiency of mNGS and the antibiotic therapy. Thus, our study aimed to explore the advantages of mNGS in the detection of pneumonia pathogens and its guiding significance for diagnosis and antibiotic treatment of CAP.

PATIENTS AND METHODS

Patient Selection and Study Design

We retrospectively reviewed 346 cases diagnosed as CAP at the 3rd Xiangya Hospital of Central South University from May 2019 to April 2021. With our inclusion/exclusion criteria (**Figure 1**), 346 samples were included for analysis and categorized into two groups defined as mNGS group and control group. mNGS group was subjected to regular BALF culture as well as mNGS testing (ID: PRJNA756706, https://www.ncbi.nlm.nih.gov/sra/PRJNA756706) in a pairwise manner, and control group only did the BALF or sputum culture. This study was approved by Institutional Review Board of the 3rd Xiangya Hospital of Central South University (No. 21030).

Sample Processing and Nucleic Acid Extraction

BALF were collected from patients according to standard procedures. DNA was extracted using a QIAamp® UCP Pathogen DNA Kit (Qiagen) following the manufacturer's instructions and 600 μL of the processed specimens was mixed with glass beads of 0.1–0.2 mm diameter. A vortex mixer (Crystal, TX, United States) was used to disrupt the bacterial cell wall at 1,600 g for 10 min. The tubes were then heated at 99°C for 10 min before DNA extraction. Human DNA was removed using Benzonase (Qiagen) and Tween20 (Sigma) (16). The differential lysis method was used to remove host DNA. we first use physical hypotonic lysis and chemical lysis to break human cells, and then obtain microbial cells by enzymatic hydrolysis, followed by wall breaking and nucleic acid extraction. Total RNA was extracted with a QIAamp® Viral RNA Kit (Qiagen) and ribosomal RNA was removed by a Ribo-Zero rRNA Removal Kit (Illumina). The concentration of extracted DNA/RNA was measured using a Qubit Fluorometer before library preparation. cDNA was generated using reverse transcriptase and dNTPs (Thermo Fisher).

Library Preparation and Sequencing

Libraries were constructed for the DNA and cDNA samples using a Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA) (17). The initial input of DNA is 5–100 ng. Firstly, DNA needs to be fragmented to obtain 150–250 bp inserts, followed by terminal repair and adapter connection, and finally, library amplification to construct a library that meets the requirements of sequencing. Library was quality assessed by Qubit dsDNA HS Assay kit followed by High Sensitivity DNA kit (Agilent) on an Agilent 2100 Bioanalyzer. Library pools were then loaded onto an Illumina Nextseq 550Dx sequencer for 75 cycles of single-end sequencing to generate ∼20 million reads for each library. For negative controls, we also prepared PBMC samples with 10^5 cells/mL from healthy donors in parallel with each batch, using the same protocol, and sterile deionized water was extracted alongside the specimens to serve as non-template controls (NTC).

Bioinformatics Analyses

Trimmomatic was used to remove low quality reads, adapter contamination, and duplicate reads, as well as those shorter than 50 bp (18). Low complexity reads were removed by Kcomplexity with default parameters (19). Human sequence data were identified and excluded by mapping to a human reference genome (hg38) using Burrows-Wheeler Aligner software.

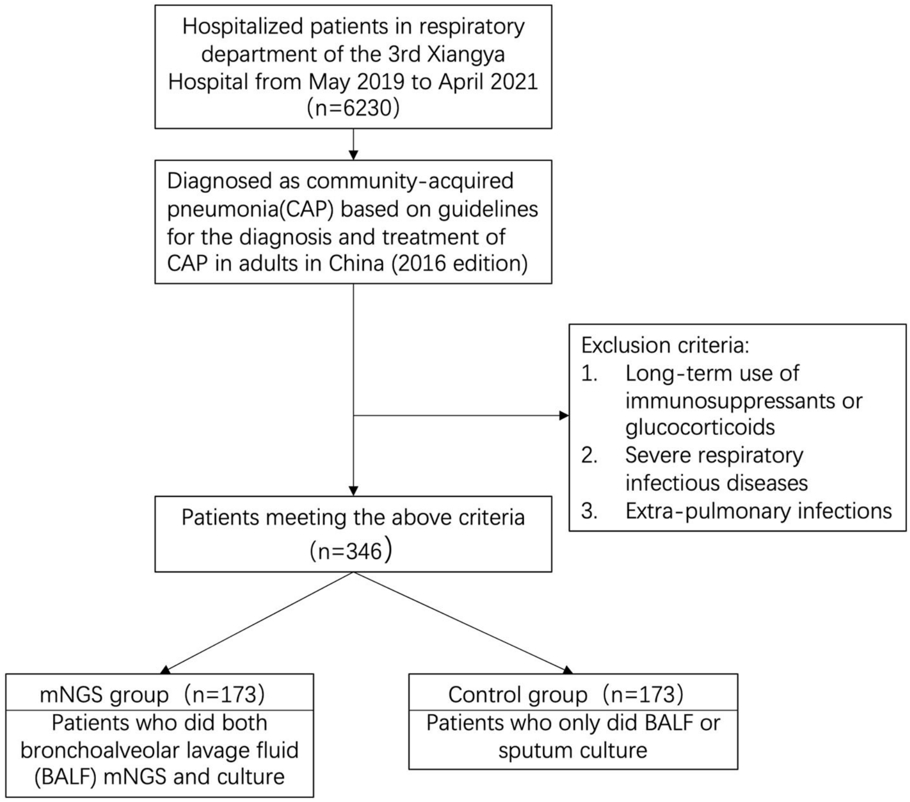

FIGURE 1 | Flowchart of case selection. From 6230 cases, a total of 370 community-acquired pneumonia cases were selected for further analysis. Patients in mNGS group did the mNGS and BALF culture at the same time. Patients in control group only did the BALF or sputum culture. mNGS, metagenomic next-generation sequencing.

We designed a set of criteria similar to the National Center for Biotechnology Information (NCBI) criteria for selecting representative assembly for microorganisms (bacteria, viruses, fungi, protozoa, and other multicellular eukaryotic pathogens) from the NCBI Nucleotide and Genome databases. Pathogen lists was selected according to three references: (1) Johns Hopkins ABX Guide (https://www.hopkinsguides.com/hopkins/index/Johns_Hopkins_ABX_Guide/Pathogens), (2) Manual of Clinical Microbiology (https://www.clinmicronow.org/doi/book/10.1128/9781683670438.MCM), and (3) clinical case reports or research articles published in current peer-reviewed journals. The final database consisted of about 18,562 genomes. Microbial reads were aligned to database with SNAP v1.0beta.18. Virus-positive detection results (DNA or RNA viruses) were defined as the coverage of three or more non-overlapping regions on the genome. A positive detection was reported for a given species or genus if the reads per million (RPM) ratio, or RPM-r was ≥5, where the RPM-r was defined as the RPM_{sample} / RPM_{NC} (i.e., the RPM corresponding to a given species or genus in the clinical sample divided by the RPM in the NC/negative control). In addition, to minimize cross-species misalignments among closely related microorganisms, we penalized (reduced) the RPM of microorganisms sharing a genus or family designation, if the species or genus appeared in non-template controls. A penalty of 5% was used for species (12) (**Figure 2**).

Statistical Analysis

Continuous variables were compared using the Mann–Whitney U-test; categorical variables were compared using the chi-square test. $P < 0.05$ was considered significant. Statistical analyses were performed using SPSS version 23.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Clinical Characteristics of Patients With CAP

Finally, a total of 346 patients with CAP was enrolled. One hundred and seventy-three patients were in the mNGS group, and the rest 173 patients were in the control group. The clinical characteristics of patients between these two groups were different as follows (**Tables 1**, **2**). The rates of patients with comorbidities including hypertension, neoplastic, diabetic, renal, and cerebrovascular diseases were relatively lower in the mNGS group, but they did not reach statistical significance. However, the incidence of ARDS was significantly higher in the mNGS group ($P = 0.021$). Not unexpectedly, the average hospital stay

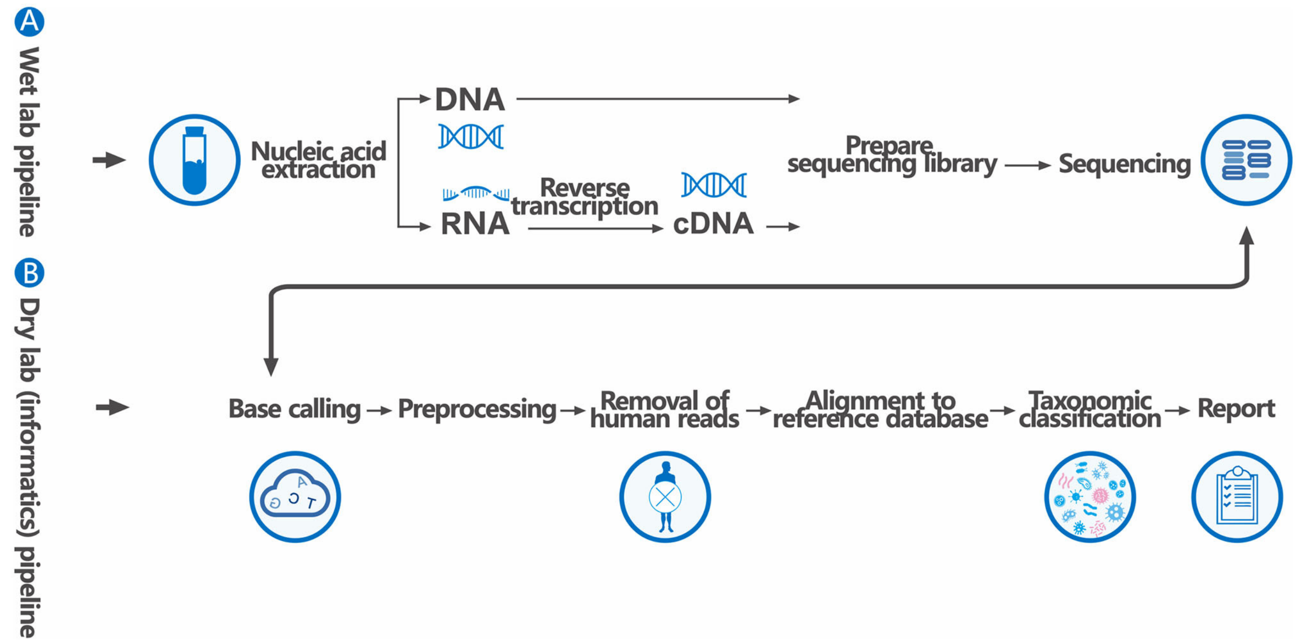

FIGURE 2 | The generalized workflow of mNGS for clinical pathogen diagnosis. The workflow has two components: **(A)** a wet lab protocol in which samples are collected, processed, extracted for nucleic acids, prepared into a sequencing library, and sequenced, and **(B)** a dry lab computational pipeline that includes microbial identification, statistical analysis, and interpretation. The sequencing library may be targeted, undergo DNA amplification, or both.

was longer in the mNGS group ($P = 0.001$). But the empirical use of antibiotics in two groups were roughly the same. The acute physiology and chronic health evaluation (Apache) II score in two groups were not very different, either. The laboratory test results were 5–7 days after the mNGS and BALF or sputum culture did. Except for the platelet-to-lymphocyte (PTL) ratio and erythrocyte sedimentation rate (ESR), there were several significant differences between the two groups, indicating that the patients in mNGS group were relatively severer. Defined daily dose (DDD) represented the strength of antibiotic application and the antibiotics use density, which was higher in the mNGS group ($P = 0.013$) indicating the use of advanced antibiotics was more frequent.

Comparison of Diagnostic Performance Between BALF mNGS and Culture

Within the mNGS group (both tested by mNGS and BALF culture), through BALF mNGS, 110 (64%) were detected positively with pathogens, and 20 (11%) were completely negative; 43 (25%) were detected with colonizing pathogens or contaminating pathogens, which we also considered as negative detection according to our experience. Furthermore, 92 (55%) were detected with bacteria, 30 (18%) with fungi, 15 (9%) with viruses, and 31 (18%) were detected with other special pathogens (**Figure 3A**). Conversely, BALF culture done at the same time with mNGS only showed 28% positive rate. Within this positive detection by BALF culture, only bacteria (91%) and fungi (9%) could be cultured, so the virus and other special pathogens could not be recognized by traditional cultures (**Figure 3B**). Thus, the sensitivity of mNGS was much higher than traditional microbiological culture. Within the mNGS group, we detected 24 cases of *Chlamydia psittaci* by mNGS, including eight female patients and 16 male patients, with the average age of 65 years old. In addition to *C. psittaci*, some of these patients were also infected with other pathogens, among which *Candida albicans* was the most (10/24). The reads of *C. psittaci* matched in these patients ranged from 16 to 270670. The relative abundance refers to the proportion of pathogen in the same type of microorganism, and the relative abundance of *Chlamydia* was: 0.1–97.9%. A higher relative abundance indicated a higher proportion of the species in the sample. Relative abundance was only a parameter that indicates the amount of pathogens, and it could not be directly judged whether it was pathogenic or not based on the value of relative abundance. Therefore, we did not compare the relative abundance of detected pathogens. In the process of clinical diagnosis, the value of relative abundance was only for reference. Sixteen out of 24 patients adjusted the use of antibiotics based on the reports of mNGS. The white blood cells and neutrophils of most patients did not increase significantly, but the increase of PCT and D-dimer and the decrease of blood calcium were related to the severity of the condition (**Supplementary Table 1**).

Comparison of Pathogens Detected by BALF mNGS and Culture

Among the 168 kinds of morbigenous microorganism, *C. psittaci* (24/168) was the most detected pathogen by mNGS, followed by *Hemophilus parainfluenzae* (19/168). We also detected virus (15/168), and other special pathogens (31/168).

TABLE 1 | Demographic and baseline characteristics of patients.

Characteristics	mNGS group (*n* = 173)	Control group (*n* = 173)	*P*
Age, mean (range), years	60 (15–87)	64 (18–90)	0.045
Sex, female, *n* (%)	47 (27.2)	67 (38.7)	0.030
Hospital, mean (range), days	16 (1–66)	11 (0–70)	0.001
Comorbidity, *n* (%)			
Cardiovascular disease	34 (20.0)	35 (20.2)	1.000
Hypertension	43 (24.9)	51 (29.5)	0.398
Chronic obstructive pulmonary disease	19 (11.0)	17 (9.8)	0.860
Neoplastic disease	17 (9.8)	25 (14.5)	0.249
Diabetes	27 (15.6)	30 (17.3)	0.772
Kidney disease	8 (4.6)	12 (6.9)	0.490
Bronchiectasis	16 (9.2)	16 (9.2)	1.000
Cerebrovascular disease	22 (12.7)	26 (15.0)	0.641
ARDS, *n* (%)	49 (28.3)	30 (17.3)	0.021
On empiric antibiotics at time of sample collection, *n* (%)	173 (100)	171 (98.8)	0.499
Apache II score, mean ± standard deviation	14.0 ± 5.4	12.2 ± 6.4	0.051
30-day mortality, *n* (%)	14 (8.1)	12 (6.9)	0.839

mNGS, metagenomic next generation sequencing; ARDS, acute respiratory distress syndrome.

TABLE 2 | Laboratory findings of patients.

	mNGS group (*n* = 173)	Control group (*n* = 185)	*P*
White blood cell count, x 10^9/L	9.41 (0.56–57.27)	8.44 (1.68-29.47)	0.035
Percentage of neutrophils, %	77.9 (9.8–97)	72.5 (38.5–98.4)	0.001
Lymphocyte count, x 10^9/L	1.07 (0.11–4.14)	1.28 (0.12–9.51)	0.005
NLR	11.18 (0.21–86.47)	9.05 (0.82–121.20)	0.001
PLR	282.82 (9.42–1925.00)	270.68 (6.00–1103.80)	0.803
Cre, umol/L	89 (30–855)	79 (26–527)	0.027
PCT, ng/mL	2.40 (0.01–75.57)	1.51 (0.01–70.62)	0.004
CRP, mg/L	82.65 (0.43–320.04)	50.14 (0.01–314.69)	0.001
ESR, mm/h	63.49 (2–120)	54.73 (2–120)	0.081
Ca^{2+}, mmol/L	2.04 (0.99–2.49)	2.15 (1.15–3.34)	0.001
Albumin, g/L	30.0 (16.5–44.7)	32.5 (17.1–47.2)	0.001
DDD	116 (36–255)	109 (0–300)	0.013

mNGS, metagenomic next generation sequencing; NLR, neutrophil-to-lymphocyte ratio; Cre, creatinine; PLR, platelet-to-lymphocyte ratio; DDD, defined daily dose; PCT, procalcitonin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.
Data are presented as means (range).

The positive rate of mNGS for pathogenic bacteria was almost twice than that of BALF culture (**Figure 4**). Except for *K. pneumoniae* and *Acinetobacter baumannii*, the positive rate for mNGS of other pathogens were relatively higher. Not only that, *Haemophilus parainfluenzae*, *Mycobacterium tuberculosis*, *Nocardia*, *Legionella*, *Streptococcus parasanguis*, and *Tropheryma whipplei* were only detected by mNGS, and the BALF cultures of these microorganism were all negative. The positive rate of mNGS for fungi was seven times than that of BALF culture, and *C. albicans* was one of the most detected fungi in BALF culture. For *Pneumocystis jirovecii*, *Aspergillus*, and *Cryptococcus*, the positive rate of mNGS was much higher than that of BALF culture. Viruses and some special pathogens can only be detected by mNGS. *Human herpesvirus 1*, *4,* and *5* were only considered pathogenic when the reads and abundance was relatively high. mNGS can detect rare pathogens such as *Orientia tsutsugamushi* and *Leptospira interrogans*, which was of great guiding significance for antibiotic adjustment. In addition, we also counted other pathogens in the mNGS report of 173 patients that we thought were not pathogenic (**Figure 5**). Among the non-pathogenic pathogens, *C. albicans* was the most common. But the *A. baumannii*, *K. pneumoniae*, etc. were also included which were "usually" considered to cause pneumonia. As there was no detailed uniform criteria or authoritative guide for the interpretation of mNGS reports, we always determined whether the pathogens were pathogenic, colonized, or contaminated based on clinical experience, patient's imaging findings and inflammation indicators.

The Influence of mNGS on Treatment and Prognosis

The correct use of antibiotics was extremely important for the treatment of CAP. Among the 116 patients with pathogen-positive pneumonia, 46 (40%) cases were completely covered by antibiotics before the pathogens were detected. These 46 cases were not adjusted for antibiotics after the pathogens were detected. Forty (34%) cases were partially covered by antibiotics before the pathogens were detected. After the pathogens were detected, 37 cases adjusted their antibiotics, and three cases did not adjust, of which one case were transferred to specialist hospitals for further treatment, and two cases died. Thirty (26%) cases were not covered by antibiotics at all, of which 25 cases were adjusted after the pathogens were detected, and the remaining five cases were transferred to other hospitals or death (**Figure 6**). All the pathogens detected by mNGS and culture, and the details of antibiotics therapy had been listed in **Supplementary Table 2**. As for pathogens were not detected or pathogens that were colonized and contaminated, we could only rely on laboratory test results, imaging findings, and empirical treatment to adjust antibiotics.

DISCUSSION

We retrospectively reviewed 346 CAP cases. Based on the patients' clinical characteristics and inflammatory indices, the patients in the mNGS group were relatively severer in our study, including a higher incidence of ARDS, WBC, PCT, neutrophils, ESR elevation, and peripheral blood calcium concentration decrease. We suggested that mNGS would be recommended to

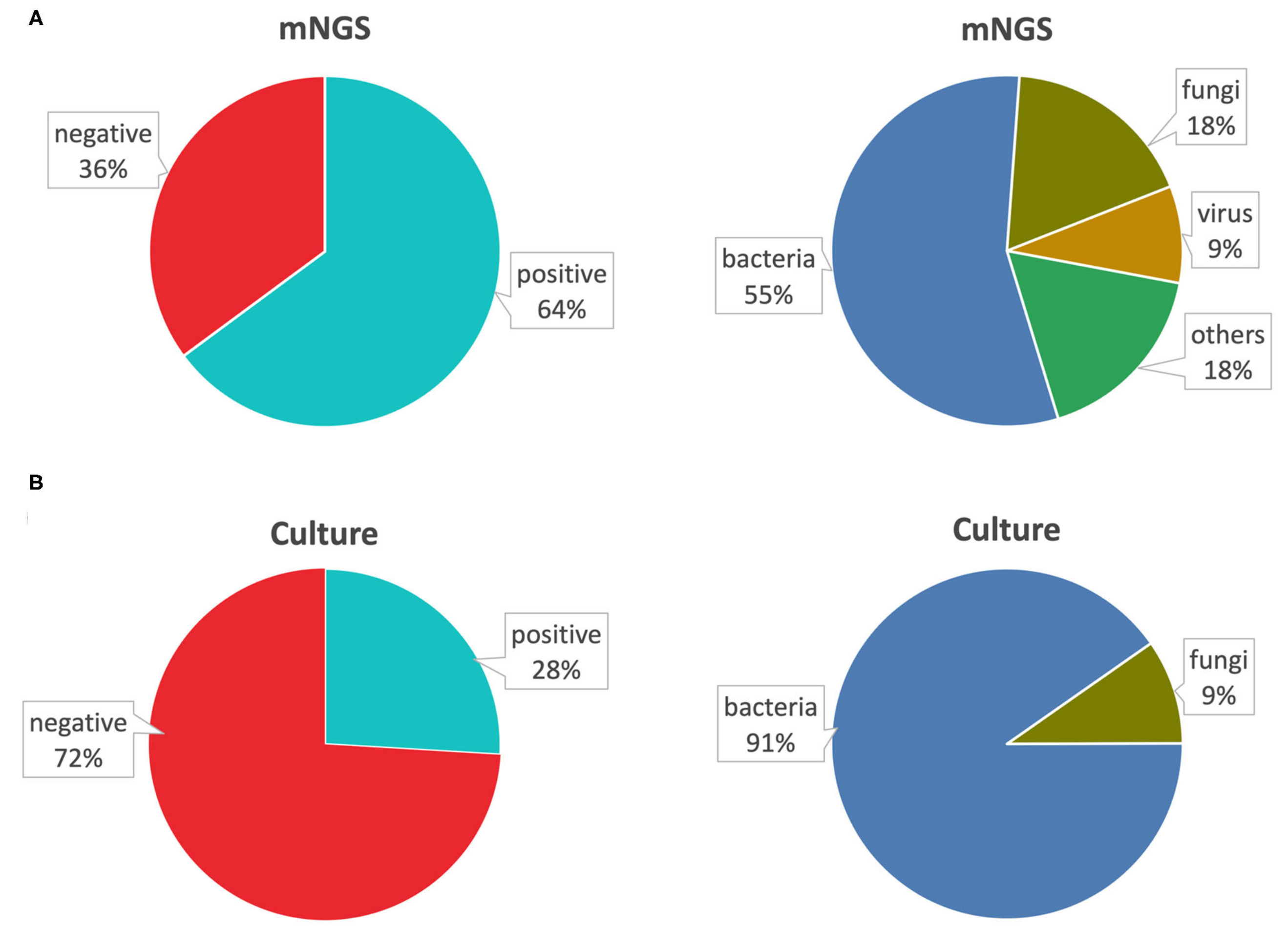

FIGURE 3 | Comparison of positive rate of pathogens by BALF mNGS and culture. **(A)** The positive rate of mNGS and the types of pathogens detected. **(B)** The positive rate of BALF culture and the types of pathogens detected. BALF, bronchoalveolar lavage fluid; mNGS, metagenomic next-generation sequencing.

patients with more complex and severer conditions to accurately identify the pathogens and timely adjust antibiotics. The DDD reflects the density of antibiotic application. The patients in the mNGS group treated more antibiotics because their conditions were more serious and complicated. However, we have also controlled it at a relatively low level which was benefited from the timely detection of pathogens by mNGS. The patients received timely targeted anti-infection treatment, and most of them had better prognosis. In addition, although patients in the mNGS group were with severer conditions, the 30-day mortality rate was basically the same as that in the control group, which indicated that the early diagnosis and promptly treatment of patients through mNGS could reduce the mortality. Moreover, mNGS can detect many pathogens that cannot be detected by traditional microbiological culture. During the incipient stage of the COVID-19 pandemic, mNGS supplied a quick and accurate identification for pathogenic virus (20).

In the past, we thought that *C. psittaci* was relatively rare. However, we detected 24 patients infected with *C. psittaci* through mNGS. Most of the cases were also accompanied by the infection of other pathogens (*C. albicans* was the top one), but many of the pathogens were the microbiota in oropharynx and colonized bacteria in respiratory tracts. Meanwhile, we found that after mNGS had been fully applied in our clinical practice from the year 2018, the incidence of *C. psittaci* pneumonia had been greatly increased, which meant that *C. psittaci* was widespread in the past, but it was difficult to be detected. Although miost patients infected with *C. psittaci* have severe pneumonia, clinicians can adjust the dosage and classes of antibiotics in time according to mNGS reports, and the overall outcome of the patients is better. Previous studies also confirmed that mNGS could improve the outcome of patients with severe pneumonia of *C. psittaci* and played a positive role in diagnosis and treatment of CAP, as well as adjustment of antibiotics (21).

Traditionally, the detection of tuberculosis could only be based on methods such as acid-fast staining of sputum smears, culture of *M. tuberculosis*, and interferon gamma release assay (IGRA), etc. These methods have disadvantages such as low sensitivity, low positive rate, time-consuming, and not direct enough. However, mNGS can detect as low as 1–2 reads of *M.*

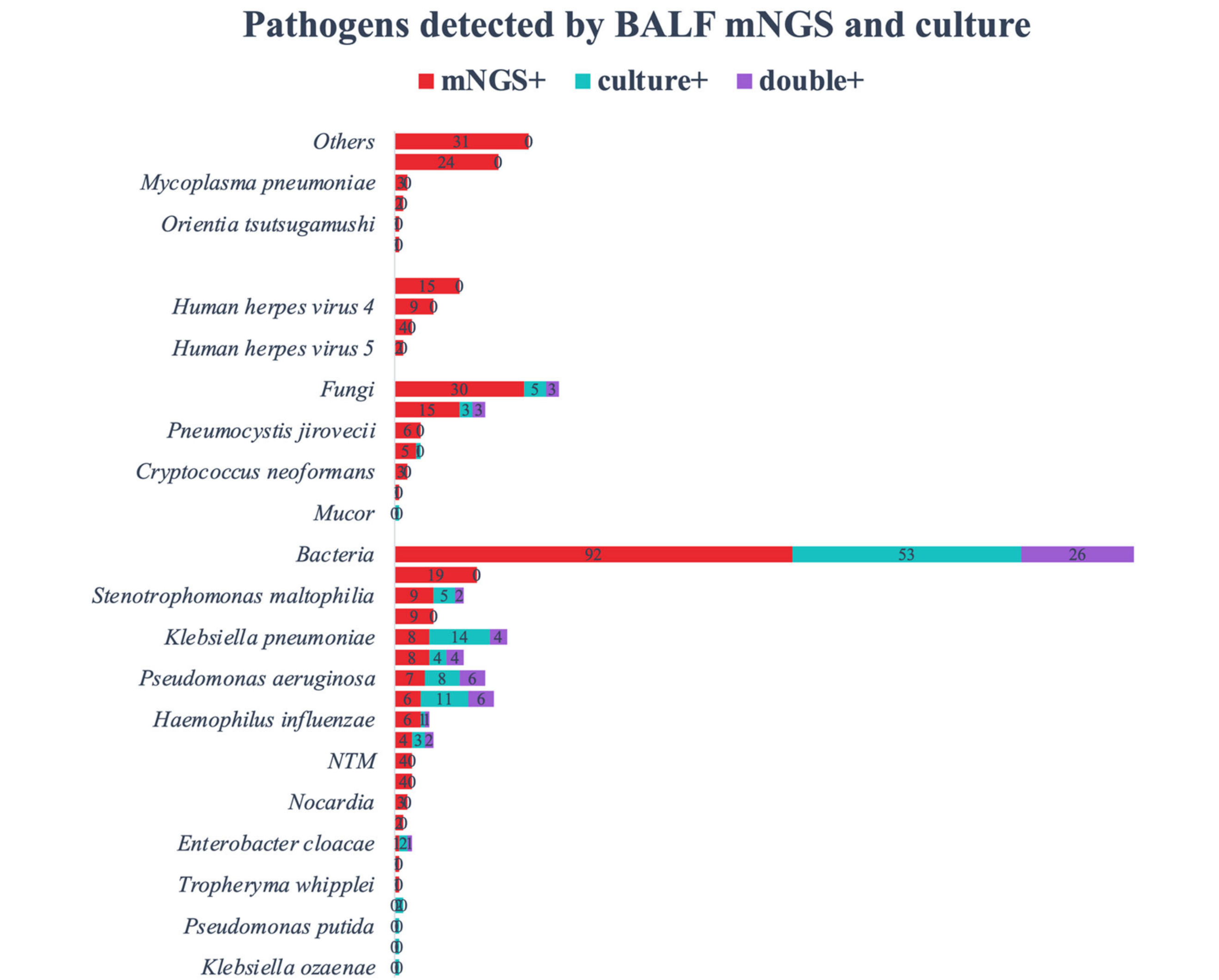

FIGURE 4 | The overlap of positivity between BALF mNGS and BALF culture. Bacteria were the most detected, followed by fungi, special pathogens, and viruses. The positive rate of BALF culture in detecting *Klebsiella pneumoniae* and *Acinetobacter baumannii* was higher than that of mNGS. *Chlamydia psittaci* was the most detected by mNGS, followed by *Haemophilus parainfluenzae* and *Candida albicans.* All viruses, special pathogens, MTB and NTM were only detected by mNGS, and the BALF culture of these pathogens were negative. BALF, bronchoalveolar lavage fluid; mNGS, metagenomic next generation sequencing; MTB, *Mycobacterium tuberculosis*; NTM, *nontuberculous mycobacteria*.

tuberculosis which provides an opportunity for patients to be transferred to specialist hospitals for tuberculosis therapy in time. In the past few decades, the detection of *O. tsutsugamushi* and *L. interrogans* could only rely on microscopic examination, but mNGS can currently detect these special pathogens sensitively, which provides a good guidance for clinicians to adjust treatment protocols. Among the 116 pathogen-positive cases, the positive rate of mNGS for bacteria and fungi were significantly higher than that of BALF culture, additionally mNGS can detect viruses and special pathogens sensitively.

Due to regional differences, the pathogens of our CAP cases were quite different from those in northern China. Chen et al. (22) reported that the bacteria such *as Citrobacter freundii*, *Salmonella enterica*, and *Aeromonas hydrophila* detected by mNGS are common pathogens in CAP. But those bacteria were not detected in any of our cases, which was possibly because the patients in Peking University People's Hospital was more complicated since the difficult and complicated patients around the country would like to go there for further treatment, so the pathogenic pathogens could have a multiple source.

Among the non-pathogenic pathogens detected by mNGS, *Human herpesvirus 4, 1,* and *5* were the most common viruses. These viruses can be latent in the host when the patient is immunocompetent (23). Most studies believed that they had no pathogenic significance when detected in BALF. But when the patient's immune function was low or suppressed, these viruses will be pathogenic (24). *C. albicans* was also detected relatively frequently, but pneumonia with *C. albicans* was relatively

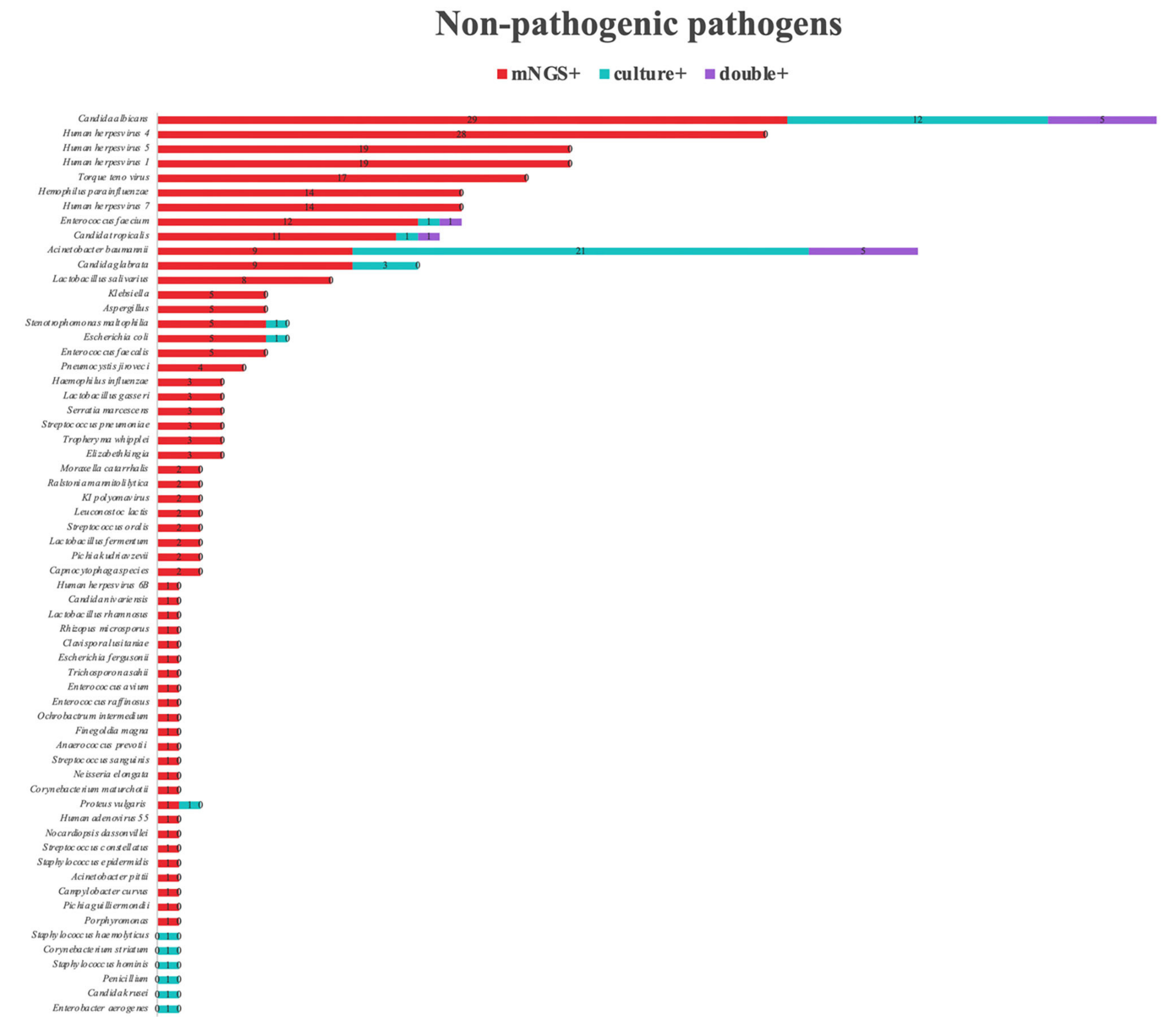

FIGURE 5 | Non-pathogenic pathogens detected by mNGS. A total of 64 different non-pathogenic pathogens were detected in the mNGS group. *Human herpesvirus 5*, *Candida albicans*, *Human herpesvirus 4* are the most common non-pathogenic pathogens detected by mNGS. *Acinetobacter baumannii*, *Candida albicans*, *Candida glabrata* are the most common non-pathogenic pathogens detected by sputum culture. mNGS, metagenomic next-generation sequencing.

rare (25), and most of it were hematogenous dissemination. Additionally, *Enterococcus faecium* and *Enterococcus faecalis* pneumonia were also very rare (26). When interpreting the mNGS reports, some clinicians thought that *C. albicans* was pathogenic. We believed that anti-*C. albicans* treatment must be determined based on the patient's imaging findings and inflammatory indicators. *Torque teno virus* is widely present in the human body, animals, air, and solid surfaces (27), which caused pneumonia only when patients were under immunosuppression condition (28). Therefore, the positive report of *Torque teno virus* may be sample contamination or in immunosuppression condition. When the reads of *H. parainfluenzae* are not high, it is necessary to comprehensively determine whether it is pathogenic based on clinical features, infection sites, inflammation indicators, and lung imaging findings. Some cases of *A. baumannii* and *K. pneumoniae* are in-hospital infection (29, 30). Patients with long-term use of antibiotics and longer hospital stays are prone to in-hospital infection. The impaired intestinal barrier, long-term use of acid inhibitors, long-term bed rest, and nasogastric reflux will cause bacterial translocation (31). The primary lesions of these patients are not in the lungs. Therefore, *A. baumannii* and *K. pneumoniae* detected in the BALF of these patients cannot be regarded as the pathogenic bacteria for pneumonia. The use of mNGS in patients with immunosuppression not only identifies pathogens, but also reflects the patient's immune status and microbiota

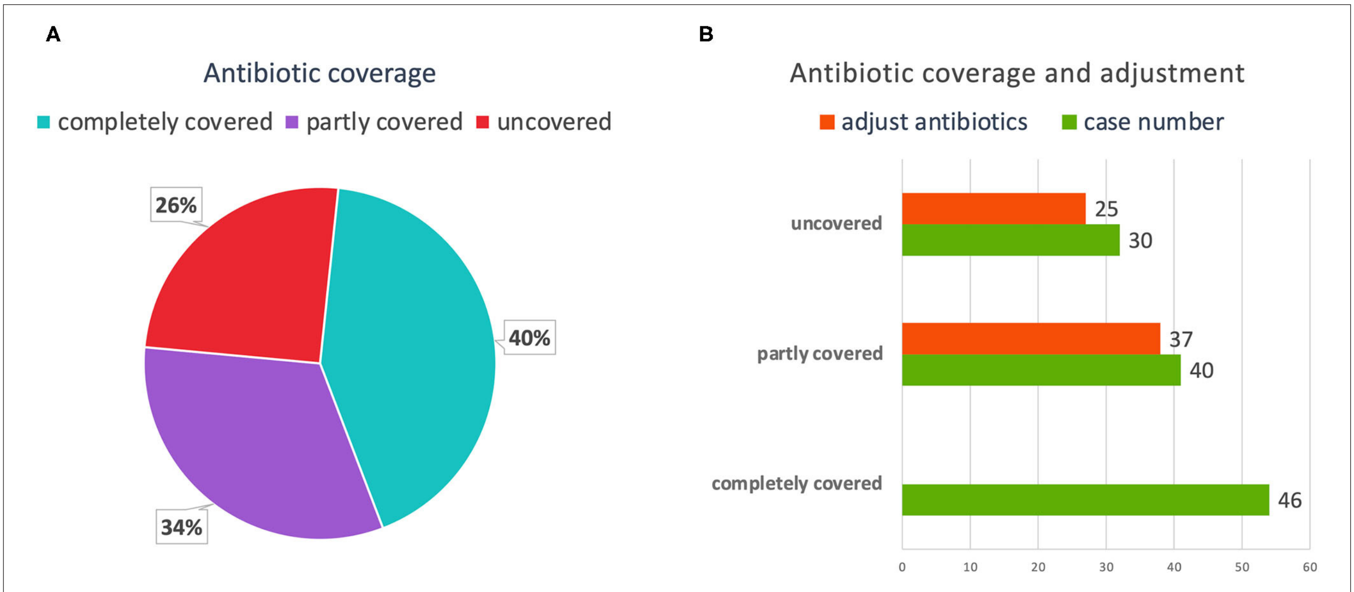

FIGURE 6 | The coverage and adjustment of antibiotics in the mNGS group. **(A)** Among patients in the mNGS group, complete antibiotic coverage was 54 (43%); partial coverage was 41 (32%); and no coverage was 32 (25%). **(B)** Antibiotics were adjusted for 38 partially covered patients, and for 27 uncovered patients. mNGS, metagenomic next-generation sequencing.

distribution. A small number of cases in which the detection was negative with mNGS, but the BALF culture was positive, which was possibly because: (1) the specimen may have been contaminated. For example, the positive *Aspergillus* BALF culture may be caused by contamination. (2) The low pathogens load in the specimen. Under suitable culture conditions, active micro bacteria or fungi could also be cultured, but micro pathogens could not reach the minimum threshold of mNGS detection. (3) Detection of some fungi requires breaking the cell wall to obtain DNA, but conventional sputum culture does not. Therefore, if this process was badly handled, negative results of mNGS may occur. Nevertheless, mNGS showed significant advantages for detecting fastidious bacteria.

Normal sputum culture takes 5–7 days. *M. tuberculosis* culture even takes about 6 weeks. But mNGS can detect pathogens within 48 h and even shorter, which greatly improves the timeliness of treatment. In the use of antibiotics, mNGS can be used to determine whether the current antibiotic therapy covered the pathogens and the reads of pathogens detected by mNGS will guide clinicians to adjust the dosage of antibiotics. Moxifloxacin was the common choice for the severe CAP regularly. However, after the *C. psittaci* was detected by mNGS, we adjusted the moxifloxacin to the doxycycline. Additionally, after the rare pathogens such as *L. interrogans* detected, adjusted to penicillin G was essential for the initial treatment stage. After the fungi detected by mNGS, we should take a comprehensive consideration between the specific reads and abundance of the pathogens to determine whether use the antifungal agents such as fluconazole or voriconazole.

However, there are still some disadvantages of mNGS. Due to the high sensitivity of mNGS, some colonized and contaminated pathogens will also be detected. Because of the differences in the ability of clinicians to interpret the mNGS reports, it may lead to the abuse of antibiotics. The mNGS performed during the early stage of hospitalization could clarify what the main pathogen is. After a period of hospitalization, patients with underlying diseases or immunocompromised patients may have in-hospital infections or colonization of some special bacteria, which will affect the interpretation of mNGS results. Besides, some clinicians are not strict enough on the indications for mNGS, which leads to a waste of medical resources. Otherwise, some laboratories will delete background bacteria, but occasionally the main pathogenic bacteria may be deleted. mNGS has some shortcomings, and it is not a routine pathogen detection method in the guideline. However, due to the large population and a vast extent of land, medical resources are not evenly distributed to a certain extent. The technology of pathogen detection in some remote areas is poor, and even the PCR technology for single pathogens is lacking. Therefore, it is necessary to use mNGS to detect pathogens in an appropriate and timely manner.

Therefore, we suggest that mNGS would be mainly used: (1) the infection is complicated and severe; (2) pathogens which are hard to detected by traditional microorganism test or culture, such as *M. tuberculosis* and *Cryptococcus*. We need to improve clinicians' ability of interpretation of mNGS reports to prevent the abuse of antibiotics caused by mNGS.

The limitation in our study is that this is a single-center retrospective study with a relatively small sample size, thus there was selection bias. Since we tended to compare the positive rate of mNGS and BALF culture, as well as the characteristics of the detected pathogens, the 173 control patients who only underwent BALF or sputum culture were not used for subsequent analysis of pathogen characteristics.

As the antibiotic coverage of pneumonia patients was close to 100%, the positive rate of BALF or sputum culture would be affected by antibiotics, possibly resulting in a relative higher positive rate of mNGS. All mNGS reports were interpreted by a senior clinician, so the consistency was ensured.

CONCLUSION

The positive rate of mNGS for pathogens in patients with CAP was higher than that of traditional BALF culture. mNGS can detect pathogens in a more timely and accurate manner and assist clinicians to adjust antibiotics in time. With available indications, we recommend mNGS for the complementary diagnosis of severe or complicated pneumonia.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the SRA repository, accession number PRJNA756706.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the 3rd Xiangya Hospital of Central South University (No. 21030). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

The study was conceived, designed, and supervised by CL and QZ. Statistical analyses were performed by YZ. Clinical data collection was done by HL. Sample collection was done by SS. Manuscript was written by HL and YZ. Figures and tables were drawn by GC. mNGS testing data was uploaded by FC. Manuscript editing was done by JW, HL, and YZ contributed equally to this study. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.807174/full#supplementary-material

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REVIEW
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Recent Advances in Fungal Infections: From Lung Ecology to Therapeutic Strategies With a Focus on *Aspergillus* spp.

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Fungal infections are estimated to be the main cause of death for more than 1.5 million people worldwide annually. However, fungal pathogenicity has been largely neglected. This is notably the case for pulmonary fungal infections, which are difficult to diagnose and to treat. We are currently facing a global emergence of antifungal resistance, which decreases the chances of survival for affected patients. New therapeutic approaches are therefore needed to face these life-threatening fungal infections. In this review, we will provide a general overview on respiratory fungal infections, with a focus on fungi of the genus *Aspergillus*. Next, the immunological and microbiological mechanisms of fungal pathogenesis will be discussed. The role of the respiratory mycobiota and its interactions with the bacterial microbiota on lung fungal infections will be presented from an ecological perspective. Finally, we will focus on existing and future innovative approaches for the treatment of respiratory fungal infections.

Keywords: chronic respiratory disease, microbiome, mycobiome, aspergillosis, live biotherapeutic products, disease management, environmental interference

RESPIRATORY FUNGAL INFECTIONS

Fungal pathogens are estimated to lead to more than 1.5 million deaths every year worldwide, with a global burden exceeding one billion (1). Despite this, the issue of fungal pathogenicity has been largely neglected (2, 3). Over the past two decades, the prevalence of invasive fungal diseases has increased considerably (1). This has also been acknowledged in the case of healthcare-associated invasive fungal infection (4, 5), for which a call to action was recently issued by the scientific community (6). Moreover, the increased prevalence of invasive fungal diseases correlates with an increasing number of vulnerable at-risk patients, which include among others, immunosuppressed individuals due to transplants, AIDS, cancer, corticosteroid therapies or autoimmune diseases, or patients undergoing major surgery (1, 7).

The most prevalent human fungal pathogens are the airborne opportunists *Aspergillus* spp., *Cryptococcus* spp., and *Pneumocystis* spp., as well as the human-associated commensal and polymorphic fungal species *Candida albicans* (7, 8). These fungi are responsible for more than 90%

of all reported fungal disease-related deaths (9). The latest estimates of the annual burden of fungal diseases amount to more than 14 million cases for all diseases within the pulmonary aspergillosis spectrum, over 200,000 cases of cryptococcal meningitis, 500,000 cases of *Pneumocystis jirovecii* pneumonia, 700,000 cases of invasive candidiasis, and over 10 million cases of asthma with fungal sensitisation (1, 9, 10).

In the current review, we will first look at the full spectrum of diseases caused by fungi of the genus *Aspergillus*, with a particular focus on the pathogenesis and the underlying immunological mechanisms. Then, lung ecology, and more specifically the interaction of the respiratory mycobiota (fungal composition) with the bacterial microbiota and the virome will be discussed in the context of fungal infection. Finally, we will discuss the current therapeutic approaches, as well as future perspectives in therapeutic strategies for the fight against pulmonary aspergillosis.

Pulmonary Aspergillosis: A Wide Spectrum of Diseases

Aspergillus spp. are the most frequently isolated filamentous fungi in humans and animals (11–13). These primarily saprotrophic fungi are widespread in the environment and can be found in soil and decaying biomass, especially in compost piles, where they participate in the degradation of organic matter (14–16). Their wide environmental distribution can be explained by the competitiveness and adaptability of the *Aspergillus* genus (17). Indeed, *Aspergillus* spp. are able to use multiple organic substrates and adapt to a broad range of environmental conditions (12). These fungi show a remarkable phenotypic plasticity in their ecology and stress-responses, which are believed to be at the basis of the success of *Aspergillus* spp. as opportunistic pathogens.

Fungi of the genus *Aspergillus* are associated with a large variety of clinical manifestations ranging from allergic reactions to life-threatening invasive infections. Such infections are generally caused by *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans*, and *Aspergillus terreus*, with *Aspergillus fumigatus* being responsible for 90% of the reported cases (12). Respiratory infections due to *Aspergillus* spp. are caused by inhalation of airborne conidia, i.e., asexual spores (14). These fungi produce large quantities of small airborne conidia with a size of 2–5 μm in diameter (15, 18), whose concentration can range from 1 to 100 per m^3 in air, but can reach up to 10^8 per m^3 in some environments (13). A human inhales approximately 100–1,000 conidia per day, which can reach the lung alveoli due to their small size (15). In immunocompetent individuals, inhaled conidia are usually efficiently cleared either by mucociliary movement or through phagocytosis by macrophages (**Figure 1**) (18). However, depending on the immunological status of the host, *Aspergillus* spp. can lead to a variety of pathologies (12).

Pulmonary aspergillosis is classified into three different groups with distinct clinical manifestations (11, 13, 19). The disease spectrum of pulmonary aspergillosis spans from hypersensitivity responses (asthma or allergic bronchopulmonary aspergillosis—ABPA), to colonization (i.e., presence of the fungus without any clinical, radiological or laboratory indications of active fungal disease), to infection (chronic or invasive aspergillosis). **Figure 2** presents a diagram showing the disease spectrum of pulmonary aspergillosis depending on the host status.

Hypersensitivity Responses

Although other fungi can cause allergic bronchopulmonary mycoses (ABPM), the vast majority of hypersensitivity responses are associated to *Aspergillus* spp. These can range from fungal asthma to allergic bronchopulmonary aspergillosis (ABPA), the latter being a complex type I, III, and IV hypersensitivity response observed notably in patients with cystic fibrosis (CF) or chronic asthma (13). Hypersensitivity to *Aspergillus* is characterized by high levels of *Aspergillus*-specific IgE (19). ABPA affects close to 5 million patients worldwide (1, 10).

Colonization

In immunocompetent hosts, *Aspergillus* spp. may colonize the lungs without any clinical manifestations (10). In a study from Soubani and colleagues where *Aspergillus* spp. were isolated from sputum samples of 66 elderly hospitalized patients, 92% cases were determined to be *Aspergillus* colonization and only 4.5% fulfilled the criteria of invasive aspergillosis (20). Appropriate diagnostics and close monitoring should be considered in order to discriminate simple colonization from invasive infection (10, 11, 19). In immunocompromised patients, prior fungal colonization in the lower respiratory airways is considered an important risk factor for the development of invasive aspergillosis (10).

Chronic Pulmonary Aspergillosis

Aspergillus spp. can also cause a chronic, non-invasive form of infection called chronic pulmonary aspergillosis (CPA). One form of CPA is aspergilloma, which is characterized by the proliferation of the fungus inside a pre-existing cavity, leading to the development of a fungus ball (21). Aspergilloma typically occurs in immunocompromised patients previously suffering from lung pathologies such as tuberculosis, lung abscess, cysts, or tumors (21). Another form is chronic cavitary pulmonary aspergillosis, also called chronic necrotizing aspergillosis or complex aspergilloma. This is an inflammatory form of the infection characterized by the production of serum IgG antibodies directed to *Aspergillus*, elevated acute-phase inflammation markers, and the absence of pulmonary or vascular invasion. CPA usually occurs in immunocompetent or mildly immunosuppressed patients. CPA, including aspergilloma, is estimated to affect more than 3 million people worldwide (1).

Invasive Pulmonary Aspergillosis

On the other side of the disease spectrum, invasive pulmonary aspergillosis (IPA) is the most severe and life-threatening form of *Aspergillus* infection occurring in immunosuppressed patients. IPA affects more than 300,000 patients annually and its mortality rate ranges from 30 to 80% (1, 10). IPA is characterized by the invasion of the lung tissue by *Aspergillus* hyphae, which can be followed by angioinvasion and dissemination to other organs in patients with prolonged neutropenia (22). Other at-risk patients include individuals who underwent hematopoietic

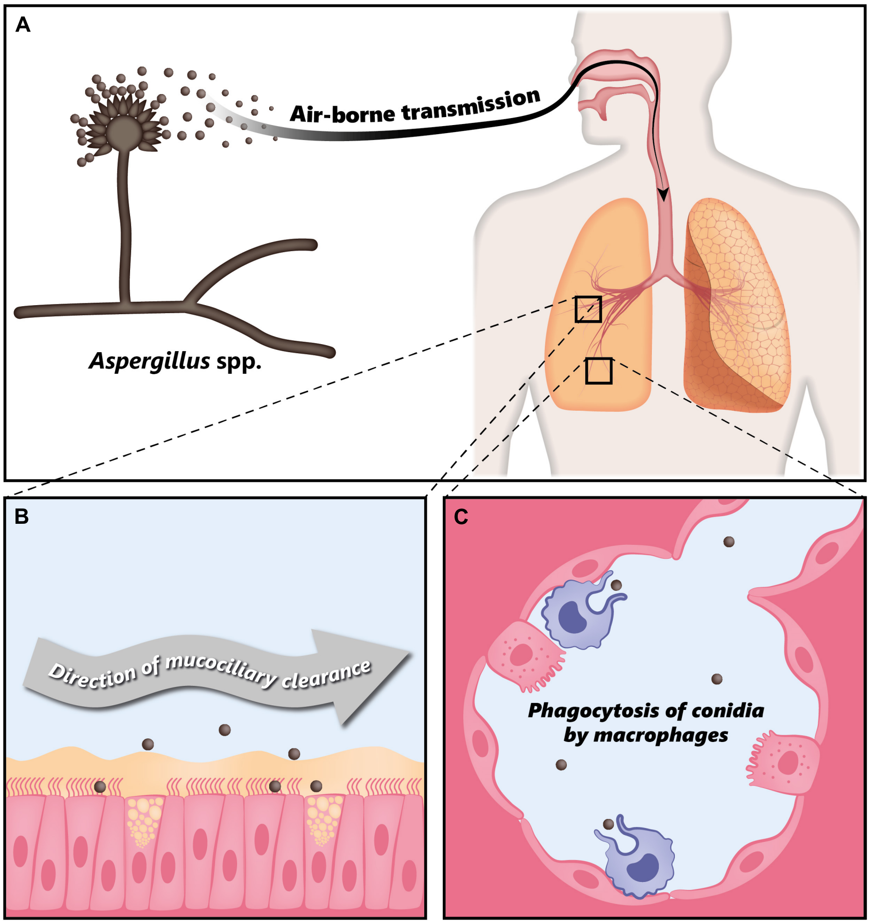

FIGURE 1 | Clearance of *Aspergillus* conidia in the immunocompetent host. **(A)** *Aspergillus* spp. conidia are transmitted through air and every individual inhales thousands of conidia every day. **(B)** In the immunocompetent host, most of the inhaled conidia are trapped by the mucus layer secreted by the tracheal and bronchial epithelium, and are efficiently eliminated through mucociliary clearance. **(C)** Due to their small size, conidia can eventually reach the alveoli, where they are phagocytosed by alveolar macrophages. Modified from Palmieri (170).

stem cell transplantation, solid-organ transplantation, prolonged corticosteroid therapy, or those who have AIDS (11, 13, 19). *Aspergillus* spp. are the most common opportunistic fungal pathogens causing invasive pulmonary aspergillosis in lung transplant recipients, with an incidence of 40.5 cases per 1,000 patients annually, despite the use of prophylactic antifungal treatments (23). Invasive aspergillosis most commonly occurs within 1 year after transplantation, with the majority of the cases reported within the first 6 months (23, 24).

Fungal Infections in Chronic Respiratory Diseases

In healthy individuals, innate immune responses and specifically macrophages and ciliated bronchial epithelial cells contribute to the efficient clearance of fungal conidia. In patients with chronic respiratory diseases, such as chronic obstructive pulmonary disease (COPD), asthma, and CF, these clearance mechanisms are impaired predisposing to fungal colonization and infection. In the following paragraphs, we will summarize the role of fungal pathogens in specific pulmonary diseases, focusing especially on *Aspergillus* spp.

Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease patients are often affected by exacerbations due to bacterial infections. The use of long-term inhaled corticosteroids and courses of oral steroids to treat exacerbations can predispose to fungal colonization and infection, as these treatments lead to impaired host immunity (25–27). In addition, in COPD, environmental fungal sensitization has been associated with frequent exacerbations (28). Bafadhel and colleagues reported that positive cultures for filamentous fungi are common in COPD, however, this finding was not related to exacerbations (26). Moreover, *A. fumigatus* sensitization was associated with poor lung function and, interestingly, patients with a positive *A. fumigatus* culture were on higher inhaled corticosteroid doses and had higher total and percentage sputum neutrophil counts (26). Recently, Tiew and colleagues evaluated the airway mycobiome in COPD patients in a multicenter study and observed that COPD patients

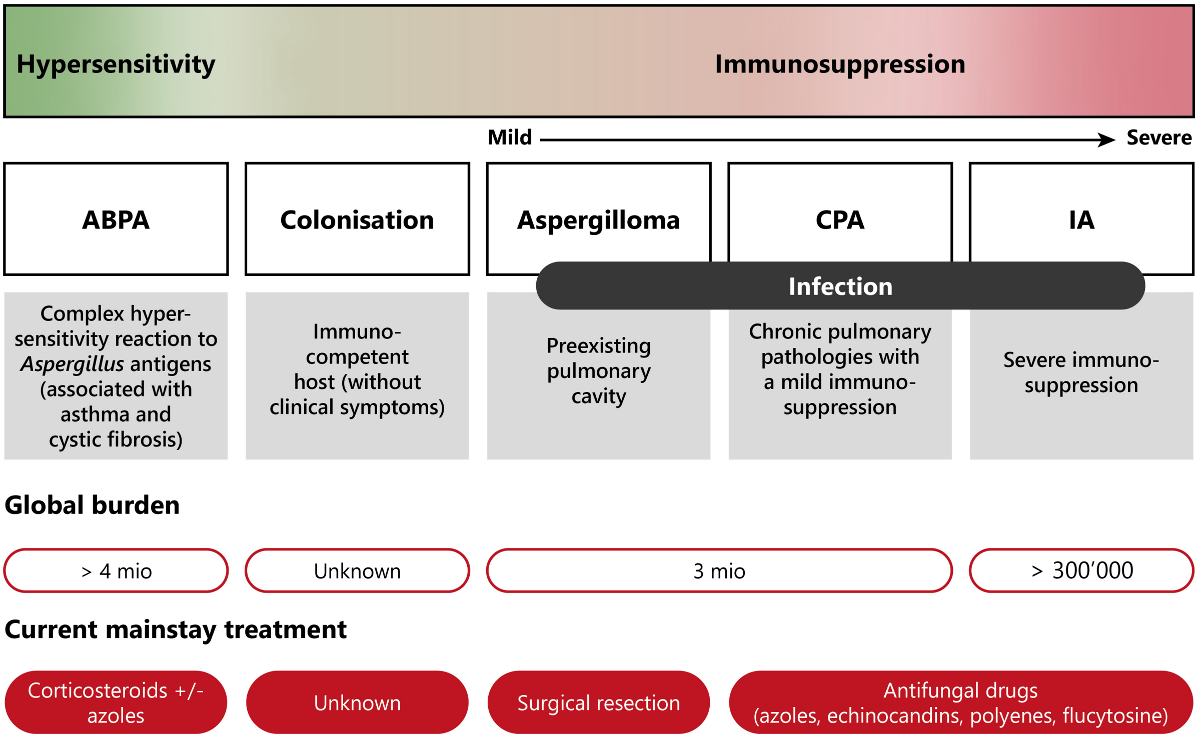

FIGURE 2 | Disease spectrum of pulmonary aspergillosis. This diagram summarizes the diverse pathologies caused by *Aspergillus* spp., due to improper elimination of conidia by mucociliary clearance or macrophage phagocytosis following inhalation. Depending on the immune status of the host, pulmonary aspergillosis can range from allergic reaction (hypersensitivity) to life-threatening invasive infection (severe immunosuppression). Moreover, current burden and treatment information are indicated. ABPA, allergic bronchopulmonary aspergillosis; CPA, chronic pulmonary aspergillosis; IA, invasive aspergillosis. Modified from Palmieri (170).

with very frequent exacerbations (≥3 per year) had an increased number of fungal interactions (29), which is suggestive of a more complex mycobiome. Using unsupervised hierarchical clustering of the COPD mycobiome, the authors reported two distinct patient clusters with variable clinical outcomes: the first cluster was characterized by *Saccharomyces* and increased symptoms, whereas the second cluster was characterized by *Aspergillus*, *Curvularia*, and *Penicillium* and demonstrated poorer clinical outcomes with increased exacerbations and higher mortality (29).

Innate immune cells and macrophages in particular play an important role in the first line of pulmonary host defense. In COPD patients, it has been demonstrated that alveolar macrophages exhibit reduced phagocytic capacity (30, 31). Monocyte-derived macrophages from both smokers and COPD patients were shown to be defective in their phagocytic and pro-inflammatory cytokine responses following *A. fumigatus* exposure (32). This impairment in macrophage function may consequently contribute to fungal germination, dissemination and infection, and lung damage in COPD patients. There is an increasing interest in understanding the direct influence of fungal colonization and infection on COPD pathogenesis and exacerbations. However, future research should continue to consider the indirect effects of bacterial composition alterations on fungal community composition in the lung through inter-kingdom interactions, and the potential consequences for COPD patients.

All diseases within the aspergillosis spectrum can be found in COPD patients although their prevalence differ. Tiew and colleagues have recently reviewed this topic in depth (33).

Asthma

Genetic and environmental factors drive asthma development, progression and risk for exacerbation. Changes in fungal community composition in the gut are associated with susceptibility to develop asthma in humans (34, 35), however, causality has not been established yet. Analysis of the airway mycobiome in asthma patients (fungal-sensitized and non-fungal sensitized) and healthy controls indicated that both the sputum and bronchoalveolar lavage (BAL) mycobiome was dominated by three species: *A. fumigatus, C. albicans*, and *Mycosphaerella tassiana*, irrespective of health status (36). Interestingly, other fungi such as *Aspergillus tubingensis,* a member of the *A. niger* species complex, was also prominent in the BAL fluid. Alterations in the balance of fungi detected in the lung were found to be associated with several disease markers, including asthma status and duration, and inflammatory biomarkers.

Fungi can play an important role in asthma development as fungal colonization and sensitization often take place in early life. Moreover, fungi are predominant triggers of asthma exacerbations. Environmental presence of fungi, such as *A. alternata,* in house dust has been associated with active asthma symptoms (37). In addition, a meta-analysis of seven studies revealed that indoor presence of *Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium* was associated with enhanced asthma exacerbations in both children and adults (38). Descriptive and mechanistic studies have started to reveal the influence of alterations in the gut bacterial and fungal composition on asthma development (discussed in section "Gut-Lung Axis"). However, the influence of a change in the composition of the pulmonary bacterial community due to asthma, on the susceptibility to develop a respiratory fungal infection, and subsequent asthma exacerbation, is currently unexplored.

Cystic Fibrosis

Cystic fibrosis is a rare autosomal recessive disorder that causes severe damage to the lungs, digestive system and other organs. The microenvironment in the lungs of CF patients, which is characterized by depletion of the airway surface liquid layer leading to impaired mucociliary clearance, is ideal for microbial colonization (39). Bacterial pathogens, and most commonly *Pseudomonas aeruginosa,* are known contributors to disease progression and exacerbations (40). Although fungi are often isolated from the lower airways of CF patients, the clinical impact of their presence and especially for the development of non-allergic fungal disease is poorly understood (41, 42). The most frequently detected fungi in the airways of CF patients are *Aspergillus* spp., notably *A. fumigatus*, and *Candida* spp. (43). The prevalence of *Aspergillus* colonization was highest in adolescents and young adults (44). Risk factors for *Aspergillus* colonization in people with CF include age and the use of inhaled corticosteroids and antibiotics (44). Moreover, aggressive use of antibiotics have been suggested to contribute to the increase in fungal colonization (45). This suggests that inter-kingdom interactions may be important in containing fungal colonization in the respiratory tract.

Interestingly, *A. fumigatus* colonization in people with CF is often preceded by *P. aeruginosa* infection (46). In patients infected with both *A. fumigatus* and *P. aeruginosa,* more severe clinical outcomes have been observed when compared to those infected with *P aeruginosa* alone (47). *P. aeruginosa* can have both antifungal and growth stimulating effects on *A. fumigatus* resulting in: (1) inhibition, (2) reciprocal antagonism, and (3) cooperation (48), which we will briefly describe here.

The main mechanisms by which *P. aeruginosa* can inhibit *A. fumigatus*' growth is via the release of phenazines, including pyocyanin, phenazine-1-carboxamide, 1-hydroxyphenazine, and phenazine-1-carboxylic acid. Phenazines are small diffusible quorum-sensing molecules, which easily penetrate *A. fumigatus* conidia, and are considered a strong virulence factor of *P. aeruginosa* (49–51). The quorum-sensing system allows bacteria to assess cell density and to regulate physiological activities accordingly, which consequently modulate the pathogenicity of the microorganisms (48, 52). Moreover, nutrient availability and competition is also involved in this inhibitory process. As an example, *P. aeruginosa* and *A. fumigatus* can both compete for the utilization of iron as a central nutrient for their survival (53). Although *P. aeruginosa* has a variety of fungicidal mechanism, alterations in these fungicidal capacities have been observed in clinical isolates from CF patients (52, 54), indicating that there can be shifts between inhibition and cooperation. Several cooperative interactions have been described which stimulate growth and potentially contribute to disease progression. Phenazines can stimulate fungal growth via increasing iron bioavailability (50). Moreover, pyochelin, a siderophore, can be used by *A. fumigatus* as a ferrochelator. Interestingly, dimethyl sulfide, a volatile organic compound released by *P. aeruginosa*, can communicate with *A. fumigatus* and create a positive growth environment resulting in stimulation of fungal growth (48, 55).

The Climax-Attack model (CAM) is a theory which has been proposed for CF a few years ago grounded on basic ecological principles (56). In this theory it is postulated that there are two major functional communities in CF pulmonary disease. The attack community consists of transient viral and microbial populations that induce strong innate immune responses. Consequent alterations in the immune response create a microenvironment that facilitates a climax chronic community having a reduced growth rate and being inherently resistant to antibiotic therapy (56, 57). Soret and colleagues provided important information on the involvement of the mycobiome in the CAM model in CF pulmonary exacerbations. They inferred an inter-kingdom network by plotting bacterial genera significantly correlated with at least one fungal genus and vice versa. Network analysis revealed three main clusters organized around *Aspergillus*, *Candida* and *Scedosporium* genera (57). The positively correlated OTUs predicted interactions of these three fungal genera with bacteria belonging to *Capnocytophaga*, *Parvimonas*, *Streptococcus*, or *Veillonella*. In addition, these interactions were assessed using *in vitro* co-cultures between *A. fumigatus* and *Streptococcus mitis* or *Streptococcus oralis* and confirmed that both *S. mitis* and *S. oralis* enhanced *A. fumigatus* growth. Such translational studies in which principles from ecology are used to understand disease can potentially form the basis for the future development of therapeutic strategies to combat exacerbations.

SARS-CoV-2 Co-infection

The current SARS-CoV-2 coronavirus pandemic created the perfect arena for the establishment of opportunistic fungal co-infections. The use of high dose systemic glucocorticoids, which are widely used as an anti-inflammatory medication for COVID-19 (58–60), together with epithelial cell damage in the lung following SARS-CoV-2 infection, expose patients to opportunistic fungal infections, such as COVID-19 associated invasive pulmonary aspergillosis (CAPA) (61). To date, over 100 cases of CAPA have been reported (61). Moreover, invasive mucormycosis, also known as "black fungus," has been largely reported in convalescent COVID-19, particularly in India and other Asian countries, in patients with uncontrolled diabetes mellitus or immunosuppression (62). The main reason for the increase in invasive mucormycosis cases has been pointed out to be the elevated iron levels in the serum of convalescent COVID-19 patients (62). Complementary to dexamethasone, Tocilizumab, an IL-6 receptor antagonist, has been widely used to treat COVID-19 in critically ill patients (63–65). IL-6 plays a critical role not only in the cytokine storm in severe COVID-19

(63, 65), but also in the innate immunity against fungal pathogens such as *A. fumigatus* (66). Accordingly, IL-6 inhibition has been associated with an increase in secondary infections in COVID-19 patients (67). A recent case report described an invasive *Aspergillus* infection in a COVID-19 patient following treatment with tocilizumab (65). Moreover, IL-6 inhibiting drugs may predispose COVID-19 patients to invasive mucormycosis (62).

MECHANISMS OF FUNGAL INFECTIONS: FROM INNATE IMMUNE RESPONSE TO pH MODULATION

Innate Immunity Against *Aspergillus* spp.

Despite constant exposure to *A. fumigatus* conidia, most people do not develop fungal disease. This suggests an efficient clearance of the conidia by the innate immune system in immunocompetent individuals before the adaptive immune system is activated (18). Upon inhalation, most resting conidia arriving in the respiratory tract are deposited against the airway fluid, due to turbulent airflow caused by the branching pattern of the respiratory tract (18). The trapped conidia are then removed by the ciliary action of the respiratory epithelium, which is the first line of defense in the lung (18, 68).

Host Recognition of Fungal Pathogen-Associated Molecular Patterns

Due to their small size, some of the inhaled conidia can reach the respiratory alveoli. After 4–5 h, resting conidia become swollen, and if not cleared, germinate and form hyphae within 12–15 h after arrival into the lungs (18). The maturation of conidia triggers a morphological change leading to the loss of the thin hydrophobic RodA protein layer, thus exposing the immunogenic components of the inner cell wall (69–71). These cell wall pathogen-associated molecular patterns (PAMPs) include polysaccharides such as β-D-glucan, mannan, chitin, and galactomannan, all of which are recognized by different pattern recognition receptors (PRRs) (15, 72). *A. fumig*atus conidia and hyphae can be recognized by the host via both soluble (pentraxins, complement proteins, and pulmonary collectins) and cell-associated microbial PRRs [Toll-like receptors (TLRs) and C-type Lectin receptors (CLRs)] (73, 74).

Pentraxins, such as pentraxin-3 (PTX3), are secreted by various cells, including neutrophils, dendritic cells, mononuclear phagocytes, and pulmonary epithelial cells (75). They bind to galactomannan on *A. fumig*atus conidia and facilitate recognition by phagocytes such as alveolar macrophages (76, 77). In addition, pulmonary collectins include lung surfactant proteins A and D and serve as opsonins. They bind to *A. fumig*atus conidial carbohydrate structures in a calcium-dependent manner. Surfactant proteins A and D have also been shown to promote the agglutination of conidia and their binding to neutrophils and alveolar macrophages, and improve the phagocytosis and killing of conidia by neutrophils (78).

Toll-like receptors recognition of pathogens triggers a signaling cascade leading to the activation of transcriptional factors such as NF-κB, which controls the expression of pro- and anti-inflammatory cytokines and chemokines (79). The universal adaptor molecule MyD88 has been shown to play a significant role in the signaling of TLRs, which induce the production of various inflammatory cytokines and reactive oxygen species (79). TLR2 and TLR4 have been implicated in the recognition of *A. fumig*atus conidia and hyphae (80). However, available data concerning their roles in *A. fumig*atus-associated immunity are conflicting. Indeed, the *A. fumigatus*-associated PAMPs for TLR2 and TLR4 remain undetermined. TLR9 has also been shown to play a role in innate immunity against *A. fumigatus* by recognizing fungal unmethylated CpG DNA (81). Dectin-1 is a CLR primary receptor that recognizes fungal β-glucan and that is essential for the mediation of the proinflammatory response (80), and is widely expressed on innate immune cells including macrophages, dendritic cells, and neutrophils (82–84). Dectin-1 can also induce the expression of the anti-inflammatory cytokine IL-10, indicating its dual role in modulating the inflammatory response (85). Dectin-2, another type of CLR, has recently been shown to be implicated in the innate immune response against *A. fumig*atus. Macrophages and dendritic cells express Dectin-2 and recognizes α-mannan in the fungal cell wall's outer layer. Accordingly, in response to *A. fumigatus* infection, alveolar macrophages upregulate Dectin-2. Moreover, Dectin-2 was shown to mediate an NF-κB-dependent proinflammatory response against swollen conidia (80). Finally, DC-SIGN is expressed at the surface of dendritic cells and some macrophages, and binds to *Aspergillus* conidia via the recognition of fungal galactomannan (18). Genetic polymorphism in the above-mentioned PRRs, as well as in cytokines, chemokines, and immune receptors genes, has been associated with an increased susceptibility to pulmonary aspergillosis (86–88). For instance, the Dectin-1 variant Y238X has been shown to impair the production of several cytokines such as IFN-γ and IL-10 by human peripheral mononuclear cells, leading to an increased susceptibility to invasive aspergillosis in patients receiving hematopoietic stem cell transplantation (HSCT) (89). Furthermore, the Asp299Gly polymorphism in TLR-4 is highly associated with chronic cavitary pulmonary aspergillosis (86).

Cellular Immune Responses

Clearance of Aspergillus Conidia by Innate Immune Cells and Epithelial Cells

Alveolar macrophages (AMs), neutrophils and epithelial cells constitute the first line of defense against inhaled *A. fumigatus* conidia (18). Alveolar macrophages phagocytose and kill conidia either via oxidative mechanisms through the generation of reactive oxygen species (ROS), or by non-oxidative mechanisms through phagosomal acidification (80). Corticosteroids have been shown to impair the capacity of AMs to kill conidia (18). Neutrophils were initially thought to kill hyphae exclusively, however, they have also been essential in killing germinating conidia. Neutrophils bind and phagocytose swollen conidia to trigger respiratory burst and degranulation. While the size of the hyphae prevents phagocytosis, direct contact with neutrophils can induce oxidative and non-oxidative mechanisms to damage the hyphae (18). Moreover, respiratory epithelial cells, i.e., bronchial and alveolar epithelial cells, as well as endothelial cells, have been shown to participate actively in the innate immune response against *Aspergillus fumigatus* strains Af293 and CEA10 by phagocytosing and killing conidia *in vitro* (18, 90–94). Furthermore, human peripheral blood monocytes have also been shown to internalize *Aspergillus* conidia and inhibit their germination and hyphal growth (95). Interestingly, both classical ($CD14^+CD16^-$) and non-classical monocytes ($CD14^+CD16^+$) were found to successfully internalize conidia (96). However, only classical monocytes were able to inhibit *Aspergillus* hyphal development (96). Lastly, dendritic cells (DCs) also have well-documented roles in the defense against *A. fumigatus*. Immature DCs (iDCs) have been shown to phagocytose opsonized and non-opsonized conidia and hyphae, both of which are recognized through PRRs such as Dectin-1, among others. TNF-α, IL-6, IL-12, IL-1α, and IL-1β are the central proinflammatory cytokines produced by iDCs upon recognition of *A. fumigatus* conidia and hyphae (84, 97, 98).

Innate Lymphoid Cells and Innate-Like T Cells

Innate Lymphoid Cells (ILCs), γδ T cells, and mucosal associated invariant T cells (MAIT cells) have also been reported to have important roles in the innate immune response against *Aspergillus* spp. ILCs, and particularly type 3 ILCs (ILC3s), are commonly found in barrier epithelial surfaces, where they contribute to the maintenance of mucosal homeostasis, elimination of pathogens, regulation of the inflammatory response, as well as tissue remodeling (99). γδ T cells are innate-like T cells displaying features of both the innate and adaptive immune system. They are mainly involved in the immune surveillance and defense against pathogens in various peripheral tissues, including the lung (100). γδ T cells have been reported to produce IL-17A following challenge with *A. fumigatus* conidia (101). Moreover, mice lacking γδ T cells were more susceptible to *A. fumigatus* infection (102). Together with ILC3s, γδ T cells were shown to produce IL-22, which is critical for an efficient clearance of *A. fumigatus* (101, 103). Furthermore, deficiency in IL-22 production resulted in an impaired production of cytokines and chemokines and an impaired clearance of *A. fumigatus* in the murine lung (101, 103). MAIT cells are a subset of CD8+ unconventional T cells which are abundant in mucosal surfaces such as lung (100), and constitute up to 10% of the total T cells present in the peripheral blood (104). Upon activation, MAIT cells release proinflammatory cytokines such as IFNγ, TNFα, IL-17, and IL-22, and are able to kill pathogens through the production and release of cytotoxic compounds such as perforin and granzymes (100, 104). MAIT cells have been shown to be activated by T cell receptor (TCR)-dependent mechanisms through direct contact with antigen presenting cells such as dendritic cells in the case of bacteria and yeasts (105, 106), and TCR-independent mechanisms via cytokines in the case of viruses (107, 108). However, the activation of MAIT cells by filamentous fungal pathogens such *A. fumigatus* is not yet well-understood (104). Jahreis and colleagues showed that a fast TCR-dependent response was elicited by MAIT cells against several *Aspergillus* species, including *A. fumigatus*, *A. terreus*, and *A. flavus* (104). This response is characterized by the upregulation of activation markers, such as the CD69 antigen, and the release of cytotoxic compounds such as granzyme A and perforin (104). Finally, Natural Killer (NK) cells have also been shown to have a role in the innate immune defense against *A. fumigatus* (18).

Adaptive Immunity

Following a fungal encounter and the initial activation of the innate immune system, adaptive immunity is rapidly organized to clear the pathogen efficiently. Indeed, three different CD4+ T-helper cell lineages have been shown to play crucial roles in pulmonary aspergillosis: Th1, Th2, and Th17 (109–111). Th1 cells response is associated with protective immunity through the secretion of the pro-inflammatory cytokines TNFα and IFNγ, which promote antifungal activity of macrophages and neutrophils at the site of infection (110, 112, 113). Interestingly, Th1 cells have been shown to induce a fungus-specific Th1 immunity to an epitope of the *A fumigatus* cell wall glucanase Crf1. This antigen can be presented by DCs through three common major histocompatibility complex (MHC) class II alleles, which induces memory Th1 cells that are cross-reactive to *C. albicans* (114).

Alternatively, Th2 cells response is rather associated with non-protective immunity through the activation of M2 macrophages and decrease of Th1 response (110, 113). Th2 responses are predominant in patients suffering from ABPA, and are characterized by a decrease in IFNγ and an increase in IL-4 and IL-10 production, which in turn promotes humoral responses, through IgE production, and allergy (112, 113). Interestingly, fungal PAMPs have been shown to act as adjuvants enhancing T cell responses (115–117). This is notably the case of chitin, which is present in the fungal cell wall, in allergic disorders such as asthma (118–122). Dubey and colleagues showed that mice pre-treated with chitin before being challenged with *A. fumigatus* extract had high IgE levels (123).

The role of Th17 cell response is less clear. On the one hand, Zelante and colleagues showed that production of IL-17 and IL-23 by Th17 is not protective in a murine *A. fumigatus* infection model and inhibits Th1 cells development and antifungal activity of neutrophils *in vitro* (124). This was confirmed by blocking IL-17 and IL-23 production which showed increased clearance of *A. fumigatus*. This protective effect of IL-17 and IL-23 has been confirmed in an acute aspergillosis murine model, where mice were sensitized with *A. fumigatus* (125). The IL-17 and IL-23 -producing cells were identified by the authors as eosinophils (125). On the other hand, Jolink and collaborators showed that IL-17 provides a protective immunity by decreasing lung fungal burden in a murine infection model (126). Moreover, a cross-reactive Th17 response to *C. albicans* and *A. fumigatus* has also been described during acute ABPA in humans (127). This strong cross-reactive response is suggested to be rather induced by *C. albicans*-specific Th17 cells upon encounter with *A. fumigatus*, than by naïve T cells (127).

Lastly, regulatory T (Treg) cells have also been shown to have a protective effect in the immune response in aspergillosis. Treg cells have been shown to regulate the inflammatory response caused by a strong Th1 response in the early phase of *A. fumigatus* infection, as well as in the case of allergic reaction due to Th2 responses (113, 128, 129).

There is evidence of oligoclonal expansion of T cells upon exposure to *A. fumigatus*. For instance, stimulation with the allergen Asp f 1 induced an oligoclonal expansion of antigen-specific T cells directed to this antigen in ABPA and non-ABPA patients (130). Furthermore, the p41 epitope of the *A. fumigatus* extracellular cell wall glucanase Crf1 has also been shown to induce an antigen-specific oligoclonal T cell response in HSCT patients (114).

Virulence Factors and Immune Evasion in *Aspergillus fumigatus*

Aspergillus fumigatus has evolved several virulence factors to escape innate immune responses. These virulence factors include the rodlet layer, DHN-melanin, ROS detoxifying enzymes, and toxins. Resting conidia are surrounded by a rodlet layer composed of hydrophobic RodA proteins (18, 80, 131, 132). This protein coat masks cell wall β-1,3-glucans, and thus prevents the detection of conidia by the innate immune response (69–71). Moreover, the rodlet layer was found to participate in the adherence of conidia to the pulmonary epithelium (131). DHN-melanin is the major melanin pigment that gives a gray-green color to *A. fumigatus* (133). It protects the integrity of the genome in conidia from ultraviolet light, as well as ROS (131, 134). It also masks fungal PAMPs, similar to the rodlet layer, and permits the fungus to evade phagocytosis by interfering with the acidification of the phagolysosome notably by interfering with intracellular Ca^{2+} signaling (135, 136). Moreover, *A. fumigatus* possesses a number of different enzymes for detoxifying ROS produced by host phagocytic cells such as macrophages. These enzymes include catalases, superoxide dismutase, glutathione transferases, fatty acid oxygenases, and efflux pumps, all of them either detoxifying H_2O_2 or superoxides, or expulsing ROS extracellularly (131, 134). Finally, *A. fumigatus* secretes several toxins considered as secondary metabolites, that further enable it to evade the immune response. These toxins are virulence factors crucial for *A. fumigatus* pathogenesis. For instance, gliotoxin has been shown to have several immunosuppressive effects, including inhibition of phagocytosis and neutrophil-derived ROS production, as well as proapoptotic activities (137, 138). Moreover, gliotoxin decreases ciliary movement and angiogenesis (131, 134, 139). All these immunosuppressive effects are thought to result from inhibition of the NF-κB signaling pathway (140), which as mentioned above is a key mediator of inflammatory responses (141).

pH Modulation as a Strategy to Colonize the Host Tissues

Environmental pH is an extremely important factor influencing not only fungal growth and development, but also fungal physiology (142, 143). Indeed, pH modulation has been shown to affect fungal enzyme activity (144), and to be a crucial element controlling fungal pathogenicity. Fungal infections are often accompanied by a shift in pH in the surrounding host tissue (142), through the secretion of either acids or alkali (143). The specific roles of acidification and alkalinization in fungal pathogenesis discussed in this review are summarized in **Figure 3**.

Interestingly, fungi, including *Aspergillus* spp. *and Candida* spp. are known to produce low molecular weight organic acids (LMWOAs) such as oxalate, citrate, malate, formate, acetate, and succinate, which contribute to pH modulation (145, 146). Pathogenic fungi acidify their environment in order to enhance the activity of enzymes, as well as to damage the host tissues (143, 147). For instance, in the case of *C. albicans,* acidification through acetate excretion has been shown to allow the production of aspartyl proteases (143, 148–150), which are major virulence factors in this pathogen (151).

Oxalic acid is a known pathogenicity factor for the phytopathogenic fungi *Sclerotinia sclerotiorum* and *Botrytis cinerea* (152, 153). This acid is secreted in the host tissues and accumulates in the form of oxalate, leading to a pH decrease. Additionally, as oxalate is a strong chelator of divalent metallic cations, it can sequester calcium ions, with multiple possible structural and physiological consequences for the host (146). In the case of plant pathogens, the formation of calcium oxalate (CaOx) crystals in the middle lamella weakens the cell wall structure and facilitates infection. Moreover, oxalate can inhibit plant defenses and induce programmed cell death, which is also beneficial for necrotrophic pathogens (17, 146, 153).

The significance of a similar production of oxalic acid or other LMWOAs by *Aspergillus* spp. to infect the human lung is an active topic of research. Indeed, although many aspects of the ecology of *Aspergillus* spp. have been investigated in relation to their pathogenicity, one aspect that has been largely ignored is its ability to lower the pH of its environment. This may be necessary for their capacity to colonize or cause infection, via the secretion of oxalic acid or other low molecular weight organic acids. Several studies have reported the presence of CaOx crystals in the case of pulmonary aspergillosis (154–164), and the detection of CaOx crystals has been proposed as an easy tool for differential diagnosis (162). In most of the reported cases, oxalate deposition was associated with *A. niger* infection, but some reports also include infection caused by *A. flavus* or *A. fumigatus* (159). Oxalic acid and oxalate crystals are thought to cause host tissue damage (including in pulmonary blood vessels), as well as tissue injury via iron-dependent generation of free radicals (157, 163). A mechanical role of CaOx crystals was recently reported by Yi et al. (164) in a case of pulmonary angioinvasive aspergillosis in a Burkitt's lymphoma patient with severe neutropenia, with pathophysiological examinations showing the presence of CaOx crystals around and within the walls of blood vessel. Aside from mechanical damage to the host tissues, the formation of CaOx could also have a dramatic effect on cell physiology. Indeed, calcium is an extremely important secondary messenger in many cell types, including those of the immune system (165). During immune stimulation, Ca^{2+} mobilization from extracellular medium or cellular compartments is essential to increase intracellular Ca^{2+} concentration (166), and thus Ca^{2+} chelation has been shown to inhibit the immune response *in vitro* (167, 168). All this suggests a potential role of oxalic acid also in the inhibition of the immune response. Despite these converging indications, a potential link between oxalic acid

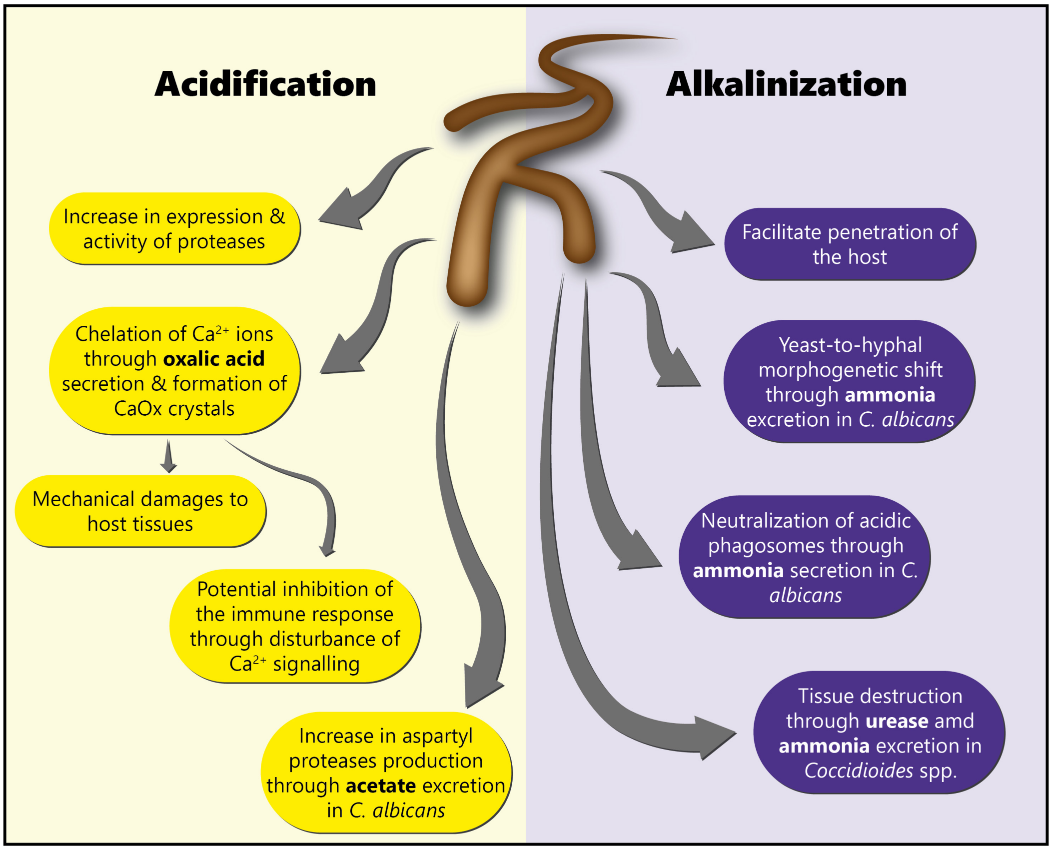

FIGURE 3 | Roles of acidification and alkalinization mechanisms in fungal pathogenesis. This scheme summarizes the different roles of acidification and alkalinization mechanisms in fungal pathogenesis for the colonization of the host tissues.

production and pathogenicity for *Aspergillus* spp. in animals has only been recently proposed (169, 170). Indeed, we demonstrated that oxalic acid production by *A. niger* led to a strong pH decrease, as well as calcium ion sequestration and precipitation in the form of CaOx crystals in differentiated 3D bronchial epithelial tissues. Moreover, we showed that the addition of the soil oxalotrophic bacterium *Cupriavidus oxalaticus* inhibited the growth of *A. niger*, and reverted pH values and free calcium concentrations back to physiological levels. Furthermore, CaOx crystals were no longer observed, suggesting the consumption of oxalic acid by *C. oxalaticus* (169, 170). However, the exact contribution of oxalic acid in the pathogenesis of *A. niger* still needs to be determined. Conversely, oxalic acid production by *Candida* spp. has never been confirmed experimentally, although these fungi are listed as oxalate-producers in the Human Metabolome Database (HMDB0002329).

Fungal pathogens are also known to manipulate the pH of their environment through alkalinization. This is notably the case of *C. albicans*. Alkalinization has been shown to facilitate the invasion of the host tissues, and the evasion of the immune system through neutralization of acidic macrophages phagosomes (150). Alkalinization of the host environment occurs through the excretion and accumulation of ammonia (NH_3), which is then converted into ammonium ions (NH^{4+}) by the urease (143, 150). Moreover, a lack of carbon is required for ammonia-mediated alkalinization to occur (142). Furthermore, *C. albicans* has been shown to auto-induce its switch to the hyphal growth form through the release of ammonia (171). *Coccidioides* spp. has been reported to excrete urease and ammonia to destroy the host tissue (172, 173). St Leger and colleagues have shown that *A. fumigatus* produced small amounts of ammonia in minimal medium, leading to a pH increase and allowing the production of active proteases (174). However, to the best of our knowledge, the role of environmental alkalinization in the pathogenesis of *A. fumigatus* has never been investigated.

LUNG ECOLOGY

Lung Homeostasis

Contrary to the gut, whose microbiota has been extensively studied, the lungs were considered sterile for a long time (175). However, the lung is now known to harbor a diverse microbiota composed of bacteria, fungi and viruses (176, 177). The bacterial composition in the lung has been studied in depth over the past years, although many studies have remained descriptive and in-depth mechanistic analyses are scarce. In the healthy lung, the microbiota is dominated by the genera *Prevotella*, *Streptococcus*, and *Veillonella* (178, 179). In patients with an acute or chronic respiratory disease the composition differs (180–183). Recent data provide clear evidence for the role of the airway microbiota (184), for instance, in the modulation of the host immune response or mucus production (185).

Whereas, the influence of bacterial community composition on lung homeostasis and disease has been a very active area of investigation, our knowledge on the involvement of fungi in these processes is still limited. Using fungal ribosomal RNA gene sequencing of BAL and sputum samples, it has been demonstrated that the mycobiota of the healthy lung mainly consists of environmental agents *Davidiellaceae*, *Cladosporium, Aspergillus, Eurotium, Penicillium,* and *Candida* (181). Epithelial cells of inner and outer body surfaces (e.g., intestine, skin, and lung) are the first physical barrier which interacts with commensals and are also important producers of antimicrobial peptides and immune mediators that regulate immune homeostasis and host defense (186, 187). Fungi colonize all barrier surfaces, and investigations into the influence of fungi on host immunity during homeostasis and disease are more advanced in the intestine and the skin. The influence of fungal seeding and colonization on immune maturation in the lung remains to be investigated, and we can learn from the findings in the intestine and skin and translate those to the lung in the future (188–190).

Gut-Lung Axis

In-depth analysis of the microbiome has revealed profound differences in composition between various body compartments (191, 192). The importance of inter-organ communication and the role of the microbiome herein has been increasingly recognized (193). Respiratory diseases have not only been associated with microbial dysbiosis in the lung, but also in the gut (194). The gut microbiota composition has been shown to influence the immune responses of distant organs, including lungs, through the systemic dissemination of metabolites such as short chain fatty acids (SCFAs), or through the direct seeding of bacteria from the gut to the lungs by gastro-oesophageal reflux and microaspiration. This crosstalk between the gut and the lung compartment is called the gut-lung axis (195). The metabolites or bacteria can consequently have a stimulatory effect on the local immune cells (194–196), and ultimately impair or contribute to the development or progression of respiratory disease.

Recent studies in which fungal composition in the gut was altered using antifungal drugs, provide evidence for an immunoprotective role of the gut mycobiota (197, 198). For example, prolonged oral administration of antifungal drugs led to an exacerbation of allergic airway inflammation in an experimental mouse model. Antifungal treatment led to alterations in several fungal species, with an increase in *Aspergillus, Wallemia* and *Epicoccum* spp., and an observed decrease in *Candida* spp. The enhanced allergic airway inflammatory response was recapitulated when orally supplementing mice with a mixture of the three enriched fungi. This indicates that disruption of commensal fungi can influence both local and distal immune responses. Whether oral antifungal drugs also affect lung mycobiome composition is currently unexplored.

Disruption of bacterial communities using antibiotics can induce fungal dysbiosis and vice versa, suggesting an important role for inter-kingdom interactions (197). Indeed, the *bacterial* microbiome is likely to have an influence on the composition of the *fungal* microbiome, either directly through bacterial-fungal interactions or indirectly through its impact on host immunity, thus making the lung environment more permissive or restrictive to fungal growth (196). Interestingly, using a gnotobiotic approach, van Tilburg and colleagues demonstrated that the presence of gut bacteria, but not fungi, in early life could reduce allergic airway inflammation in a respiratory OVA sensitization and challenge model. These outcomes suggested that homeostatic control of allergic airway inflammation is dependent on bacterial presence, and that intestinal colonization with fungi can skew this inflammatory response (199).

Although a link between the lung bacterial microbiota composition and the disease outcome in patients with invasive pulmonary aspergillosis has been recently demonstrated (200), many knowledge gaps still exist regarding the link between the pulmonary microbiota and *Aspergillus* spp. infections. For instance, alteration of the air-blood barrier, and in particular enhanced access to the extracellular matrix, is a known risk factor for fungal infection (201), especially in the case of *Aspergillus* (202). Therefore, investigating the role of the lung microbiota and specific bacterial community compositions on the strengthening of the air-blood barrier is of clinical importance. The integration of principles from ecological theory will be key to elucidate the bacterial—fungal interactions of the gut and pulmonary microbiota and their human host. This will contribute to identifying microbial groups from within the airway microbiota, or their metabolites, for the development of therapeutic tools to control *Aspergillus* (179, 196).

CURRENT THERAPEUTIC STRATEGIES

The prompt diagnosis and treatment of invasive fungal pulmonary infections such as pulmonary aspergillosis is crucial to prevent associated complications and fatal outcomes. As the clinical presentations and radiological changes are non-specific, biopsy and histopathological analysis remains the gold standard for securing the diagnosis, but is frequently contraindicated in clinically marginal patients. Moreover, colonization is difficult to discriminate from a true invasive infection, and fungal blood cultures are insensitive (10, 203, 204). Therefore, serum and BAL biomarkers such as galactomannan and β-D-glucan, or PCR to detect fungi are being increasingly employed to establish the diagnosis (205).

Current available treatments for pulmonary aspergillosis are limited. They include the use of antifungal drugs—such as azoles, echinocandins, polyenes, and flucytosine, as primary treatment in the case of CPA or IPA, surgical resection in the case of patients suffering from aspergilloma and presenting associated complications such as severe hemoptysis, and corticosteroids in the case of ABPA, with or without the administration of antifungal drugs (206). The standard of care for fungal pulmonary infection is similar across different underlying lung diseases. Treatment would rather depend on the clinical manifestations: for example, no treatment in the case of colonization, corticosteroids and/or antifungal and/or newer biologics in case of allergic sensitisation/ABPA, and antifungal agents for invasive infection. The frequency by which these clinical manifestations are observed would depend on the underlying lung disease. For example, invasive infection is seen almost exclusively in immunocompromised patients, ABPA in patients with asthma or CF, whereas colonization can be observed in any lung disease associated with structural damage (COPD, CF, and non-CF bronchiectasis).

Resistance to antifungal drugs has increased dramatically in the last decades. While antibiotic resistance has been widely recognized in bacteria, antifungal resistance in opportunistic fungal pathogens has not yet received sufficient consideration. The reasons for the rise in antifungal resistance are multiple. Indeed, there is a limited arsenal of active antifungal compounds available on the market that are being used in both agriculture and human health, thus fostering the emergence and rapid spread of cross-resistance in human opportunistic fungal pathogens (2).

This is particularly well highlighted in the case of *A. fumigatus* resistance to azoles. Azoles are frontline antifungal compounds used in crop protection and in human and animal health (207, 208). They have a fungistatic effect on yeasts such as *Candida albicans*, while acting as fungicides against filamentous molds such as *A. fumigatus*. Their fungicidal effect against *A. fumigatus* is linked to defects in cell wall remodeling, resulting in loss of cell wall integrity and death (209). However, despite the fungicidal effect of triazoles in filamentous fungi, their application in agriculture at sub-inhibitory concentrations has led to the emergence and rapid spread of resistance among natural populations of *A. fumigatus* in soil (2, 210). This resistance to azoles may compromise the success of treatment in human patients suffering from *A. fumigatus* infections (211). Azole fungicides such as propiconazole, difenoconazole, or tebuconazole have a very similar structure when compared to those used in clinical practice, and their use is correlated with the increased emergence of clinical azole-resistant *A. fumigatus* strains (208). The emergence of this resistance led to the hypothesis that the extensive use of azole fungicides in agriculture selected for azole resistant *A. fumigatus* in the environment (212). This is supported by several studies reporting the presence of azole-resistant *A. fumigatus* strains in patients never treated with azoles (16, 213). This accounts for two thirds of patients suffering from azole-resistant aspergillosis. Resistance has been attributed to specific mutations in the tandem repeat (TR) of the *cyp51A* gene promoter region, which is involved in the biosynthesis of ergosterol in fungi (16, 214). Clinical azole-resistant *A. fumigatus* strains have also shown cross-resistance to azoles commonly used in agriculture (16). Thus, considering an integrated disease management approach through the One Health initiative that brings together scientists, medical doctors, veterinarians, and plant pathologists, is needed to reduce our reliance on chemical control alone and to stop the spread of resistance among opportunistic pathogens (2).

In case of pulmonary aspergillosis refractory to azoles, echinocandins, such as caspofungin or micafungin, or polyenes, such as amphotericin B, are used as second-line antifungal treatments (211, 215). Echinocandins inhibit the synthesis of the fungal cell wall component β-(1,3)-D-glucan by targeting the β-(1,3)-glucan synthase (216). Little is known on the resistance mechanism to echinocandins in *A. fumigatus*, due to its limited use in the treatment of Aspergillosis (216). Echinocandin resistance in *A. fumigatus* has been attributed to mutations in FKS genes encoding the β-(1,3)-glucan synthase (216). The same has been reported for *C. albicans* (217). Amphotericin B (AmB) has been shown to bind to ergosterol in the fungal cell membrane and form pores, thus disrupting the cell membrane integrity (218, 219). AmB has also been reported to induce endogenous production of ROS leading to oxidative stress and fungal death (219). Although resistance to AmB is rare, *A. terreus* has been shown to be intrinsically resistant (218). Moreover, AmB resistance has also been reported in *A. fumigatus* (220) and *A. flavus* (221). AmB resistance in *A. terreus* may be due to high endogenous production of catalase (218). Finally, flucytosine (5-FC) is a synthetic antifungal compound which, when it is taken up in fungal cells, is first converted into 5-fluorouracil (5-FU). 5-FU is then converted into metabolites which inhibits DNA and RNA synthesis (222). 5-FC is rarely administered as a monotherapy to treat fungal infections such as aspergillosis, but rather in combination with AmB (222, 223).

ADVANCES IN THERAPEUTIC STRATEGIES AND FUTURE PERSPECTIVES

Current approaches used for the treatment of pulmonary aspergillosis focus on attacking the pathogen directly via the use of antifungal compounds. New antifungal drugs have been recently developed against *Aspergillus* spp. infection. Newly developed drugs, as well as drugs currently in development, are listed on the Aspergillosis website.[1] Several of these have shown promising results and are being tested in phase 3 clinical trials (224). Olorofim (F901318, ClinicalTrials.gov Identifier: NCT05101187), from a new class of antifungal drugs called orotomides, is highly active against *Aspergillus* spp. It targets the dihydroorotate dehydrogenase (DHODH) which is involved in the pyrimidine biosynthesis pathway. Olorofim has been shown to inhibit conidia germination, as well as polarized hyphal growth (225). Biafungin, or Rezafungin (CD101, ClinicalTrials.gov Identifier: NCT04368559), is a novel echinocandin targeting the 1,3-β-D-glucan synthase. It shows high in vitro and *in vivo* activity against *Aspergillus* spp. (224, 226). Ibrexafungerp (SCY-078, MK-3118, ClinicalTrials.gov Identifier: NCT03059992) is a triterpenoid antifungal also inhibiting the biosynthesis of β-(1,3)-D-glucan (227). Ibrexafungerp has been shown to be effective against aspergillosis in an *in vivo* murine model (228). Finally, another interesting compound is ASP2397 (VL-2397), which is a novel natural antifungal compound currently in phase 2 clinical

[1] https://www.aspergillus.org.uk/

trial (ClinicalTrials.gov Identifier: NCT03327727). ASP2397 is a cyclic hexapeptide siderophore with a similar structure than ferrichrome, which has a high affinity for iron (224, 229). ASP2397 showed high antifungal activity against *A. fumigatus*, *A. terreus* and *A. flavus*. It was shown to inhibit conidia germination as well as hyphal growth. Moreover, ASP2397 has been reported to have a higher efficacy than posaconazole *in vivo* in an IPA mouse model (229). However, new therapeutic approaches are needed in order to slow down the pace of antifungal resistance emergence in fungal pathogens.

In order to cause disease, a pathogen needs a susceptible host, as well as suitable environmental conditions for its growth. These three factors, i.e., pathogen, host and environment, constitute the so-called disease triangle, which dictates the occurrence of a disease caused by a particular pathogen in a susceptible host in a particular environmental setting (152). The disease triangle concept has already been used in plant disease management for decades (230). However, one aspect that has been completely overlooked in this original disease triangle is the presence of the host microbiota and its role in disease development. A recent paper from Bernardo-Cravo et al. (231) highlighted the necessity to include the host microbiome as a fourth factor influencing the onset of a disease, as it plays a very important role in host immunity. Therefore, the disease triangle should become a disease pyramid (**Figure 4**) and one should consider all four factors, i.e., the host, the host microbiome, the environment, and the pathogen, when thinking about disease management.

We recently proposed to focus on the "environment" corner of the disease pyramid to control the growth of fungal pathogens such as *Aspergillus* spp. through a process we named "environmental interference" (169, 170). We demonstrated that the soil oxalotrophic bacterium *Cupriavidus oxalaticus* is able to control the growth of the fungal pathogen *A. niger*. This is achieved by degrading the oxalic acid produced by *A. niger*, preventing acidification of the local environment favorable to infection. Therefore, *C. oxalaticus* inhibits the growth of the pathogenic fungus *A. niger* by manipulating the pH and restoring it back to a more neutral, physiological value. This environmental interference principle could be potentially extended to other important environmental factors for fungal pathogenesis, such as iron. Indeed, iron is an essential nutrient that is generally limiting, and thus its acquisition is crucial for pathogen's virulence (232). Ghio et al. (157) reported ferric iron (Fe^{3+}) complexation at the surface of CaOx crystals associated with *A. niger* infection, resulting in lung tissue injury via the generation of oxidants. However, fungal, as well as bacterial pathogens, are well-known to acquire iron through the secretion of siderophores (233, 234). *A. fumigatus* is genetically equipped for efficient iron acquisition, encoding four different siderophores: fusarinine C (FsC), triacetylfusarinine C (TAFC), ferricrocin (FC), and hydroxyferricrocin (HFC) (235, 236). Experimental data showed that the initial phase of lung infection with *A. fumigatus* is accompanied by upregulation of iron acquisition genes (237). Loss in the ability to produce siderophores, and thus to acquire iron, has been shown to be detrimental for *A. fumigatus in vivo* (238). Moreover, interfering with the acquisition of iron by the use of chelators inhibited the growth of *A. fumigatus* in a murine cornea infection model (239). Therefore, developing a bacterial biocontrol strategy based on the interference of iron acquisition by *Aspergillus* would provide a further option to exploit the principle of interfering with the environment. This could be achieved either by direct competition between *Aspergillus* spp. and the biocontrol bacteria, where these latter would produce a siderophore with a higher affinity for iron than the fungal siderophore, allowing them to acquire iron better than the fungus, or by bacterial "cheating" through stealing the siderophores produced by *Aspergillus* spp. Indeed, non-siderophore producing bacteria are known to steal other species' siderophores through the use of a matching receptor (240). On the other hand, host cells internalize iron through a global process called nutritional immunity, aimed at controlling infection (241, 242). However, unlike for the gut microbiota (243), the interplay between the regular members of the airway microbiota and nutritional immunity is still largely undetermined. Indeed, while essential metals, such as iron or zinc, have been found to be altered in several respiratory diseases, the exact causes and mechanistic consequences of this metal dysregulation on the immune system and respiratory microbiota still need to be further explored (242). Interestingly, gallium, a group IIIA metal, has been proposed as an antifungal agent. Gallium is used in several medical applications ranging from cancer to calcium disorders and bone metabolism (244). Moreover, gallium nitrate III [$Ga(NO_3)_3$] has been widely used as an antibacterial agent against bacterial pathogens such as *Klebsiella pneumoniae*, *Staphylococcus aureus* or *Pseudomonas aeruginosa* (244). Furthermore, gallium nitrate IV has been tested in a phase 2 clinical trial for intravenous administration as an anti-infective agent against *P. aeruginosa* infection in CF patients (ClinicalTrials.gov Identifier: NCT02354859). Gallium is known to disrupt the iron homeostasis in bacteria and cancer cells (244). Its antifungal inhibitory effect has recently been demonstrated by Bastos and colleagues against azole-resistant *A. fumigatus* and multidrug-resistant *Candida* spp. (244). Finally, it is worth investigating whether gallium could be used to treat bacterial-fungal polymicrobial infections, which are frequent in CF patients (245, 246).

The role of the microbiota as a keystone factor influencing the onset and development of a disease provides a powerful incentive for the use of microorganisms to prevent and treat illness. These microorganisms, also referred to as live biotherapeutic products (LBPs), are defined by the FDA as "*a biological product that contains live organisms, such as bacteria; is applicable to the prevention, treatment, or cure of a disease or condition of human beings; and is not a vaccine*" (247). They are not intended to reach the systemic circulation, but rather exert their action through interaction with resident members of the microbiota and/or by modulating complex host-microbiota interactions. This implies a multifactorial mode of action, which is in strong contrast to the reductionist approach traditionally used in medical research (247).

The large majority of LBPs correspond to single species products. There are examples in which single species LBPs appear to confer protection against an invading pathogen, a phenomenon known as colonization resistance [i.e., *Lactobacillus* *murinus* protection against *Streptococcus pneumoniae* lung colonization (248); *Clostridium scindens* providing colonization resistance against *Clostridium difficile* (249)]. However, the use of single-species LBPs likely fails to capture the complexity of the multifactorial and multi-species role of the microbiota in health homeostasis. A similar concept to colonization resistance is widely used in soil ecology, namely that of disease-suppressive and conducive soils (250). This concept links the composition of the soil microbiota with the natural protection against plant fungal infections. Mendes et al. (251) showed that a specific assemblage of rhizospheric bacteria lead to disease-suppression and confer protection against the root fungal pathogen *Rhizoctonia solani* in sugar beet seedlings. In the same way, a specific microbiota composition could lead to more pathogen-suppressive communities, or conversely, dysbiosis of the microbiota could lead to pathogen-permissive communities. Translating soil ecological concepts into human medicine could be of great benefit, as it would provide key insights into the complex interspecies interaction dynamics within the human microbiome and between itself and the host (252, 253). Such lung microbiota-based biocontrol strategy could be applied against aspergillosis, as lung microbiota has been suggested to prevent the establishment of *Aspergillus* spp. in the lungs (196).

Additionally, LBPs have been shown to restore the epithelial barrier and modulate the immune response. These effects have been mostly studied in the case of administration of probiotics in the gastrointestinal tract (254). However, their potential beneficial effect on the airway epithelial barrier restoration, as well as in the immune homeostasis have been suggested by Martens et al. (254). This is notably the case of the bacterial strains *Lactobacillus plantarum* MB452, *Lactobacillus rhamnosus* GG and *Streptococcus thermophiles* ATCC 19258, among others

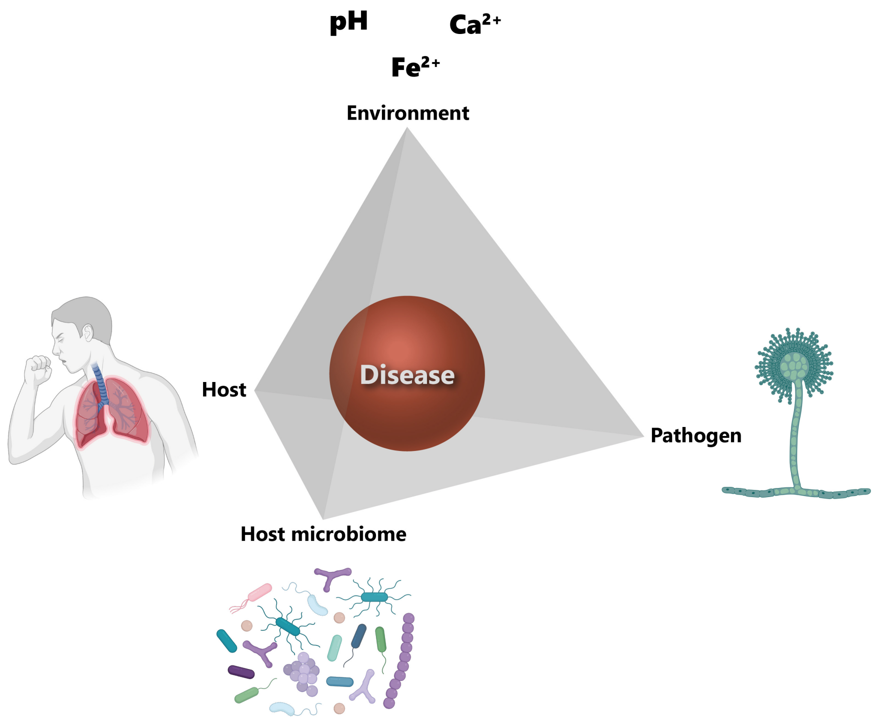

FIGURE 4 | Disease pyramid. The onset of a disease depends on the interplay between the host, the pathogen, the environment, and the host microbiome. Host susceptibility mainly depends on genetic factors and immune status. The main factor for the successful colonization of the host tissues by the pathogen is its virulence. The environment corner refers to the host lung microenvironmental parameters and include among others pH, calcium (Ca^{2+}) and iron (Fe^{2+}) concentrations. Host microbiota community structure can also influence the establishment of the pathogen. Icons have been created using Biorender.com. Modified from Palmieri (170).

BOX 1 | Key unanswered questions.

- What comprises a healthy lung mycobiome?
- What is the role of the mycobiome component of the respiratory microbiota in the colonization resistance to fungal pathogens?
- What is the role of commensal fungi in microbiota community stability?
- What is the involvement of commensal fungi in the maintenance of lung barrier homeostasis/function?
- How do commensal fungi contribute to pulmonary immune maturation in early life and what are the consequences of early life fungal dysbiosis for respiratory disease development?
- Which mechanisms underlie the bacterial -induced predisposition to invasive pulmonary aspergillosis and other respiratory fungal infections?
- How does nutrient availability contribute to lung microbial community composition and consequently influence fungal infection?

(254). Recently, the use of nasal probiotic strains such as *Lacticaseibacillus casei* AMBR2 and *Lactococcus lactis* W136 has shown promising results. Indeed, *Lacticaseibacillus casei* AMBR2 showed a beneficial effect on the epithelial barrier function and modulation of the immune response in *in vitro* Calu-3 differentiated tissue co-cultured with donor-derived nasal microbiota and macrophage-like cells (255, 256). Cho and colleagues reported that *Lactococcus lactis* W136 suppressed the growth of patient-derived strains of the bacterial pathogen *Pseudomonas aeruginosa* in *in vitro* co-cultures (257).

Although large efforts have been made to enhance our understanding of respiratory fungal infections, the treatment of fungal pathogens poses a series of unique challenges. Fungal infections are difficult to diagnose in a timely manner, antifungal drugs and treatments are limited, and given the emergence of resistance to antifungal drugs there is a reduced effectiveness of these treatments. Moreover, severe infections often occur in subjects with significant comorbidities, including chronic respiratory diseases and a state of immunosuppression. It is thus imperative to develop novel treatment strategies for fungal infections. In order to do so, we have identified several *key unanswered questions* (**Box 1**). In view of the variability of fungal infection outcomes, and in particular those with *Aspergillus* spp., there is an urgent need to understand the conditions that make the respiratory tract permissive to conidial germination in susceptible individuals, and in particular, to determine whether the composition of the airway microbiota plays a role in this regard.

Future research in this field should focus on moving toward the complete characterization of the lung microbial ecosystem by shotgun metagenomic sequencing and gene expression analysis (258). Moreover, technical advances are required to enhance our ability to culture fungi from respiratory tract samples in the setting of prophylactic anti-fungal treatment, and in particular move toward building a collection of lung commensal fungal strains which can be implemented in mechanistic studies.

AUTHOR CONTRIBUTIONS

FP and NU conceptualized and drafted the manuscript. AK, EB, PJ, and CG provided valuable discussion and input. FP, AK, EB, PJ, CG, and NU revised the manuscript. All authors approved the final version of the manuscript.

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Risk of Pleural Empyema in Adult Patients With Asthma: A Nationwide Retrospective Cohort Study

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Background: Respiratory system infections commonly occur among individuals with asthma. However, whether asthma patients have a higher risk of pleural empyema development remains unclear.

Methods: This is a retrospective cohort study based on data from the National Health Insurance Research Database of Taiwan. The asthma cohort consisted of 48,360 newly diagnosed adult individuals from 2000 to 2012. The comparison cohort consisted of the same number of adults who did not have asthma and was matched for age, gender, comorbidity, and the year of diagnosis. The development of pleural empyema was followed up to 2013.

Results: Pleural empyema incidence was 2.03-fold higher in the asthma cohort compared to the comparison cohort (8.65 vs. 4.25 per 10,000 person-years), with an adjusted hazard ratio (HR) of 2.12 [95% confidence interval (CI) = 1.76–2.56]. Stratified analyses by age, gender, comorbidity, and corticosteroid use revealed that the crude and adjusted HRs of pleural empyema associated with asthma were all significant. Among patients with asthma, the risk of pleural empyema elevated with increased frequency of annual asthma-related emergency room visits and hospital admissions ($\geq$1 vs. $<$1, aHR = 8.07, 95% CI = 4.31–15.1 and aHR = 9.31, 95% CI = 5.56–15.6).

Conclusion: An increased risk of pleural empyema occurrence was observed in adult patients with asthma than those without asthma. Furthermore, the risk of pleural empyema may increase with poor control of asthma.

Keywords: empyema, asthma, pneumonia, cohort study, retrospective study

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INTRODUCTION

Asthma is a heterogeneous disease manifesting with airway inflammation (1). This disease is defined by the presence of respiratory symptoms that vary over time and in intensity, together with variable expiratory airflow limitation (2). Inadequate control of asthma may lead to frequent exacerbations, worse health status, and poor quality of life (3). Asthma is also found to be associated with respiratory system infections, but information on the underlying mechanism of this predisposition is limited (4). Altered epithelial microenvironment and impaired immune function may contribute to the susceptibility of respiratory system infections (5, 6).

Pleural empyema indicates the occurrence of frank pus within the pleural space (7). It is most commonly caused by respiratory system infections, such as pneumonia (8). Mortality rate among individuals having pneumonia with pleural empyema is far higher than in patients who do not have pleural empyema (9, 10). The crucial nature of pleural empyema and the need for removal have been identified for several centuries (11). Delayed diagnosis and poor drainage have been found to be linked with high mortality (12). Alcohol consumption, substance use, diabetes mellitus, immunosuppression, malignancy, pulmonary disease, and prior occurrence of pleural effusion are predictors of the development of pleural empyema (13, 14).

Several studies have investigated the association between asthma and respiratory system infections (15–18). However, the studies did not examine the incidence of pleural empyema in asthma patients. Pleural empyema is a noticeable infection of the respiratory system and requires timely treatments, such as antibiotic therapy, pleural drainage, intrapleural fibrinolysis, and surgery. Thus, investigating the risk of pleural empyema in patients with asthma is necessary. This study aimed to examine whether patients with asthma have a higher risk of pleural empyema development. In addition, we attempted to assess the effect of asthma control on the occurrence of pleural empyema.

MATERIALS AND METHODS

Data Source

The Taiwan National Health Insurance (NHI) program was established in 1995. The National Health Insurance Research Database (NHIRD) is a nationwide database housing medical claims data of over 99.5% of people living in Taiwan (https://nhird.nhri.org.tw/en/). The database is updated and maintained by the National Health Research Institutes. The Longitudinal Health Insurance Database 2000 (LHID2000, a subset data of NHIRD) was used for this study. The database contains medical claims data of one million persons randomly selected from users registered in 2000. Data on their demographic characteristics, diagnostic codes, procedure claims, and medication claims were available from 1995 to 2013. All data were deidentified to protect their privacy; therefore, written informed consent from the participants involved was unnecessary. This study was approved by the Research Ethics Committee of the China Medical University and Hospital (CMUH-104-REC2-115).

Study Cohorts

Patients with newly diagnosed asthma [International Classification of Disease, 9th Revision, Clinical Modification (ICD-9-CM) code 493 and asthma medication (bronchodilator or corticosteroid)] from January 1, 2000, to December 31, 2012, were selected in the asthma cohort. The date of the diagnosis was defined as the index date. Individuals with a diagnosis of pleural empyema before the index date and those with incomplete data were excluded from the analysis. The individuals in the comparison cohort included people free from asthma. The exclusion criteria for the comparison cohort were the same as for the asthma cohort. The comparison cohort was 1:1 frequency-matched with the asthma cohort by age, gender, comorbidity, and index year. All subjects were monitored until any of the following occurred: (1) development of pleural empyema, (2) withdrawal from NHI system, (3) death, and (4) the date of 31st of December 2013.

Outcome and Variables

All diseases were recorded in accordance with the ICD-9-CM in the NHIRD. The primary outcome was pleural empyema (based on ICD-9-CM code 510 and related antibiotic treatment). We also determined the related baseline comorbidities between 1995 and index date, including chronic obstructive pulmonary disease (COPD, ICD-9-CM code 496), diabetes mellitus (ICD-9-CM code 250), chronic kidney disease (CKD, ICD-9-CM code 585), chronic liver disease and cirrhosis (CLD, ICD-9-CM code 571), rheumatic disease (ICD-9-CM codes 446.5, 710.0–710.4, 714.0–714.2, 714.8, and 725), stroke (ICD-9-CM codes 433–438), cancer (ICD-9-CM codes 140–209), and malnutrition (ICD-9-CM codes 260–269). We selected only diagnoses from the outpatient department that appeared at least twice within 1 year or had a diagnosis of hospitalization to increase the accuracy for asthma, pleural empyema, and all comorbidities. In addition, we evaluated the related medication, corticosteroid use.

Statistical Analysis

We used the Chi-squared test to examine the proportion distribution of age group, gender, comorbidity, and medication between asthma and comparison cohorts. The means of age in the two cohorts were compared using a student's *t*-test. The estimation of cumulative incidence of pleural empyema in asthma and comparison cohorts was performed by the Kaplan–Meier method. A log-rank test was utilized to determine the significance. The incidence rates of pleural empyema were calculated by asthma, age group, gender, comorbidities, and medication. Univariable and multivariable Cox proportional hazard regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). Moreover, we calculated the incidence rates and relative risk of pleural empyema by stratification with age, gender, comorbidities, and medication between asthma and comparison cohorts. Among patients with asthma, we further evaluated the impact of the annual number of asthma-related emergency room visits and hospital admissions and cumulative corticosteroid doses on pleural empyema development. Data analysis was performed with the SAS statistical software (Version 9.4 for Windows; SAS Institute, Inc., Cary, NC, USA). Statistical significance was considered at a p-value < 0.05.

RESULTS

We recruited an asthma group comprising 48,360 patients and a comparator group of 48,360 individuals (**Table 1**). Age and gender did not significantly differ between asthma and comparator group. The mean age ± standard division of asthma and comparator group was 54.9 ± 18.7 and 54.1 ± 18.5 years, respectively. Approximately 52% of the individuals were women in both groups. The major comorbidities of the asthma group were COPD (24.8%), CLD (21.7%), followed by diabetes mellitus

TABLE 1 | Baseline characteristics between asthma and non-asthma cohorts.

	Asthma				*p*-value
	No		Yes		
	***N* = 48,360**		***N* = 48,360**		
	n	**%**	***n***	**%**	
Age					0.63
20–49	19,720	40.8	19,680	40.7	
50–64	11,918	24.6	11,826	24.5	
≥65	16,722	34.6	16,854	34.9	
Mean ± SD	54.1	±18.5	54.9	±18.7	0.001
Gender					0.46
Women	24,873	51.4	24,988	51.7	
Men	23,487	48.6	23,372	48.3	
Comorbidity					
Diabetes mellitus	5,254	10.9	5,269	10.9	0.88
CKD	1,195	2.47	1,066	2.20	0.01
CLD	10,337	21.4	10,513	21.7	0.17
COPD	11,880	24.6	11,988	24.8	0.42
Rheumatic disease	1,360	2.81	1,346	2.78	0.78
Stroke	3,549	7.34	3,350	6.93	0.01
Cancer	1,598	3.30	1,527	3.16	0.20
Malnutrition	401	0.83	339	0.70	0.02
Medication					
Corticosteroid	7,355	15.2	11,392	23.6	<0.001

CKD, chronic kidney disease; CLD, chronic liver disease and cirrhosis; COPD, chronic obstructive pulmonary disease; SD, standard deviation.

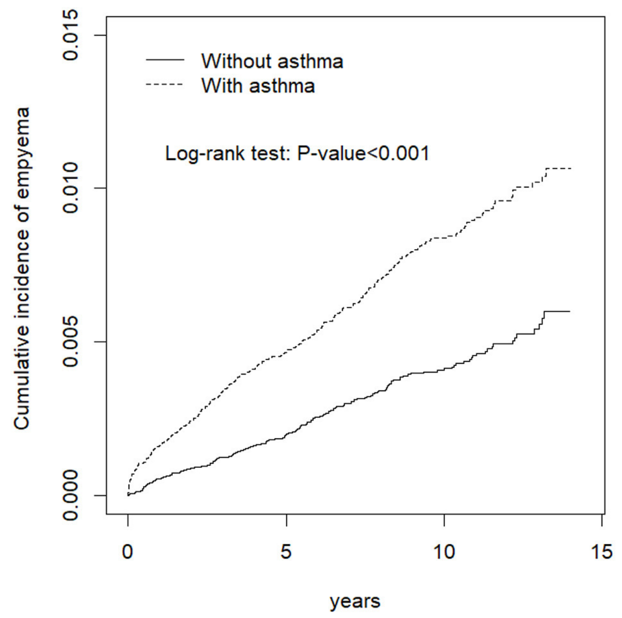

FIGURE 1 | Cumulative incidence of pleural empyema in the asthma group (dashed line) and in the comparison group (solid line).

(10.9%), stroke (6.93%), and cancer (3.16%). The mean follow-up periods were 7.72 ± 4.15 years in the asthma group and 8.03 ± 4.02 years in the comparator group. The proportion of corticosteroid use were 23.6% in the asthma group and 15.2% in the comparator group. **Figure 1** showed that patients with asthma had a higher cumulative incidence of pleural empyema than individuals without asthma throughout the 14-year study period.

The overall incidence density rates of pleural empyema were 8.65 and 4.25 per 10,000 person-years in asthma and comparator groups, respectively (**Table 2**). Compared with the comparator group, the corresponding adjusted HR (aHR) of pleural empyema was 2.12 (95% CI = 1.76–2.56) in the asthma group after adjusting for age, gender, COPD, diabetes mellitus, CKD, CLD, stroke, cancer, and corticosteroid use. Compared with persons aged 20–49, the aHRs of pleural empyema were 2.43-fold higher in those aged 50–64 (95% CI = 1.80–3.29) and 4.10-fold higher in those aged ≥65 (95% CI = 3.09–5.44). The aHR of pleural empyema was 2.55-fold higher for men relative to women (95% CI = 2.09–3.12). Moreover, the risk of pleural empyema was higher in persons with stroke (aHR = 2.68, 95% CI = 2.12–3.38), diabetes mellitus (aHR = 1.86, 95% CI = 1.50–2.31), and COPD (aHR = 1.48, 95% CI = 1.22–1.79) compared with subjects without these comorbidities.

Further analyses revealed that the incidences and aHRs of pleural empyema for the asthma group were all significantly higher compared with the comparator group after stratification for age, gender, presence of comorbidities, and corticosteroid use (**Table 3**). In addition, we assessed the impact of the frequency of asthma-related emergency room visits and hospital admissions on the development of pleural empyema among the asthma group (**Table 4**). A higher frequency of annual asthma-related emergency room visits and hospital admissions raised the risk of pleural empyema development (both p for trend <0.001). Moreover, we analyzed cumulative corticosteroid doses on pleural empyema development among the asthma group (**Table 4**). The incidence of pleural empyema was higher in those with corticosteroid use; however, the p value for trend was not significant ($p = 0.25$).

DISCUSSION

We believed that this investigation is the first population-based retrospective cohort study to evaluate the incidence of pleural empyema in patients with asthma. Our findings revealed that patients with asthma have a significantly higher risk of developing pleural empyema than those without asthma. The risk of pleural empyema was also larger in older people, in males, those with comorbidities. Furthermore, the hazards of pleural empyema were significantly larger in the asthma cohort compared with the comparison cohort under stratification by age, gender, comorbidity, and corticosteroid use. Moreover, we found that the risk of pleural empyema was higher in asthma patients with an increased number of asthma-related emergency medical demands and hospital admissions, indicating that the level of asthma control may influence the occurrence of pleural empyema.

TABLE 2 | Incidences and hazard ratios of pleural empyema by asthma, age, gender, comorbidity, and corticosteroid use among all participants.

	Event	PY	Rate[#]	Crude HR (95% CI)	Adjusted HR[†] (95% CI)
Asthma					
No	165	388,334	4.25	1.00	1.00
Yes	323	373,337	8.65	2.03 (1.68–2.45)***	2.12 (1.76–2.56)***
Age					
20–49	69	347,688	1.98	1.00	1.00
50–64	121	196,168	6.17	3.10 (2.31–4.16)***	2.43 (1.80–3.29)***
≥65	298	217,815	13.7	6.76 (5.20–8.79)***	4.10 (3.09–5.44)***
Gender					
Women	135	404,727	3.34	1.00	1.00
Men	353	356,945	9.89	2.95 (2.42–3.60)***	2.55 (2.09–3.12)***
Comorbidity					
COPD					
No	278	569,143	4.66	1.00	1.00
Yes	210	165,529	12.7	2.68 (2.24–3.21)***	1.48 (1.22–1.79)***
Diabetes mellitus					
No	372	694,873	5.35	1.00	1.00
Yes	116	66,799	17.4	3.17 (2.57–3.91)***	1.86 (1.50–2.31)***
CKD					
No	470	749,761	6.27	1.00	1.00
Yes	18	11,911	15.1	2.30 (1.43–3.68)***	1.22 (0.75–1.96)
CLD					
No	350	599,220	5.84	1.00	1.00
Yes	138	162,451	8.49	1.45 (1.19–1.77)***	1.16 (0.95–1.41)
Rheumatic disease					
No	471	743,383	6.34	1.00	
Yes	17	18,288	9.30	1.44 (0.89–2.33)	
Stroke					
No	390	727,905	5.36	1.00	1.00
Yes	98	33,766	29.0	5.19 (4.15–6.49)***	2.68 (2.12–3.38)***
Cancer					
No	466	746,246	6.24	1.00	1.00
Yes	22	15,425	14.3	2.17 (1.41–3.33)***	1.39 (0.91–2.14)
Malnutrition					
No	484	756,482	6.40	1.00	
Yes	4	5,189	7.71	1.19 (0.45–3.18)	
Medication					
Corticosteroid					
No	370	634,551	5.83	1.00	1.00
Yes	118	127,120	9.28	1.56 (1.27–1.92)***	1.02 (0.82–1.26)

CI, confidence interval; CKD, chronic kidney disease; CLD, chronic liver disease and cirrhosis; COPD, chronic obstructive pulmonary disease; HR, hazard ratio; PY, person-years.
[#]Incidence rate per 10,000 person-years.
*[†]Multivariable analysis including age, gender, COPD, diabetes mellitus, CKD, CLD, stroke, cancer, and corticosteroid use. ***$p < 0.001$.*

The mechanism between asthma and pleural empyema remains largely unknown. Patients with asthma who are susceptible to pneumonia may still play a major role. This condition may be driven by the following: (1) a large prevalence of carriage of the bacteria, (2) a disordered immune response from exposure to the bacteria, (3) impaired bacterial clearance, and (4) a suboptimal response to vaccination (5). Parapneumonic pleural effusions are known to represent a common complication of pneumonia and can be found in approximately 40% of bacterial pneumonia cases (11). In addition, inhaled or systemic corticosteroid use, shared comorbidities, cigarette smoking, alcohol consumption, unhealthy lifestyle, poor self-care, and poor physical health are commonly noted among asthma patients. These factors are also related to the development of pneumonia and pleural empyema (9).

Inconsistent evidence was found between chronic inflammatory airway disease and the development of pleural empyema. Lu et al. (19) conducted a case-control study to

TABLE 3 | Incidences and hazard ratios of pleural empyema by age, gender, comorbidity, and corticosteroid use between asthma and non-asthma cohorts.

	Asthma						Crude HR (95% CI)	Adjusted HR† (95% CI)
	No			Yes				
	Event	PY	Rate#	Event	PY	Rate#		
Age								
20–49	23	174,025	1.32	46	173,662	2.65	2.01 (1.22–3.31)**	1.98 (1.20–3.27)**
50–64	31	99,658	3.11	90	96,510	9.33	3.00 (1.99–4.51)***	3.06 (2.03–4.62)***
≥65	111	114,651	9.68	187	103,164	18.1	1.86 (1.47–2.36)***	1.91 (1.51–2.43)***
Gender								
Women	36	205,289	1.75	99	199,437	4.96	2.83 (1.93–4.14)***	2.78 (1.89–4.08)***
Men	129	183,045	7.05	224	173,900	12.9	1.82 (1.47–2.26)***	1.94 (1.56–2.42)***
Comorbidity§								
No	34	212,837	1.60	98	210,361	4.66	2.92 (1.98–4.31)***	2.95 (1.99–4.36)***
Yes	131	175,497	7.46	225	162,975	13.8	1.84 (1.48–2.28)***	1.91 (1.54–2.37)***
Corticosteroid								
No	139	338,472	4.11	231	296,079	7.80	1.90 (1.54–2.34)***	2.05 (1.66–2.53)***
Yes	26	49,862	5.21	92	77,258	11.9	2.28 (1.48–3.53)***	2.43 (1.61–3.86)***

CI, confidence interval; HR, hazard ratio; PY, person-years.
#Incidence rate per 10,000 person-years.
†Multivariable analysis including age, gender, COPD, diabetes mellitus, CKD, CLD, stroke, cancer, and corticosteroid use.
§Individuals with any comorbidity of COPD, diabetes mellitus, CKD, CLD, rheumatic disease, stroke, cancer, and malnutrition were classified into the comorbidity group.
$^{**}p < 0.01$, $^{***}p < 0.001$.

TABLE 4 | Incidences and hazard ratios of pleural empyema by emergency room visits, hospital admissions, and cumulative corticosteroid doses among asthma cohort.

	Event	Incidence#	Crude HR (95% CI)	Adjusted HR† (95% CI)
Annual emergency room visits				
<1	311	8.34	1.00	1.00
≥1	12	334.9	19.7 (10.6–36.4)***	8.07 (4.31–15.1)***
p for trend				<0.001
Annual hospital admissions				
<1	302	8.10	1.00	1.00
≥1	21	362.7	24.6 (15.0–40.5)***	9.31 (5.56–15.6)***
p for trend				<0.001
Cumulative corticosteroid doses (mg)				
None	231	7.80	1.00	1.00
<115	9	10.3	1.31 (0.67–2.55)	0.98 (0.50–1.91)
115–335	35	15.4	1.93 (1.35–2.75)***	1.53 (1.07–2.19)*
≥335	48	10.6	1.30 (0.95–1.78)	0.93 (0.68–1.28)
p for trend				0.25

CI, confidence interval; HR, hazard ratio.
#Incidence rate per 10,000 person-years.
†Multivariable analysis including age, gender, COPD, diabetes mellitus, CKD, CLD, stroke, cancer, and corticosteroid use.
$^{*}p < 0.05$, $^{***}p < 0.001$.

evaluate the potential risk factors of pleural empyema. They enrolled 1,851 pleural empyema cases and 7,404 non-empyema controls and found significant factors that lead to pleural empyema include the following: aspiration history [odds ratio (OR) = 7.28, 95% CI = 5.00–10.6)], human immunodeficiency virus infection (OR = 5.66, 95% CI = 1.38–23.2), malnutrition (OR = 2.86, 95% CI = 2.07–3.95), cancer (OR = 2.74, 95% CI = 2.28–3.30), diabetes mellitus (OR = 2.25, 95% CI = 1.96–2.59), stroke (OR = 1.99, 95% CI = 1.70–2.34), CKD (OR = 1.78, 95% CI = 1.42–2.25), chronic obstructive pulmonary disease (COPD, OR = 1.72, 95% CI = 1.47–2.01), asthma (OR = 1.34, 95% CI = 1.15–1.57), and CLD (OR = 1.20, 95% CI = 1.06–1.35). In another study, Lu et al. evaluated COPD and the subsequent development of pleural empyema (20). They enrolled 55,136 COPD cases and 98,769 non-COPD controls and found that the incidence of pleural empyema was 3.64-fold higher in the COPD cohort than in the comparison cohort (15.8 vs. 4.34 per 10,000 person-years), with a corresponding aHR of 3.25 (95% CI = 2.73–3.87). These findings may suggest that chronic inflammatory airway disease may contribute to the development of pleural empyema. By contrast, Dusemund et al. (21) performed a case-control study in Switzerland to investigate outcomes of community-acquired pneumonia in patients with chronic lung disease. They found that the incidence of pleural empyema was insignificant in asthma [0.5% (asthma) vs. 0.9% (controls), $p = 0.141$] and COPD [0.5% (COPD) vs. 0.5% (controls), $p = 0.817$]. In addition, Elemraid et al. (22) assessed predictors of pleural empyema development in children. They found that age, sex, mother's age, smoking among the child's parents, poverty, nursery attendance, asthma, and household characteristics (bedrooms and number of occupants) were not significantly related. Therefore, we may need additional investigations to clarify this issue.

Strength

The strength of this study lies in the establishment of a population-based asthma cohort to assess the risk of developing pleural empyema. Conducting a prospective cohort study is expensive. Therefore, a retrospective cohort study based on insurance claims data may be a suitable and economical alternative. Regardless of socioeconomic background and/or residential location, the universal coverage by the NHI program lowers access barriers to health care all citizens (23). This study was able to reflect a "real world" scenario in which asthma, pleural empyema, and comorbidities were assessed during medical evaluation.

Limitation

Several limitations exist and need to be considered in interpreting the study findings. First, the ICD-9-CM algorithm was used to define asthma, pleural empyema, and comorbidities. All diagnoses were dependent on the competence of clinical physicians in diagnosing; however, asthma has been carefully validated in the NHIRD (24). In addition, an *ad hoc* committee established by the insurance authority monitored the evaluation of claims data to prevent errors and violations. We selected only diagnoses from the outpatient department that appeared at least twice within 1 year or had a diagnosis of hospitalization to increase the accuracy. In addition, we applied the medication used to improve the diagnosis of asthma and empyema. Second, the NHIRD does not provide detailed information regarding smoking habits, drinking habits, and other environmental factors, which are potentially confounding factors in the current study. In addition, relevant clinical variables, such as serum laboratory data, image reports, and culture results, were unavailable in the database.

CONCLUSION

An increased risk of pleural empyema occurrence was observed in adult patients with asthma compared to those without asthma. Furthermore, the risk of pleural empyema may increase with the degree of asthma control.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

W-CL, T-CS, C-YT, T-CH, and W-HH: conception and design. C-YT, T-CH, and W-HH: administrative support. W-CL, C-LL, and T-CS: collection and assembly of data and data analysis and interpretation. All authors: manuscript writing and final approval of manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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BRIEF RESEARCH REPORT
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Healthcare Utilization and Medical Cost of Gastrointestinal Reflux Disease in Non-tuberculous Mycobacterial Pulmonary Disease: A Population-Based Study, South Korea, 2009–2017

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Gastroesophageal reflux disease (GERD) is a common non-respiratory comorbidity in patients with non-tuberculous mycobacterial pulmonary disease (NTM-PD). However, little is known about the association between GERD and healthcare utilization and medical costs of NTM-PD. Thus, we evaluated this association using the Health Insurance Review and Assessment Service National Patient Sample. NTM-PD patients with GERD had significantly higher healthcare use and spent a higher total on medical costs (5,098 vs. 2,675 USD/person/year) than those without GERD ($P < 0.001$ for all). Therefore, an appropriate management of GERD in NTM-PD patients can be an important factor to reduce the disease burden.

Keywords: NTM, non-tuberculous mycobacteria, GERD (gastroesophageal reflux disease), population base study, medical cost, disease burden

INTRODUCTION

The prevalence and incidence of non-tuberculous mycobacterial pulmonary disease (NTM-PD) has been increasing worldwide (1). Furthermore, both the attributable mortality and financial burden associated with this disease are high (2). Thus, clinical studies that identify preventable or treatable factors to address the high disease burden of NTM-PD are urgently needed to determine strategies for reducing the burden. Gastroesophageal reflux disease (GERD) is a common non-respiratory comorbidity in NTM-PD patients, with a prevalence of 26–44% (3, 4). GERD is associated with an increased number of aspiration symptoms, higher bacterial burden, and more severe radiologic findings (3, 4). Although these results indicate that the disease burden in NTM-PD patients with GERD can be more substantial than in those without GERD, there is limited information on this issue. Thus, we aimed to evaluate whether GERD is associated with a higher disease burden, specifically healthcare use, medical costs, and in-hospital mortality, in patients with NTM-PD.

METHODS

Data Source

We used the 2009–2017 Health Insurance Review and Assessment Service, National Patient Sample (HIRA-NPS), which includes ∼1,400,000 individuals each year drawn by 3% stratified random sampling by age and sex from the entire population with claims records during the year. The dataset included patient diagnosis, treatment, procedures, surgical history, and prescription drugs. Diagnosis was coded according to the 10th edition of the International Classification of Diseases (ICD-10). Generic drugs were coded according to the Korean National Code System (5).

Study Population

NTM-PD was defined by ICD-10 diagnosis codes of A31.0, A31.8, and A31.9; GERD was defined by ≥2 claims under the ICD-10 code K21 and proton pump inhibitors prescribed for ≥2 weeks (6). We included 6,589 patients with NTM-PD from January 2009 to December 2017 using the ICD-10 code, of which 1,407 patients had GERD. The Institutional Review Board of Chungbuk National University Hospital approved the study and waived the requirement for informed consent because the HIRA-NPS data were deidentified (application no. CBNUH 2021-03-020).

Outcomes

Respiratory-related healthcare use was defined as healthcare use under the ICD-10 codes of respiratory diseases (J00–J99). We compared healthcare use (outpatient department [OPD] visits, emergency room [ER] visits, or hospitalizations), medical costs, and in-hospital mortality in NTM-PD patients with GERD to those in patients without GERD. The medical cost consists of expenses related to diagnostic tests, procedures, and treatments covered by the National Health Insurance (7).

Covariables

Comorbidities were also defined using ICD-10 codes. Pulmonary comorbidities were defined as chronic obstructive pulmonary disease (COPD; J42–J44, except J43.0 [unilateral emphysema]), asthma (J45–J46), bronchiectasis (J47, excluding E84 [cystic

TABLE 1 | Characteristics of study population.

	Total (*N* = 6,589)	NTM-PD with GERD (*n* = 1,407)	NTM-PD without GERD (*n* = 5,182)	*P*-value
Age, years				<0.001
20–29	499 (7.6)	25 (1.8)	474 (9.2)	
30–39	877 (13.3)	96 (6.8)	781 (15.1)	
40–49	789 (12.0)	125 (8.9)	664 (12.8)	
50–59	1,360 (20.5)	311 (22.1)	1,049 (20.1)	
60–69	1,407 (21.4)	403 (28.6)	1,004 (19.4)	
≥70	1,657 (25.2)	447 (31.8)	1,210 (23.4)	
Sex				0.614
Male	2,458 (37.3)	533 (37.9)	1,925 (37.2)	
Female	4,131 (62.7)	874 (62.1)	3,257 (62.8)	
Type of insurance				<0.001
Health insurance	6,283 (95.3)	1,302 (92.5)	4,981 (96.2)	
Medical aid	301 (4.6)	102 (7.3)	199 (3.8)	
Veteran status	5 (0.1)	3 (0.2)	2 (0.0)	
Comorbidities				
COPD	1,720 (26.1)	528 (37.5)	1,192 (23.0)	<0.001
Asthma	1,909 (29.0)	575 (40.9)	1,334 (25.7)	<0.001
Bronchiectasis	1,653 (25.1)	406 (28.9)	1,247 (24.1)	<0.001
Cerebrovascular disease	626 (9.5)	221 (15.7)	405 (7.8)	<0.001
Hypertension	2,051 (31.1)	620 (44.1)	1,431 (27.6)	<0.001
Diabetes mellitus	1,464 (22.2)	478 (34.0)	986 (19.0)	<0.001
Connective tissue disease	405 (6.2)	173 (12.3)	232 (4.5)	<0.001
Charlson comorbidity index				<0.001
0–1	2,952 (44.8)	307 (21.8)	2,645 (51.0)	
≥2	3,637 (55.2)	1,100 (78.2)	2,537 (49.0)	

Data are presented as numbers (percentages).
NTM-PD, non-tuberculous mycobacterial pulmonary disease; GERD, gastroesophageal reflux disease; COPD, chronic obstructive pulmonary disease.

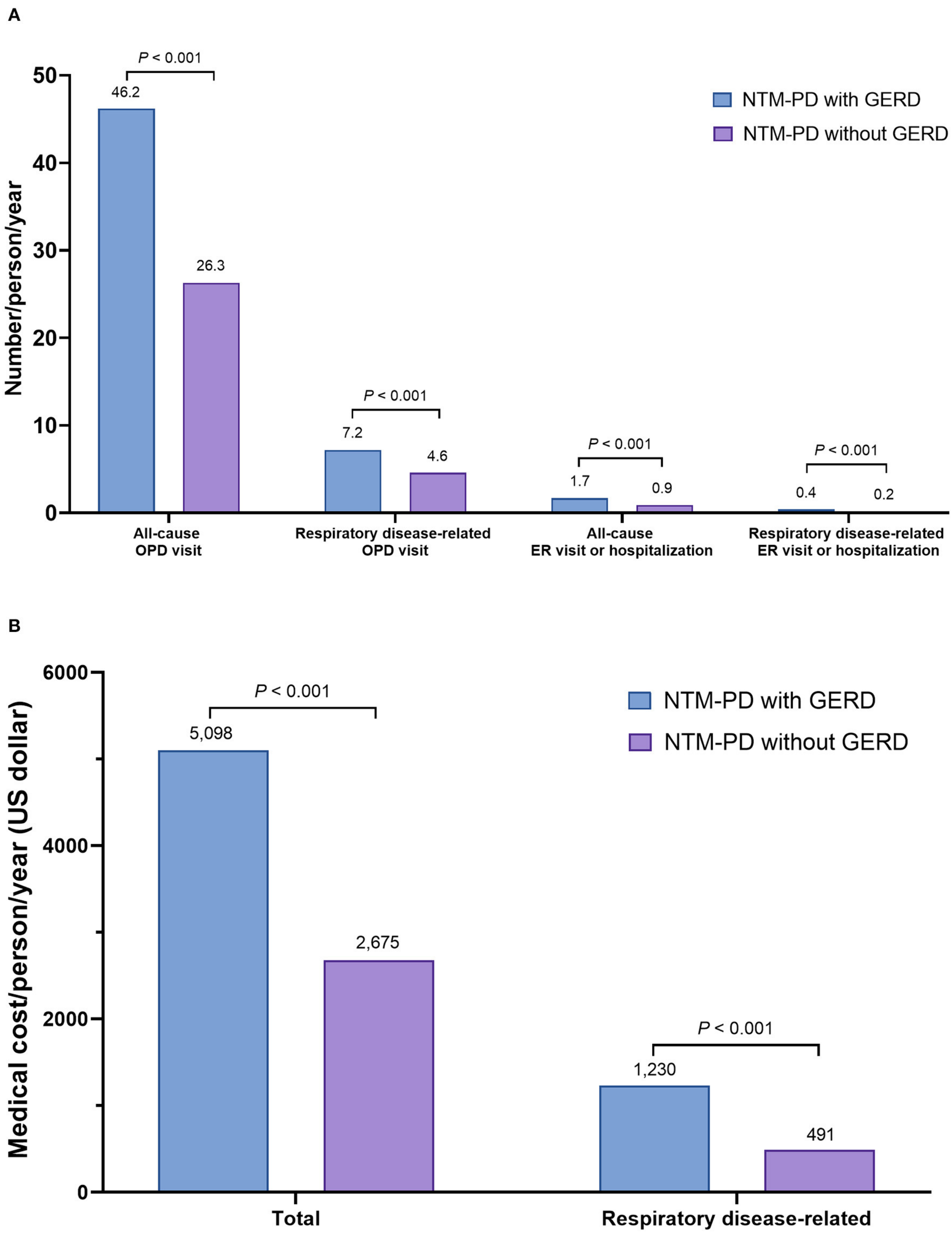

FIGURE 1 | Disease burden of GERD in patients with NTM-PD: **(A)** healthcare use of patients with NTM-PD according to presence or absence of GERD (number of events/person/year), and **(B)** medical costs of patients with NTM-PD according to presence or absence of GERD (medical cost/person/year, unit US dollar). NTM-PD, non-tuberculous mycobacterial pulmonary disease; GERD, gastroesophageal reflux disease; OPD, outpatient department; ER, emergency room.

fibrosis] or Q33.4, Q89.3 [congenital bronchiectasis]), pulmonary tuberculosis (TB; A15–19), and lung cancer (C33–C34). Extrapulmonary comorbidities were defined as cerebrovascular disease (G45–G46, I60–I69, and H34.0), hypertension (I10–I15), angina or myocardial infarction (MI; I20, I21, I22, and I25.2), congestive heart failure (I43, I50, I09.9, I11.0, I25.5, I13.0, I13.2,

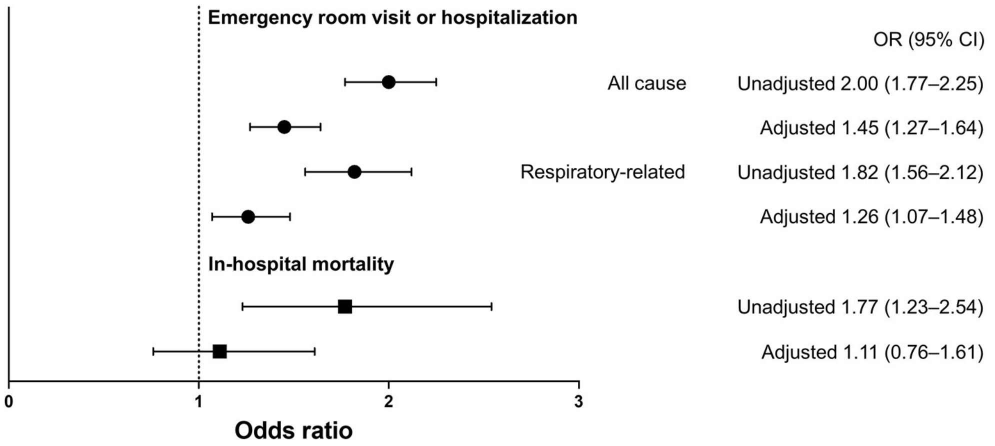

FIGURE 2 | Odds ratio and 95% confidence interval for emergency room visits, hospitalization, and in-hospital mortality in NTM-PD patients with GERD relative to those without GERD. The multivariable model was adjusted for age, sex, type of insurance (health insurance, medical aid, and veteran status), bronchiectasis, and Charlson comorbidity index (0–1 and 2). NTM-PD, non-tuberculous mycobacterial pulmonary disease; GERD, gastroesophageal reflux disease; ER, emergency room; OR, odds ratio; CI, confidence interval.

I42.0, I42.5–I42.9, and P29.0), inflammatory bowel disease (K50–K51), diabetes mellitus (E10–E14), chronic kidney disease (N18), and connective tissue disease (M05, M06, M315, M32, M33, M34, M351, M353, and M360) (5, 8). The Charlson comorbidity index (CCI) was calculated using a modified version consisting of 17 comorbidities (9).

Statistical Analysis

We calculated the age-adjusted prevalence of GERD among patients with NTM-PD by dividing the number of events by 1,000 person-years. All categorized variables were compared using Pearson's chi-squared test. To compare the medical use of NTM-PD patients with and without GERD, logistic regression analysis was used to determine the odds ratio (OR) for healthcare use (ER visits or hospitalizations) and in-hospital mortality in NTM-PD patients with GERD relative to those without GERD. A multivariable model was adjusted for age, sex, type of insurance (health insurance, medical aid, and veteran status), bronchiectasis, and CCI (0–1 and ≥2). All statistical analyses were performed with SAS 9.4 (SAS Institute, Cary, NC, USA). Statistical significance was set at $P < 0.05$.

RESULTS

Baseline Characteristics

The baseline characteristics of the patients are summarized in **Table 1**. The prevalence of NTM-PD in patients with GERD was significantly higher in older age groups (≥60 years [60.4 vs. 42.8%], $P < 0.001$). However, there was no difference in the sex ratio (∼62% of the cases were female). The proportion of patients who received medical aid was higher in the NTM-PD with GERD group than in those without GERD (7.3 vs. 3.8%, $P < 0.001$). Analysis of comorbidities indicated that the rates of asthma (40.9 vs. 25.7%), chronic obstructive pulmonary disease (37.5 vs. 23.0%), bronchiectasis (28.9 vs. 24.1%), hypertension (44.1 vs. 27.6%), diabetes mellitus (34.0 vs. 19.0%), cerebrovascular disease (15.7 vs. 7.8%), connective tissue disease (12.3 vs. 4.5%), and CCI ≥ 2 (78.2 vs. 49.0%) were significantly higher in NTM-PD patients with GERD than in those without GERD ($P < 0.001$ for all).

Prevalence of GERD in Patients With NTM-PD

Of the 6,589 patients with NTM-PD, 1,407 had GERD. During the study period, the prevalence of GERD in patients with NTM-PD was 21.4%, which was higher than that in patients aged ≥20 years (7.4%) in the HIRA-NPS database. The prevalence of GERD in patients with NTM-PD increased with age.

Healthcare Use and Medical Costs According to the Presence or Absence of GERD

NTM-PD patients with GERD had significantly higher healthcare use, including all-cause and respiratory disease-specific OPD visits and ER visits or hospitalizations, than those without GERD ($P < 0.001$ for all). Furthermore, NTM-PD patients with GERD spent a higher total on medical costs (5,098 vs. 2,675 USD/person/year), including respiratory disease-related costs (1,230 vs. 491 USD/person/year) ($P < 0.001$ for both) (**Figure 1**).

Association Between GERD and Increased Healthcare Use

GERD was independently associated with more ER visits or hospitalizations in patients with NTM-PD: all-cause (adjusted OR, 1.45; 95% confidence interval [CI], 1.27–1.64) and respiratory disease-related (adjusted OR, 1.26; 95% CI, 1.07–1.48). However, GERD was not significantly associated with in-hospital mortality (adjusted OR 1.11, 95% CI, 0.76–1.61) (**Figure 2**).

DISCUSSION

To our knowledge, this study is the first to evaluate the impact of GERD on the estimated disease burden based on healthcare use, medical costs, and in-hospital mortality in patients with NTM-PD. NTM-PD patients with GERD had significantly higher healthcare use and medical costs than those without GERD; however, there were no significant intergroup differences in in-hospital mortality.

GERD is prevalent in patients with NTM-PD, affecting up to 44% of patients (3, 4). In agreement with previous reports, 21% of patients with NTM-PD had GERD, which was significantly higher than the prevalence of GERD (7%) in the overall population aged ≥20 years during the study period. GERD has been recognized not only as an underlying disease for the occurrence of NTM-PD (10) but also as an important comorbidity that can influence the symptoms and severity of the disease (3, 10). In this regard, this study revealed NTM-PD patients with GERD are hampered by greater healthcare use and higher medical costs, which denote a significant disease burden, compared to those without GERD.

Gastro-esophageal refluxate can aggravate respiratory disease by either stimulating the sensitized esophageal-bronchial neuronal pathway or by microaspiration, which involves aspiration into the airway (11). Conversely, it has been suggested that progression of chronic lung disease can lead to deterioration of GERD (12). Consequently, it can be postulated that the interaction between the two diseases may further deteriorate the health of patients with NTM-PD and GERD. Considering the potential bidirectional process between reflux disease and chronic respiratory disease (13), appropriate treatment of GERD may lead to decreased disease burden and improvement in overall treatment outcomes in patients with NTM-PD. However, since acid reduction cannot reduce refluxate other than acid, there is a possibility that non-acid refluxate can still cause lung damage. Thus, it should be acknowledged that it is challenging to reduce reflux itself.

Our study has several strengths that support our hypothesis and was based on a large, nationally representative database. However, there are some limitations to this study. First, we used a cross-sectional study design; thus, long-term mortality was not fully evaluated based on the presence or absence of GERD. Second, there is a possibility of misclassification because we used the ICD-10 codes to define NTM-PD and GERD. Therefore, a large prospective cohort study is needed to clarify the role of GERD in NTM-PD.

In conclusion, GERD significantly increased healthcare use and medical costs in patients with NTM-PD. Thus, appropriate diagnostic and management plans to reduce the GERD-associated disease burden would bring significant improvements to patients with NTM-PD.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://opendata.hira.or.kr/op/opc/selectPatDataAplInfoView.do.

AUTHOR CONTRIBUTIONS

HL and HC were responsible for the conception and design of the study. TK, JHY, BY, JR, CKY, YK, JWS, HL, and HC undertook the analysis and interpretation of the data. TK, JHY, BY, HL, and HC drafted the manuscript. All authors made a critical revision of the manuscript, and read and approved the final manuscript.

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ORIGINAL RESEARCH
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Trends in Influenza Vaccination Rates in Participants With Airflow Limitation: The Korea National Health and Nutrition Examination Survey 2007–2018

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Background: Influenza vaccination is strongly recommended for people with chronic lung diseases, including chronic obstructive pulmonary disease, to reduce risk of exacerbation. We assess the influenza vaccination rate and its related factors in participants with airflow limitation (AFL) using nationally representative data in Korea.

Methods: We conducted a cross-sectional study from the Korea National Health and Nutrition Examination Survey from 2007 to 2018. Individuals $\geq$ 40 years who underwent spirometry and had identifiable information on influenza vaccination status were included.

Results: Overall influenza vaccination coverage was 61.2% in participants with AFL and 41.8% in participants without AFL. Age had a significant impact on the yearly vaccination rate in participants with AFL. Over the 10 years of study period, while the yearly vaccination rate steadily increased from 58.3 to 61.9% in elderly participants ($\geq$65 years) with AFL (p for trend = 0.117), the yearly vaccination rate decreased from 41.5% to 30.8% in younger participants (<65 years) (p for trend = 0.038). In multivariable analyses, younger age [adjusted odds ratio (OR) for unvaccinated = 0.88, 95% confidence interval (CI) = 0.87–0.90], male (adjusted OR = 1.64; 95% CI = 1.23–2.19), and current smokers (adjusted OR = 1.42, 95% CI = 1.01–2.00) were associated with increased odds of being unvaccinated.

Conclusions: The vaccination rate in participants with AFL affected by age. Younger age, male sex, and current smoking were associated with unvaccinated status. More attention and targeted interventions are required to improve the influenza vaccination rate in those with AFL.

Keywords: influenza, vaccination, airflow limitation, risk factor, National Health and Nutrition Examination Survey (NHANES)

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), which is a representative disease with airflow limitation (AFL), is the leading causes of death worldwide, with a prevalence of 5.6% in 2015, which is expected to increase to 7.8% by 2030 (1, 2). COPD is a chronic inflammatory airway disease characterized by fixed airflow limitation and chronic respiratory symptoms, such as cough, sputum, and progressive dyspnea. Acute exacerbation of COPD (AECOPD) can occur during the natural course of disease (3). AECOPD not only affects an individual's physical health status but also decreases lung function and increases future risk of exacerbation and even mortality (4–6). AECOPD also increases medical expense and resource use, causing increased socioeconomic burden (7).

AECOPD is a heterogeneous event thought to be caused by complex interactions between the host, respiratory viruses or bacteria, and environmental pollution (8, 9). The most common causes of AECOPD are respiratory infections, most of which are viral. Although the most frequent viruses associated with exacerbation are human rhinoviruses (8), influenza is also important, accounting for up to 28% of COPD exacerbations (10). Influenza can lead to hospitalization, frequent exacerbation, and even death in patients with COPD (11, 12). The disease course of influenza is worse in patients with COPD compared with those without COPD.

Influenza vaccination is the main strategy for prevention and control of seasonal influenza (13). The influenza vaccination has been shown to reduce AECOPD, influenza-related hospitalization, and mortality (14, 15). Thus, seasonal influenza vaccination is recommended to stable COPD patients from groups A to D classified by combining exacerbation history and severity of dyspnea suggested by Global Initiative for Chronic Obstructive Lung Disease (GOLD) (16). Despite this recommendation, it is not known whether influenza vaccination coverage has increased among COPD patients because most studies evaluated vaccination rates for a certain year (17–20). Such information is even scarcer within the past 10 years (21).

In addition to COPD, influenza vaccination should be considered in relation to other respiratory diseases including asthma and bronchiectasis. A previous meta-analysis found that influenza vaccination reduced febrile illnesses and prevented asthma attacks requiring emergency visits and hospitalizations (22). Despite limited studies regarding the efficacy of influenza vaccination in bronchiectasis, international bronchiectasis guidelines strongly recommend influenza vaccination in patients with bronchiectasis (23, 24). Consequently, more studies are needed to evaluate the factors associated with influenza vaccination coverage among individuals with AFL to develop ways to encourage vaccination and improve their health outcomes.

In this study, we measure the influenza vaccination rate among participants with AFL in a nationally representative sample of Korea, and assess factors associated with influenza vaccination.

METHODS

Study Population

For this study, cross-sectional data were used from the Korea National Health and Nutrition Examination Survey (KNHANES), which provides nationwide statistical data on the Korean population's health and diet from January 2007 to December 2018. The questionnaire on the influenza vaccination was investigated for only 3 months in 2013 and was not disclosed to the public; thus, data from 2013 were not included in this study. The KNHANES uses a complex, stratified, multistage probability cluster sampling design with sampling units of households based on geographic region, age, and gender. A health-related interview, nutrition survey, and physical examination were performed for each participant selected throughout Korea by trained interviewers. All participants agreed to participate in this study. Since publicly available data were used, ethical approval was waived. The KNHANES surveys were approved by the relevant institutional review boards, and informed consent was provided by all participants.

Because spirometry is only performed in individuals older than 40 years, we only included participants ≥40 years in the analyses. Participants who did not undergo spirometry and those who had missing data for influenza vaccination were excluded from the study.

Of the total 38,247 participants, influenza vaccination data were available for 34,464, of whom 4,873 (14.1%) had AFL on spirometry and 29,591 (85.9%) did not. Finally, this study included 4,873 participants with AFL, comprising those without influenza vaccination (n = 1,890) and those with influenza vaccination (n = 2,983) (**Figure 1**).

Exposure

The main exposure variable was influenza vaccination, which was determined by the response to a question about influenza vaccination status during the previous 1 year: "Have you been vaccinated for influenza during the previous 1 year?"

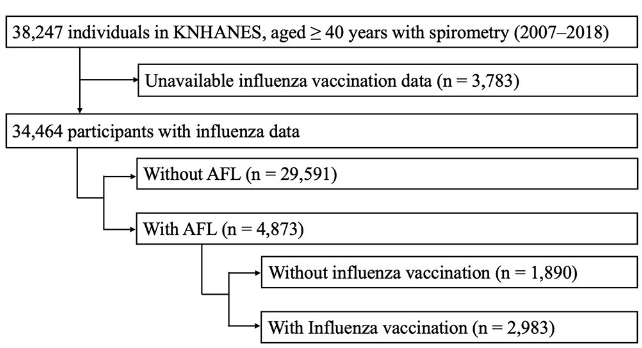

FIGURE 1 | Selection of study population. AFL, airflow limitation; KNHANES, Korea National Health and Nutrition Examination Survey.

Study Outcomes

The study outcomes were (1) influenza vaccination rate according to presence or absence of AFL during the study period; (2) influenza vaccination rate in participants with AFL stratified by age and AFL severity; and (3) factors associated with vaccination status in participants with AFL.

To reduce the disease burden of influenza, the Korean government instituted a national immunization program in 2005, which provides free influenza vaccination for people aged $\geq$ 65 years. Accordingly, we divided subjects into two age groups (65 years or older vs. under 65 years).

AFL was defined as spirometry revealing forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7. Severity of AFL was classified according to the percentage of the predicted FEV_1 (% pred): mild (FEV_1 %pred of >70), moderate (FEV_1 %pred of 60–69), moderately severe (FEV_1 %pred of 50–59), severe (FEV_1 %pred of 35–49), and very severe (FEV_1 %pred of <35) (25).

Covariates

The KNHANES provides various demographic data [age, sex, body mass index (BMI), education level, marital status, and self-perceived income status] and spirometry results. The Korean version of the EuroQol-5 dimensions questionnaire (EQ-5D), a simple health-related quality of life instrument consisting of 5 health dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression), was used to measure QoL status (26, 27). Several comorbid conditions were also included in this study. The presence of hypertension was determined by high blood pressure (mean systolic blood pressure $\geq$140 mmHg or mean diastolic blood pressure $\geq$90 mmHg on examination or current intake of antihypertensive medications). Hypercholesterolemia was defined as total cholesterol >200 mg/dL or current intake of lipid-lowering medications (28). Diabetes was defined as fasting glucose >126 mg/dL or HbA1c >6.5% or current use of oral hypoglycemic agents or insulin for glycemic control (29). The presence of other comorbid conditions was determined by a positive response to the following two questions: "Have you been diagnosed with [disease] by a doctor?" or "Do you take medicine or treatment for [disease]?" (30).

Statistical Analyses

Data are presented as medians and interquartile ranges for continuous variables and as frequencies (percentages) for categorical variables. Data were compared using the Mann–Whitney U test for continuous variables because of non-normality. Continuous variables were compared using Pearson's chi-squared test or Fisher's exact test, as appropriate. For the analyses of influenza vaccination rate by AFL severity and multivariable logistic regression to evaluate factors associated with not receiving influenza vaccination, we classified the severity of AFL into four groups [mild (FEV_1 %pred of >70), moderate (FEV_1 %pred of 60–69), moderately severe (FEV_1 %pred of 50–59), and severe to very severe (FEV_1 %pred of <50)] because there only 1.3% of participants (n = 64) had severe to very severe AFL (FEV_1 %pred of <50) in the NHANES dataset. In the multivariable logistic regression model, we adjusted for age, sex, BMI, marital status, type of medical insurance, economic activity, education level, EQ-5D, chronic bronchitis, smoking history, the severity of AFL, comorbidities (hypertension, ischemic heart disease, diabetes mellitus, and asthma). All tests were two-sided, and a p-value $\leq$ 0.05 was considered statistically significant. Statistical analyses were performed using STATA software (ver. 16; StataCorp, College Station, TX, USA).

RESULTS

Influenza Vaccination Rate According to Presence of Airflow Limitation

Influenza vaccination trends from 2007 to 2018 are shown in **Figure 2**. Compared to 61.2% of participants with AFL (2,983/4,873) being vaccinated, 41.8% participants without AFL (12,357/29,591) were vaccinated.

Figure 3 shows the influenza vaccination trends according to AFL status based on age. While the overall vaccination rate in participants 65 years or older was ~80% (**Figure 3A**), the vaccination rate in participants younger than 65 years was <40% (**Figure 3B**). AFL had a significantly different impact on the age-stratified vaccination rate. In participants 65 years or older, the overall vaccination rate (80.2% with AFL vs. 80.3% without AFL; p = 0.894) was not different by AFL. Regardless of AFL, the yearly vaccination rate steadily increased during the study period [58.3% in 2007 to 61.9% in 2018 (p for trend = 0.117) in participants with AFL; 40.2% in 2007 to 44.7% in 2018 (p for trend <0.001) in participants without AFL; **Figure 3A**].

However, among individuals 65 years or less, the vaccination rate was higher in participants with AFL than in those without AFL (34.7% with AFL vs. 29.2% without AFL; p < 0.001). Additionally, while there was a decreasing trend in the yearly vaccination rate among participants with AFL, the vaccination rate in subjects without AFL fluctuated [41.5% in 2007 to 30.8% in 2018 (p for trend = 0.038) for participants with AFL; 28.6% in 2007 to 29.9% in 2018 (p for trend < 0.001) in participants without AFL; **Figure 3B**].

Comparison of Clinical Features in Participants With AFL According to Influenza Vaccination

Differences in clinical characteristics according to influenza vaccination status in participants with AFL are presented in **Table 1**. Vaccinated participants tended to be older and were less likely to be male and current smokers compared with unvaccinated participants (p < 0.001 for both). Regarding socioeconomics, vaccinated participants had lower economic activity and lower QoL compared with unvaccinated participants (p < 0.001 for both). Comorbid conditions including hypertension, ischemic heart disease, hypercholesterolemia, diabetes mellitus, cerebrovascular disease, asthma, and osteoporosis were more common among vaccinated participants than unvaccinated participants. Except for FEV_1

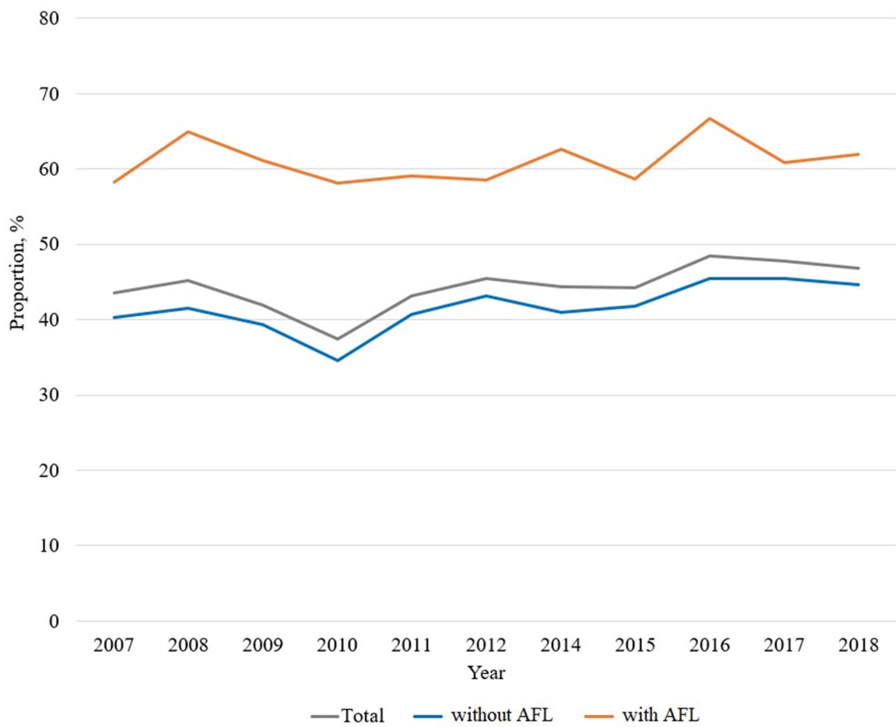

FIGURE 2 | Influenza vaccination trends in participants with AFL. AFL, airflow limitation.

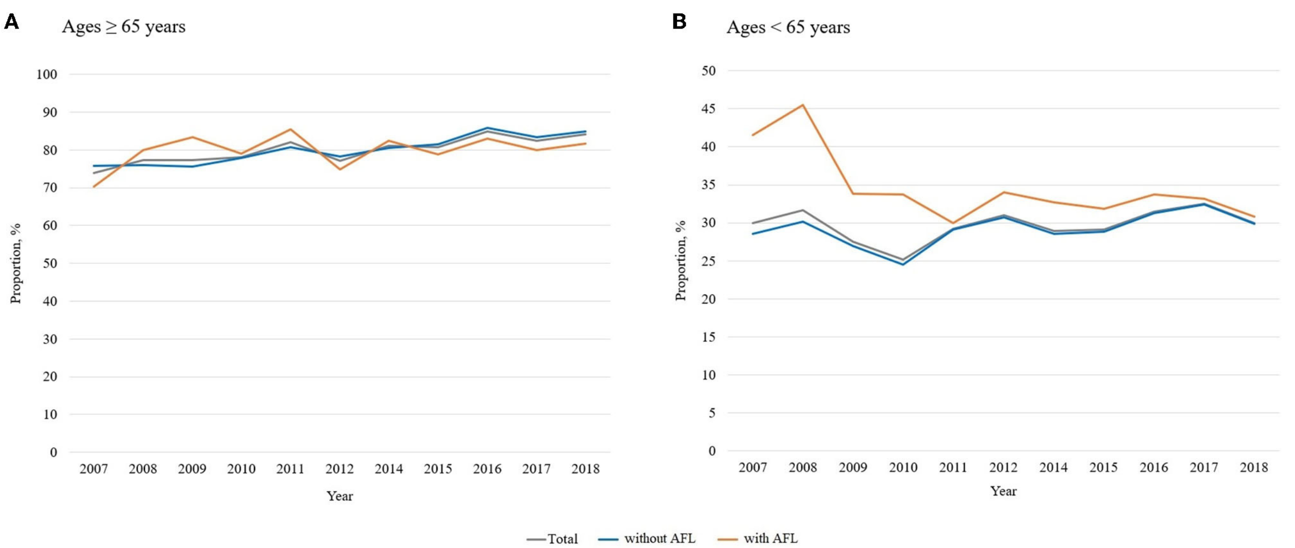

FIGURE 3 | Influenza vaccination trends according to age (≥65 vs. <65 years). AFL, airflow limitation. **(A)** Age ≥ 65 years, **(B)** Age < 65 years. AFL, airflow limitation.

% predicted, vaccinated participants had lower lung function parameters [FEV_1 (L), FVC (L), FVC % pred, and FEV_1/FVC] compared with unvaccinated participants ($p < 0.001$ for the latter four variables).

Influenza Vaccination Rate According to AFL Severity

Influenza vaccination rate was further analyzed according to AFL severity. The overall vaccination rates were 59.8% (2,027/3,392),

TABLE 1 | Comparison of clinical features in participants with airflow limitation according to influenza vaccination.

	Participants with airflow limitation (*N* = 4,873)		
	Without influenza vaccination (*n* = 1,890)	**With influenza vaccination (*n* = 2,983)**	***P*-value**
Age (years)	60 (53–66)	70 (65–75)	<0.001
Male sex	1,449 (76.7)	2,061 (69.1)	<0.001
Current smoker	883 (47.1)	1,009 (34.1)	<0.001
Smoking amount (pack-years) (*n*=3,182)	5 (0–26)	0 (0–24.5)	0.004
Body mass index, kg/m^2	23.5 (21.6–25.5)	23.5 (21.7–25.3)	0.782
Education beyond high school	1,107 (59.1)	1,149 (38.8)	<0.001
Marriage status (yes)	1,853 (98.2)	2,953 (99.2)	0.001
Economic activity status	1,257 (66.9)	1,352 (45.5)	<0.001
Health insurance	1,798 (95.8)	2,791 (94.4)	0.027
Symptoms (*n* = 3,558)			
Cough ≥3 months	45 (3.2)	88 (4.1)	0.207
Sputum ≥3 months	108 (7.8)	206 (9.5)	0.075
Chronic bronchitis	118 (8.5)	223 (10.3)	0.076
Quality of life, EQ-5D	1 (0.907–1)	1 (0.817–1)	<0.001
Comorbidities			
Hypertension (*n* = 3,576)	667 (51.5)	1,472 (64.5)	<0.001
Ischemic heart disease (*n* = 2,383)	53 (6.0)	152 (10.2)	<0.001
Hypercholesterolemia (*n* = 4,559)	298 (16.8)	571 (20.5)	0.002
Diabetes mellitus (*n* = 4,130)	853 (54.3)	1,512 (59.1)	0.003
Cerebrovascular disease (*n* = 2,354)	32 (3.6)	88 (6.0)	0.012
Asthma (*n* = 2,612)	63 (6.6)	200 (12.1)	<0.001
Osteoporosis (*n* = 1,958)	25 (3.5)	141 (11.4)	<0.001
Tuberculosis (*n* = 2,663)	49 (4.9)	87 (5.3)	0.633
Depression (*n* = 2,602)	26 (2.7)	61 (3.7)	0.151
Any cancer history* (*n* = 1,361)	33 (3.8)	52 (3.5)	0.757
Spirometry			
FEV_1, L	2.45 (1.96–2.90)	2.07 (1.60–2.49)	<0.001
FEV_1, % pred	78.2 (68.6–86.6)	77.6 (66.5–87.6)	0.287
FVC, L	3.81 (3.12–4.44)	3.32 (2.66–3.90)	<0.001
FVC, % pred	91.9 (82.3–100.6)	87.2 (76.9–96.9)	<0.001
FEV_1/FVC	66.0 (61.9–68.3)	65.0 (59.8–67.9)	<0.001

Data are presented as median (interquartile range) or number (percentage). The numbers in brackets for each comorbidity represent the number of participants who responded to the questions regarding each comorbidity.

**Includes gastric, liver, colon, breast, cervical, lung, or thyroid cancer.*

EQ-5D, EuroQol-5 dimensions questionnaire; FEV_1, forced expiratory volume in 1s; % pred, percentage of the predicted value; FVC, forced vital capacity.

61.7% (484/784), 66.6% (273/410), and 69.3% (199/287) in participants with mild, moderate, moderately severe, and severe to very severe AFL subjects, respectively (**Figure 4**; $p = 0.001$).

Factors Related to Non-vaccination in Participants With AFL

As shown in **Table 2**, in multivariable analyses, younger age [adjusted odds ratio (OR) for being unvaccinated = 0.88, 95% confidence interval (CI) = 0.87–0.90], male (adjusted OR = 1.70; 95% CI = 1.21–2.42), and current smoking (adjusted OR = 1.42, 95% CI = 1.01–2.00) were associated with increased odds for being unvaccinated. Although participants with more severe AFL were more likely to be vaccinated according to the univariable analysis, the severity of AFL was not related to vaccination status in multivariable analyses.

DISCUSSION

This study evaluated influenza vaccination trends from 2007 and 2018 among participants with AFL in Korea. During the study period, the overall influenza vaccination rate was higher in participants with AFL than in those without AFL. While the influenza vaccination rate in the elderly with AFL steadily increased similar to that of those without AFL, the rate in young participants with AFL decreased, while the rate among young participants without AFL fluctuated. In multivariable analyses,

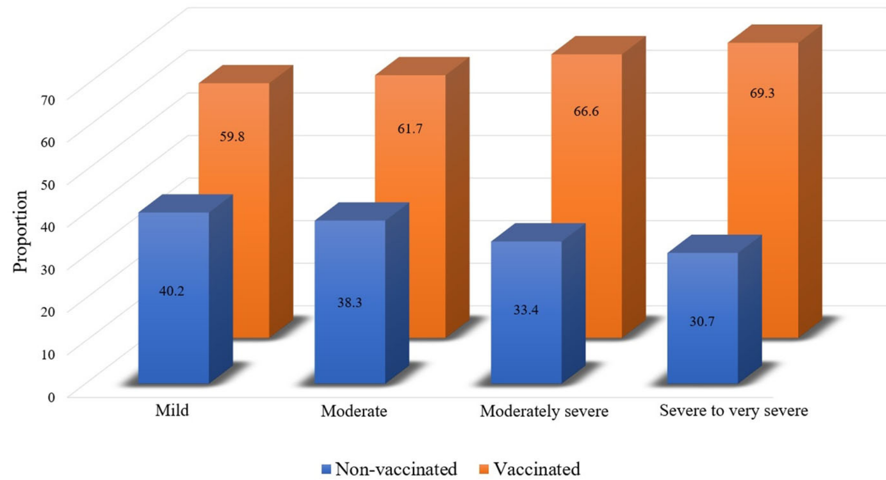

FIGURE 4 | Influenza vaccination rate according to AFL severity. AFL, airflow limitation.

younger age, male, and current smoking were associated with increased odds of unvaccinated status.

Influenza vaccination has been considered as important management in individuals with chronic airway diseases with AFL, such as COPD, asthma, and bronchiectasis. The current guidelines strongly recommend influenza vaccination in COPD. Influenza vaccination is known to reduce not only acute exacerbations but also serious lower respiratory infections in COPD participants. The benefits of influenza vaccination in participants with COPD extend beyond the prevention of respiratory illness. Studies have shown that influenza vaccination can reduce mortality and risk of ischemic heart disease, especially in the elderly (31). Furthermore, chronic comorbidities are highly prevalent in patients with COPD and are associated with frequent exacerbations and increased risk of exacerbation (32). Hence, influenza vaccination should be emphasized in patients with AFL and other comorbidities.

The vaccination rate in patients with COPD varies widely by country. While the rates of influenza vaccination in patients with COPD were relatively high in the US (∼64%) (21), Spain (∼60%) (17, 18), and Korea (∼60% in this study), the vaccination rate was relatively low in Hungary (24%) (19), Taiwan (32%) (33), and Turkey (40%) (20). Unfortunately, few studies have evaluated influenza vaccination coverage within a specific country. In our study, the influenza vaccination rate was relatively stable, suggesting more effort is needed to increase coverage in patients with COPD in Korea. Because the current data suggest that vaccination rates can vary widely, we suggest that studies evaluating vaccination rates over time would be informative to set country-specific vaccination strategies for COPD patients.

Regarding asthma, a previous meta-analysis demonstrated influenza vaccination reduced febrile illness by 72% and prevented 59–78% of cases of acute exacerbation of asthma requiring emergency visits and/or hospitalizations (22), suggesting influenza vaccination could be a cost-effective strategy to reduce acute exacerbation of asthma. Although there is limited evidence directly showing the effectiveness of vaccination in patients with bronchiectasis, influenza virus may play a crucial role in triggering exacerbation of bronchiectasis (34), which supports providing influenza vaccination to this population (23, 24). However, to provide solid evidence for influenza vaccination in patients with bronchiectasis, future studies evaluating the role of influenza vaccination in bronchiectasis are needed.

Importantly, our study results suggested whom clinicians should persuade to receive influenza vaccination, which includes younger patients, males, and current smokers. As shown in previous studies, the vaccination rate was significantly lower in the younger population with AFL than in the older population with AFL (17, 20, 21, 33, 35). Additionally, younger age was independently associated with unvaccinated status in participants with AFL. Beyond this, we further performed a trend analysis for vaccination over 10 years according to age group, which has not been performed in previous studies. While the vaccination rate in elderly subjects with AFL had a steadily increasing trend, the rate showed a decreasing trend. The reasons for this phenomenon are not clear. For a possible reason, young participants with AFL are likely to have unhealthy lifestyles, while their symptoms are mild or absent in the early stage of AFL. Accordingly, the importance of influenza vaccination is likely to be neglected in this population. This information suggests the importance of age in predicting influenza vaccination in those with AFL. We need to pay more attention to increasing the vaccination rate in the young population with AFL.

Consistent with previous studies, our study revealed that males and current smokers are more likely to be unvaccinated among participants with AFL. Generally, males are more likely to be current smokers than females, especially in Asian countries, including Korea (30). Furthermore, being a male and a smoker increases the risk of severe disease presentation, including mortality (36–38). Thus, it is plausible that such male patients who also have AFL have a much higher risk of worse prognosis

TABLE 2 | Factors associated with non-vaccination against influenza in participants with airflow limitation.

Variables	Univariable	Multivariable*
	OR (95% CI)	**OR (95% CI)**
Male sex	1.47 (1.29–1.68)	1.70 (1.21–2.42)
Age, year	0.89 (0.88–0.90)	0.88 (0.87–0.90)
Married state	0.43 (0.26–0.73)	2.33 (0.85–6.40)
Medicaid	0.73 (0.56–0.97)	0.85 (0.47–1.52)
Economic activity	2.42 (2.15–2.73)	0.96 (0.75–1.22)
Education beyond high school	2.28 (2.01–2.57)	1.15 (0.91–1.45)
EQ-5D	8.47 (5.21–13.78)	0.57 (0.21–1.50)
Body mass index, kg/m^2	1.00 (0.98–1.02)	0.97 (0.93–1.01)
Chronic bronchitis	0.81 (0.64–1.02)	0.89 (0.57–1.37)
Current smoker	1.79 (1.55–2.05)	1.42 (1.01–2.00)
Severity of airflow limitation†		
Mild (FEV_1 %pred of >70)	Reference	Reference
Moderate (FEV_1 %pred of 60–69)	0.92 (0.78–1.08)	1.07 (0.78–1.49)
Moderately severe (FEV_1 %pred of 50–59)	0.75 (0.60–0.93)	1.14 (0.77–1.69)
Severe to very severe (FEV_1%pred of <50)	0.66 (0.51–0.85)	1.22 (0.75–1.97)
Comorbidities		
Hypertension	0.58 (0.51–0.67)	0.91 (0.72–1.16)
Ischemic heart disease	0.56 (0.41–0.78)	0.80 (0.47–1.34)
Diabetes mellitus	0.82 (0.72–0.93)	0.92 (0.70–1.22)
Asthma	0.51 (0.78–0.68)	0.61 (0.33–1.13)

Data are presented as ratios (95% confidence intervals).

† *Severity of airflow limitation was graded according to ATS/ERS guideline (25), and severe and very severe were combined into one group due to the small number of participants with severe to very severe AFL (n = 64).*

* *Multivariate analysis was adjusted for age, sex, smoking PY, marriage status (married vs. unmarried or divorced), economic activity (active vs. inactive), education level (college or above vs. high school or less), EQ-5D, severity of airflow limitation, and comorbid conditions (hypertension, ischemic heart disease, diabetes mellitus, and asthma).*

OR, odds ratio; EQ-5D, EuroQol-5 dimensions questionnaire; FEV_1, forced expiratory volume in 1s; % pred; percentage of the predicted value.

than those without these risk factors. However, our study results alone cannot explain sex differences in vaccination rates. We believe that an unhealthy lifestyle in men may have influenced this observation. Additionally, there is a possibility that women might be more compliant with clinicians' recommendations for influenza vaccination than men, as shown by the sex difference in medication adherence in Korea (39).

There have been conflicting results on the relationship between AFL severity and influenza vaccination rate. While a lower rate of vaccination was observed in subjects with more severe AFL (17), other studies showed a positive association between AFL severity and influenza vaccination rate (18–20). Similarly, our study showed a positive association between AFL severity and vaccination rate, although it diminished after adjustment of covariables. Given that severe AFL can be associated with severe pneumonia in patients with AFL (40), and the protective effects of influenza vaccination seem to be correlated with AFL severity (13), strategies focusing on improving the vaccination rate in patients with severe AFL might be more cost-effective.

Our findings yield important insights that can be helpful to design targeted strategies to increase influenza vaccination coverage in patients with AFL. The identification of factors (younger age, male, and current smokers) associated with unvaccinated status can help design tailored strategies to increase influenza vaccination in patients with AFL. We also suggest a change in strategy of influenza vaccination policy for adults in Korea. Currently, the Korean government-led free influenza vaccination policy for adults is a one-size-fits-all strategy that is applied to all subjects aged 65 or older, and it does not consider risk factors other than age. Thus, a large number of young patients with AFL who are at a high risk of influenza have not been considered for the benefit of government-led free vaccination programs. However, given the broad benefits of influenza infection on respiratory diseases with AFL, a more advanced and personalized free vaccination strategy that estimates individual risk might be helpful, and it could include young patients with AFL.

There are limitations to our study that should be acknowledged. First, this study was performed in Korea, limiting the generalizability of our findings. Second, because KNHANES does not have data on post-bronchodilator spirometry, we defined AFL using pre-bronchodilator spirometry. However, prebronchodilator spirometry has been widely used to define AFL in many previous studies of COPD epidemiology (30, 41, 42). Third, this study could not evaluate the presence of bronchiectasis, which may be an important comorbidity to consider when interpreting the results of our study. This was because a questionnaire on bronchiectasis was only available in the 2007–2009 NHANES dataset. Fourth, we could not provide a reasonable explanation for our observation of the relationship between socioeconomic and influenza vaccination status in participants with AFL. Poor socioeconomic status, such as low education level, inactive economic status, and reduced QoL, showed increased odds of non-vaccination in the univariable analysis but no significant relationship in multivariable analyses. Although the reasons are not clear, it is possible that the Korean government-led free influenza vaccination program attenuated the influence of these factors on influenza vaccination. The free influenza vaccination program in Korea targets people receiving Medicaid and the elderly population whose socioeconomic status and QoL are poor, which leads to disproportionally higher influenza vaccination rates in these subjects despite their poor socioeconomic status. Thus, the simultaneous consideration of these factors (age, Medicaid, economic activity, educational level, and QoL) might have yielded the non-significance of socioeconomic variables. As socioeconomic status and government health policies might differ between countries, our study results should be interpreted with caution in other countries.

In conclusion, over the past 10 years, the influenza vaccination rate in elderly participants with AFL steadily increased, while the rate in younger participants with AFL decreased. Younger participants, males, and current smokers were most likely to have unvaccinated status among those with AFL. More attention and targeted interventions are required to improve the influenza vaccination rate in individuals with AFL.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://knhanes.kdca.go.kr/knhanes/main.do.

ETHICS STATEMENT

This study used data from KNHANES which was approved by the Institutional Review Board of the Korea Centers for Disease Control (IRB No. 1401–047-547). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HL and YJ take responsibility for the data, analysis, and wrote the first draft of the manuscript. HL, HC, and YJ designed the study. YJ performed statistical analysis of data. All authors provided critical review and approved the version for publication.

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REVIEW
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Monitoring Long Term Noninvasive Ventilation: Benefits, Caveats and Perspectives

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Long term noninvasive ventilation (LTNIV) is a recognized treatment for chronic hypercapnic respiratory failure (CHRF). COPD, obesity-hypoventilation syndrome, neuromuscular disorders, various restrictive disorders, and patients with sleep-disordered breathing are the major groups concerned. The purpose of this narrative review is to summarize current knowledge in the field of monitoring during home ventilation. LTNIV improves symptoms related to CHRF, diurnal and nocturnal blood gases, survival, and health-related quality of life. Initially, patients with LTNIV were most often followed through elective short in-hospital stays to ensure patient comfort, correction of daytime blood gases and nocturnal oxygenation, and control of nocturnal respiratory events. Because of the widespread use of LTNIV, elective in-hospital monitoring has become logistically problematic, time consuming, and costly. LTNIV devices presently have a built-in software which records compliance, leaks, tidal volume, minute ventilation, cycles triggered and cycled by the patient and provides detailed pressure and flow curves. Although the engineering behind this information is remarkable, the quality and reliability of certain signals may vary. Interpretation of the curves provided requires a certain level of training. Coupling ventilator software with nocturnal pulse oximetry or transcutaneous capnography performed at the patient's home can however provide important information and allow adjustments of ventilator settings thus potentially avoiding hospital admissions. Strategies have been described to combine different tools for optimal detection of an inefficient ventilation. Recent devices also allow adapting certain parameters at a distance (pressure support, expiratory positive airway pressure, back-up respiratory rate), thus allowing progressive changes in these settings for increased patient comfort and tolerance, and reducing the requirement for in-hospital titration. Because we live in a connected world, analyzing large groups of patients through treatment of "big data" will probably improve our knowledge of clinical pathways of our patients, and factors associated with treatment success or failure, adherence and efficacy. This approach provides a useful add-on to randomized controlled studies and allows generating hypotheses for better management of HMV.

Keywords: non-invasive ventilation, chronic hypercapnic respiratory failure, monitoring, home ventilation, long term mechanical ventilation

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INTRODUCTION

Long term noninvasive ventilation (LTNIV) is widely accepted for treatment of chronic hypercapnic respiratory failure (CHRF) related to obstructive or restrictive disorders. Its development was triggered in the early 1980's by the report by Sullivan et al. of the use of CPAP for obstructive sleep apnea syndrome (1). Since then devices have undergone major evolutions, with a progressive shift from volume-cycled to pressure-cycled then multimodal devices, and appearance of auto-titrating modes which can adapt to changes in airway resistance, reactance, or compliance of the respiratory system and aim to ensure a target tidal volume and minute ventilation (2). One of the major contributions of medical engineering for these devices has been the development of built-in software, allowing to monitor in detail items such as compliance, leaks, tidal volume, residual respiratory events, or percentage of cycles triggered/cycled by the device (3–7). Moreover, for some devices, analysis of raw data for flow, pressure and even thoraco-abdominal movements on a breath-by-breath basis is possible. Strategies for monitoring LTNIV have also evolved: historically, elective evaluations of patients on LTNIV were initially most often hospital-based or even ICU-based; at present, they rely more and more on tools such as pulse oximetry, transcutaneous capnography ($PtcCO_2$) and built-in software that can be assessed at home (4). New developments in telemedicine and the growing contribution of health care providers make these data easily available, allowing to remotely assess quality of ventilation.

In this review, we will discuss the benefits, but also the possible pitfalls of monitoring patients under LTNIV, and perspectives as to future options. This review will focus on an adult population under LTNIV.

Why Monitor LTNIV?

The goals of long-term NIV (LTNIV) are to improve symptoms related to chronic hypercapnia and—when present—sleep-related breathing disorders (SRBD), to improve health-related quality of life (HRQL), decrease hospital admissions, correct hypoventilation and optimize SpO_2 in order to prevent the development of pulmonary hypertension and cor pulmonale, and improve survival (8). In several indications (chronic obstructive pulmonary diseases (COPD), amyotrophic lateral sclerosis (ALS), restrictive thoracic disorders), improvement of ABG and control of nocturnal hypoventilation are significantly associated with a better prognosis (9–13). In order to achieve these goals, it is mandatory to ensure that: 1/ LTNIV improves daytime and nocturnal arterial blood gases, 2/ compliance to LTNIV is sufficient to have a significant physiological effect, 3/ LTNIV does not generate *per se* undesired respiratory events or side effects and discomfort, and 4/ HRQL and symptom scores improve over time. Guidelines usually recommend at least one annual elective assessment for patients under LTNIV: the frequency of evaluations depends however on the underlying pathology, its severity and rate of progression, and may be required as often as every 3 months (8, 14, 15).

Monitoring LTNIV: A Step by Step Approach

Daytime and Nocturnal Blood Gases: Capillary, Transcutaneous or Arterial Samples

Normalizing daytime arterial blood gases (ABG) is a major physiological goal for patients under LTNIV (9, 10). Thus, performing daytime measurements of ABG—by puncture of the radial artery—is a standard procedure when monitoring LTNIV. Measurements are performed without NIV, at least 30 min after interrupting NIV (when possible), preferably in a sitting position. Capillary arterialized samples, taken at the earlobe after application of a vasodilating gel, are considered reliable surrogates of arterial ABG for pH and $PaCO_2$ (16–19). Results for PaO_2 are less reliable, with a possible underestimation by the capillary technique (20).

In stable chronic respiratory failure, pH is usually within a normal range (2, 21–24). The contribution of bicarbonates to the detection of residual nocturnal hypoventilation is debated because of the impact of a large panel of drugs and comorbidities on HCO_3 levels (25, 26). HCO_3 levels should not be used as an isolated criterion for detecting nocturnal hypoventilation, but can be indicative of nocturnal hypoventilation and should lead to nocturnal capnography if available.

Daytime ABG measurements under NIV are indicative of correction of $PaCO_2$ by NIV but have been shown to be a poor reflection of correction of nocturnal hypoventilation (27–29). Thus, assessing nocturnal pCO_2 is essential for identifying periods of hypoventilation related to leaks, undesired respiratory events including patient-ventilator asynchrony or inappropriate settings.

Nocturnal arterial punctures are a source of discomfort, awaken the patient, and are not recommended. Surrogates of nocturnal ABG assessment are end-tidal CO_2 ($ETCO_2$) and transcutaneous capnography ($TcPCO_2$). Reliability of $ETCO_2$ measurements is subject to V/Q mismatch, physiological dead space and ventilatory mode. Importantly, a normal $ETCO_2$ does not exclude hypercapnia, while an elevated $ETCO_2$ is strongly suggestive of hypercapnia but may underestimate actual $PaCO_2$ value (4, 30). Thus, the use of $ETCO_2$ as a surrogate measurement for nocturnal $PaCO_2$ is not recommended in most circumstances (4).

Transcutaneous capnography ($TcPCO_2$) is considered reliable for continuous (nocturnal) monitoring of $PaCO_2$ when patients are hemodynamically stable, and devices are used by an experienced team. A probe temperature of 42°C is tolerated for 8 h without any risk of skin burns in adults (31). Several studies have reviewed the performances of commercialized capnographs and show acceptable biases and limits of agreement (27, 29). A review of the recent literature on transcutaneous capnography suggested that bias values up to $\pm$ 1 kPa (7.5 mmHg), and limits of agreement up to $\pm$ 1.33 kPa (10 mmHg) were acceptable (27). $PtcCO_2$ however has a few drawbacks: 1/ its lag time which precludes the detection of very short events (32) and 2/ the occurrence of occasional errand values, largely dependent on the expertise of the users.

Consensual—although arbitrary—definitions for hypoventilation have been published by the American

Association for Sleep Medicine (33). However, minor changes in definitions of hypoventilation can have a major impact on clinical assessment (34–36).

Pulse oximetry has the advantage of simplicity and low cost: its major drawback is the lack of specificity of desaturations, which do not allow to distinguish hypoventilation from other causes of drops in SpO_2.

Contribution of Built-In Monitoring Devices

Compliance

Compliance is directly related to efficacy of NIV (on ABG and symptom control), and prognosis; it may also reflect patient discomfort, which impairs health related quality of life. Analysis of use of LTNIV (compliance) has 3 components: total time spent with the ventilator, pattern of use and evolution of these parameters (**Figure 1**). Total time spent under NIV has to be long enough for NIV to have a beneficial impact on daytime and nocturnal ABG, and, in some cases, on total energy expenditure (37, 38). It is usually considered that using NIV <3:30 h per 24 h is insufficient and does not allow resetting of respiratory centers and resting of respiratory muscles. Borel et al. described a "U-shaped curve" relationship between compliance and prognosis (39). Too low values provide insufficient benefit, while higher values indicate more severe respiratory impairment (39). In COPD, a meta-analysis by Struik et al. suggests that a daily use of NIV for at least 5 h is required to improve $PaCO_2$ (40). In neuromuscular disorders such as amyotrophic lateral sclerosis or myotonic dystrophy, there is a clear relation between adherence to treatment and survival (41–44). Thus, the best compromise between patient comfort and efficacy must be sought for. Pattern of use (fragmentation vs. continuous use) can be an indicator of discomfort and side-effects (leaks, pain or discomfort related to interface), co-morbidities (nycturia, use of diuretics) or symptomatic patient-ventilator asynchrony (**Figure 1**) (7, 45, 46). Evolution of total time spent under NIV and changes in pattern of use may be indicative of an exacerbation with a risk of hospital admission (47).

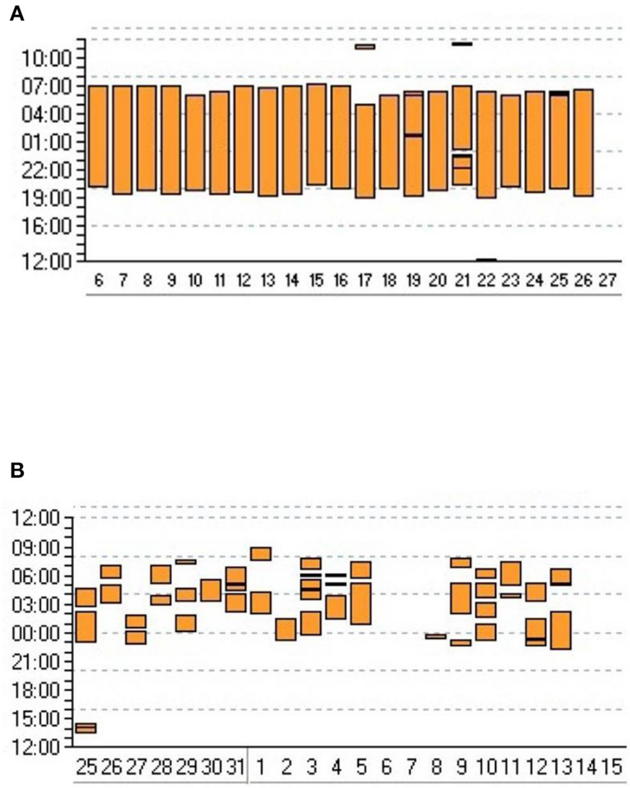

FIGURE 1 | Adherence to NIV. Graphic transcription of ventilator use provided by ventilator software. Y axis: time of the day/night; X axis: days. Each vertical bar represents time spent using the ventilator for a given day. **(A)** Very regular use suggesting that patient is adherent and comfortable with his/her treatment. **(B)** Frequent interruptions and irregular use of ventilator suggesting discomfort and/or comorbidity.

Leaks

Leaks in LTNIV are either intentional (i.e., related to exhalation valves to avoid rebreathing) or unintentional (i.e., caused for instance by a poorly adapted interface, too high insufflation pressures, or mouth leaks). Intentional leaks depend on the type of mask and insufflation pressures: interfaces have their predefined leak vs. pressure curves (48). In barometric modes, which are presently by far the most frequently used, ventilators adapt the airflow generated by the turbine to the intentional leak to ensure that the pressure attained in the airways reflects settings determined by the clinicians. Unintentional leaks are by definition unpredictable, most often variable, may differ between inspiratory and expiratory phases, and can be influenced by elements such as facial morphology, type of interface (facial *vs.* nasal mask), body position, sleep stages or ventilator settings. More importantly, they may interfere with the detection of patient's changes in inspiratory flow (which triggers pressurization or induces cycling). Leaks are thus the most frequent etiology of patient-ventilator asynchrony. They may induce significant and sometimes severe desaturations due to hypoventilation or to a "dilution effect" in patients with supplemental oxygen and can impact on quality of sleep (49). Also, leaks have a detrimental effect on reliability of data provided by ventilator software such as tidal volume, minute ventilation, or percentage of cycles triggered or cycled by the ventilator (3). This may be particularly problematic in automated modes with volume targeting or providing a continuous assessment of airway resistance.

Most built-in monitoring systems coupled to LTNIV ventilators provide data about leaks. However, leaks are estimated and reported differently according to manufacturers, which may misguide the clinician (i.e., average leaks, average leak only during expiratory phase, average leaks with or without intentional leaks) (3, 50, 51). Bench test studies have shown that reliability of reported leaks may vary considerably according to device used and to absolute value of leaks (3, 5, 52). Arbitrary threshold values for acceptable leaks are commonly reported by several manufacturers: the threshold of 24 L/min often referred to was first mentioned in a study by Teschler et al. (53): the relevance of this value is questionable today since the efficacy

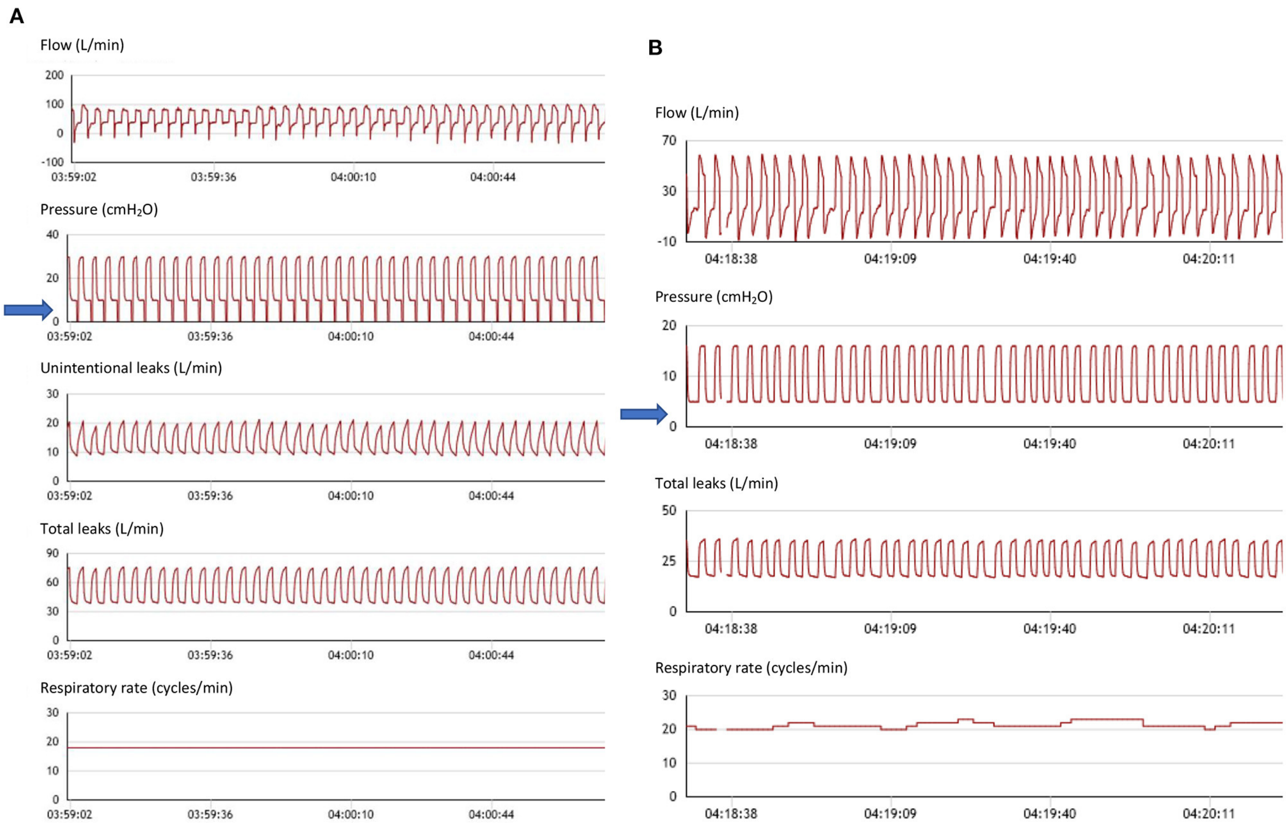

FIGURE 2 | Spontaneous vs. controlled cycles. **(A)** From top to bottom: flow (including leaks), pressure, unintentional leaks (i.e.: difference between total leak flow and estimated flow through the leak valve (small holes) of the mask), total leaks, and respiratory rate. Seventy six-year-old female subject with kyphoscoliosis, obstructive sleep apnea and obesity; ventilator settings: ST mode (spontaneous/timed); IPAP (Inspiratory positive airway pressure): 30 cmH_2O; EPAP: 10 cmH_2O (Expiratory positive airway pressure); Back-up respiratory rate (BURR): 18 cycles/min. Nasal mask (facial mask not tolerated). Flow tracing shows intermittent flattening of inspiratory curve suggesting persisting flow limitation (upper airway collapse to be compensated by increasing EPAP). Blue arrow: pressure tracing shows vertical marks associated with each cycle indicating controlled cycle (i.e., delivered by ventilator). On this segment, patient is continuously on back-up respiratory rate. Low level of intentional leaks. **(B)** From top to bottom: flow (including leaks), pressure, total leaks, and respiratory rate. Fifty four-year-old male subject with restrictive disorder. Ventilator settings: ST mode (spontaneous/timed); IPAP (Inspiratory positive airway pressure): 16 cmH_2O; EPAP: 5 cmH_2O (Expiratory positive airway pressure); Back-up respiratory rate (BURR): 16 cyc/min; nasal mask. Blue arrow: as opposed to **(A)**, all cycles are triggered by the patient (i.e., spontaneous). Normal aspect of flow and pressure tracings. DirectView software, Philips Respironics.

of ventilator turbines and their capacity to compensate for leaks has improved substantially. A statement by the French GAV-O_2 group suggested that leaks are considered troublesome essentially 1/ if they impact on patient comfort and compliance and 2/ if they cause recurrent nocturnal desaturations and episodes of hypercapnia (46).

For the patient, the variability of leaks and peak levels even of short duration may be a more important source of discomfort than the mean or median leak level.

Tidal Volume and Minute Ventilation

Tidal volume (V_T) and minute ventilation (MV) are important goals for the clinician when determining ventilator settings: a usual target for V_T is 8–10 ml/kg of ideal body weight. The accuracy of V_T, and thus MV, depends on the reliability of ventilator software, on pressure values and on leaks (54, 55). In a bench study of 7 home ventilators, bias between measured and reported V_T ranged from 66 to 236 ml (3). This may be problematic in automated volume-targeted modes. A high variability of tidal volume may *per se* be suggestive of leaks.

Cycles Triggered or Cycled by the Device

Quantifying the percentage of cycles triggered by the patient is theoretically an important information. It allows to determine to what extent the patient's respiratory rate (RR) is captured by the ventilator, i.e., to what extent ventilatory support approaches a controlled mode (**Figure 2**). This may be a goal for clinicians in neuromuscular disorders for instance, to rest inspiratory muscles, or to decrease residual apneas in obesity hypoventilation (56). However, in the presence of leaks, and/or upper airway obstruction, a low percentage of triggered cycles may reflect the fact that the ventilator does not detect the patient's inspiratory efforts. Conversely, a high percentage of cycles triggered by the patient, i.e., a spontaneous mode, may be an indicator of patient-ventilatory synchronization. This parameter must

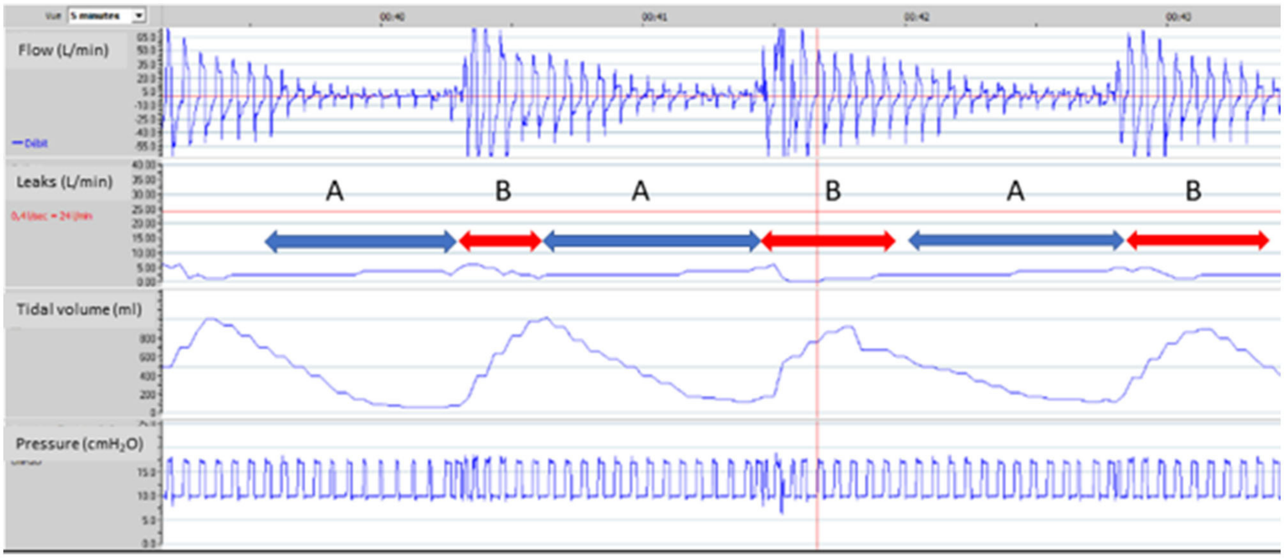

FIGURE 3 | Upper airway obstruction under NIV. 65-year-old male subject, obesity-hypoventilation with obstructive sleep apnea syndrome (OSAS). Bi-level pressure support ventilator, ResScan software, ResMed. Facial mask. Ventilator settings: ST Mode (spontaneous/timed), IPAP (Inspiratory positive airway pressure): 20 cmH_2O; EPAP (Expiratory positive airway pressure): 10 cmH_2O; BURR (Back-up respiratory rate): 16 cycles/min. From top to bottom: flow, unintentional leaks (i.e.: difference between total leak flow and estimated flow through the leak valve of the mask), tidal volume, pressure. 5 min window. Red line on leaks tracing (24L/min) is a threshold value suggested by manufacturer for upper limit of acceptable leaks (see text for comments). Event **A**: marked decrease in flow with tracing suggesting increase in upper airway resistance (leading to intermittent complete obstruction); simultaneous decrease in tidal volume without increase in leaks. Event **B**: sudden transient resumption of flow with a simultaneous increase in tidal volume. Increase in upper airway resistance could be related to an insufficient "pneumatic splint" effect in spite of rather high insufflation pressures, or to glottic closure (further characterization would require respiratory polygraphy). In a patient with a known OSAS, a pragmatic trial of increasing EPAP is an option. Value of IPAP should be increased accordingly to maintain same level of pressure support (if tolerated). Use of a facial mask may also contribute to these events, and may be replaced by nasal mask with chin strap if tolerated.

therefore be interpreted with caution, especially in the presence of leaks.

Ventilators also provide the percentage of respiratory cycles cycled by the patient i.e., for which the predefined percentage of peak inspiratory flow is reached. A high percentage of respiratory cycles cycled by the patient may be considered as evidence of appropriate patient ventilator synchronization. Conversely, a high percentage of cycling by the device may reflect either a "controlled mode" or leaks with or without patient ventilator asynchrony.

Residual Respiratory Events Under LTNIV

The presence of residual respiratory events is frequent and has a documented impact on quality of sleep and sleep structure (23, 57–60) (**Figures 3–5**). Severe patient-ventilator asynchrony may compromise the efficacy of NIV. Also, events such as recurrent upper airway obstruction may affect survival in ALS (9). It is thus important to evaluate, and correct these events.

Most devices report residual respiratory events occurring under NIV: they are labeled as apnea, hypopnea, and sometimes even classified as "central" or "obstructive" according to complex algorithms and estimation of upper airway resistance using the force oscillation technique, the shape of the flow curves or both. The relevance of these parameters in LTNIV is not well-established: these parameters are derived mainly from the use of CPAP in sleep-related disordered breathing (SRDB), and do not take into account the higher level of complexity of undesired residual (or *de novo*) respiratory events associated with LTNIV. Very few studies have assessed the reliability of these data. Georges et al. showed that the apnea-hypopnea index (AHI) reported by one specific device in patients with obesity-hypoventilation was well-correlated with a manually read polysomnographic assessment, and that a threshold value of 10/hour for AHI allowed to discriminate with a high sensitivity and specificity subjects who were correctly ventilated vs. those for whom adjustments were required (61). In a similar study, Alvarez et al. compared manually scored polygraphy with built-in software data in 26 patients with obesity-hypoventilation: automated analysis of residual events was well-correlated with polygraphy; however, manually scored tracings from ventilator software provided more consistent results than automated software (62). More recently Aarrestad et al. compared manually scored polygraphy and data from ventilator software in 67 subjects on LTNIV for restrictive disorders: a value of AHI above 7.2/hour reported by ventilator software had a sensitivity of 93% (95%CI: 68–100) and a specificity of 92% (95%CI: 81–98) for detecting patients with an AHI > 10/hour scored by polygraphy (34).

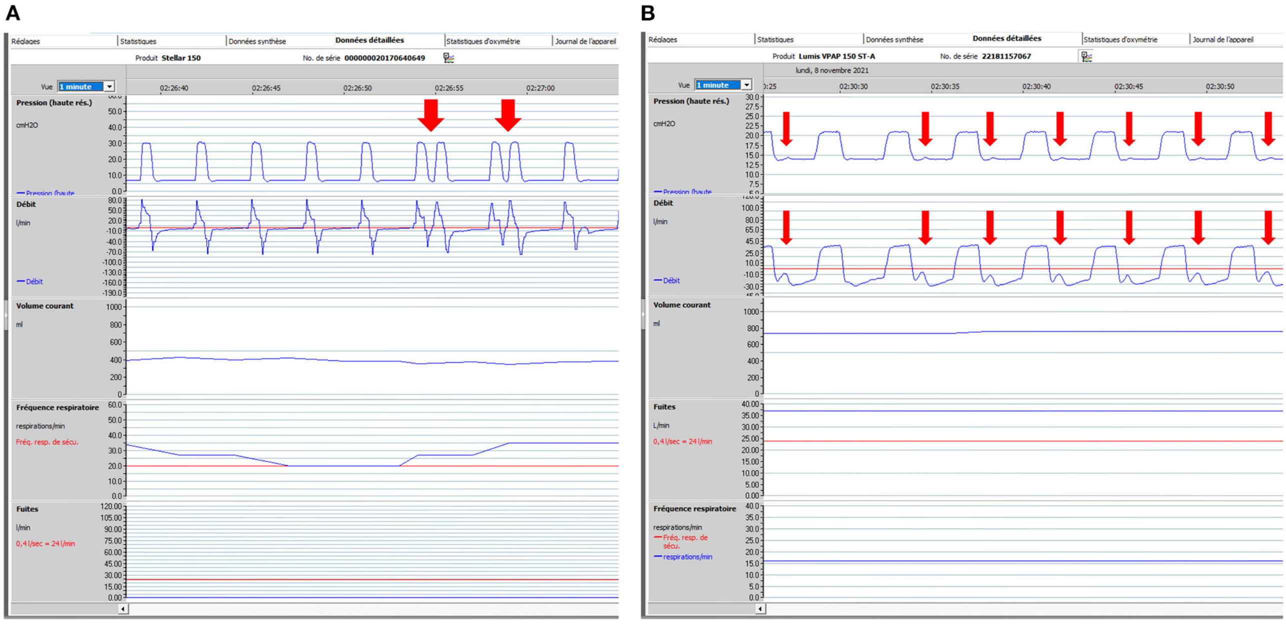

FIGURE 4 | Patient ventilator asynchrony (PVA): illustrative ventilator tracings. One-minute windows. From top to bottom: pressure, flow, tidal volume, respiratory rate and unintentional leaks (i.e., without intentional leak through exhalation valve of mask). Rescan software, ResMed. **(A)** 74-year-old male subject with severe COPD (FEV1: 20% of predicted). Bi-level pressure support ventilator, Rescan software, Resmed. Ventilator settings: ST (spontaneous/timed) mode; IPAP (Inspiratory positive airway pressure): 30 cmH_2O; EPAP (Expiratory positive airway pressure): 7 cmH_2O; BURR (back-up respiratory rate) 20 cycles/min. Red arrows show intermittent double-triggering. In this case, leaks are not in cause. Possible causes are dysfunction of ventilator, too high inspiratory trigger sensitivity, too short minimal inspiratory time (TI_{MIN}) with prolonged inspiratory efforts. Adjustments of settings (if required) to be considered are: to increase TI_{MIN}; to adapt cycling sensitivity (at a lower percentage of peak inspiratory flow, which delays cycling); to increase IPAP and reduce rise time; or to decrease sensitivity of inspiratory trigger. **(B)** 67-year-old male subject with obesity hypoventilation and severe OSAS. Bi-level pressure support ventilator, Rescan software, Resmed. Ventilator settings: ST (spontaneous/timed) mode; IPAP (Inspiratory positive airway pressure): 21 cmH_2O; EPAP (Expiratory positive airway pressure): 14 cmH_2O; BURR (back-up respiratory rate) 16 cycles/min. Red arrows show low amplitude repeated increases in flow and pressure which represent unrewarded efforts (i.e., inspiratory efforts by the patient which do not trigger the ventilator). Among possible causes are: inappropriate setting of inspiratory trigger sensitivity, increase in upper airway resistance, leaks, intrinsic PEEP (Positive end expiratory pressure), decrease in inspiratory muscle function. For all PVA, control of leaks is mandatory before adjusting other settings.

Detailed Analysis of Flow and Pressure Curves and Coupling With Pulse Oximetry and Leaks

Ventilator software provides the possibility of a detailed cycle-by-cycle analysis of pressure and flow curves, while simultaneously providing curves of estimated leaks and tidal volume. Oximetry can be added to most devices to complete this analysis. This allows the detection of residual obstructive events with or without persistence of respiratory drive (63), may show periodic breathing, and patient ventilator asynchronies (rate asynchrony such as multiple triggering, auto-triggering, ineffective efforts, or intracycle asynchronies such as underassistance, or overshoot) (49). Coupling the detailed analysis of ventilator curves with oximetry and leaks allows a better assessment of relevance and mechanisms involved, and provides a rationale to adapt ventilator settings. Illustrative cases of contribution of pressure and flow curves are shown in **Figures 2–5**.

Contribution of Respiratory Polygraphy or Polysomnography

Respiratory polygraphy (PG) or polysomnography (PSG), preferably coupled with $TcPCO_2$, are considered as gold-standard for documenting undesired respiratory events occurring under LTNIV. These events have been reported by several studies (34, 45, 57, 58, 64, 65) and defined in detail by the SomnoNIV group (63). However, PG or PSG under NIV requires expertise. PSG is not easily available in many countries, and thus use of PSG for titrating and/or monitoring LTNIV, although recommended by the AASM (American Association of Sleep Medicine), is not standard practice (66). Some specialized centers use PSG for titration and follow-up (67). Proposed algorithms recommend PSG or PG only after a thorough analysis of symptoms, $PtcCO_2$, ventilator software and correction of leaks (4, 68) (**Figure 6**).

Symptom Scores and Assessment of Health-Related Quality of Life (HRQL)

Specific HRQL questionnaires have been designed for patients under LTNIV, such as the SRI (Severe Respiratory Insufficiency Questionnaire) which is the most widely used in this setting, with 16 translations and an "App" version available (69, 70). This type of questionnaire is however mostly designed for clinical studies, and not for follow-up of individuals.

A disease-specific symptom score (S^3-NIV) has been recently developed (71) and is undergoing several translations: it is an

FIGURE 5 | Impact of major leaks. Seventy five-year old male subject with severe COPD (GOLD D; FEV1: 17% of predicted). Bi-level pressure support ventilator, S/T mode (spontaneous/timed); IPAP (Inspiratory positive airway pressure): 24 cmH_2O; EPAP (Expiratory positive airway pressure): 4 cmH_2O; Back-up respiratory rate (BURR): 18 cycles/min. Facial mask. Five-minute window, Rescan software, ResMed. **(A)** normal tracing albeit for a few cycles with decrease in flow; **(B)** Vertical arrow marks appearance of major leaks (could be related to transient displacement of interface). Pressure tracing shows episodes of auto-triggering, and double triggering. Marked drop in pressure, flow and V_T (explained by magnitude of leaks). **(C)** As leaks progressively decrease, breathing pattern becomes more regular; pressure and flow increase progressively.

11-item Lickert scale type questionnaire which can be auto-administered, and provides 2 sub-scores: "respiratory symptoms" and "sleep and NIV-related side effects." This score is perfectly suitable for clinical practice. It can be coupled with simple tools for assessing mood disturbances such as the Hospital Anxiety and Depression scale (72). These tools do not replace however the necessary face-to-face exchange with the patient and/or his/her caregivers to appreciate the relevance of findings downloaded from ventilator software and mentioned in symptom scores such as the S3-NIV.

Strategies to Detect Ineffective Control of Nocturnal Hypoventilation by NIV

NIV should be systematically monitored (4, 46). As mentioned previously, residual nocturnal hypoventilation, unintentional leaks, patient-ventilator asynchrony or abnormal respiratory events are frequent under NIV and have a negative impact on patient-related outcomes such as symptoms, HRQL and survival. It has been estimated that approximately one third of ventilated patients with normal daytime ABG and nocturnal SpO_2 had residual nocturnal hypoventilation under NIV (28, 35, 73). Withholding from performing regular testing of NIV efficacy could therefore be detrimental.

Optimal modalities for monitoring of long-term ventilated patients remain a matter of debate. As previously mentioned, complete polysomnography (PSG) under NIV is performed by some groups, but is not feasible in most centers on a routine basis (74).

Alternative tools (such as oximetry, $TcPCO_2$, or ventilator software) can be used alone or in combination. A step-by-step strategy starting by ABG and nocturnal SpO_2 has been proposed by the SomnoNIV group (4) (**Figure 6**).

Over the past years, the use of $TcPCO_2$ has been simplified (less frequent changes of probes, improved software and estimation of drift). Failure to retrieve data is rare (75) and instrumental drift of $TcPCO_2$ is a minor problem when used by an experienced team (27). Several studies have shown that continuous $TcPCO_2$ recording provides an accurate picture of the overnight time course of $PaCO_2$ in CHRF under NIV. Experts propose different thresholds to define significant nocturnal hypercapnia: maximal $TcPCO_2$ >49 mmHg, $TcPCO_2$ >49 mmHg for >10% of recording time, $TcPCO_2$ >55 mmHg for ≥10 min or an increase in $TcPCO_2$ ≥10 mmHg above awake supine value to a value exceeding 50 mmHg for ≥10 min (33). The choice of a clinically relevant threshold may be influenced by 1/the bias between arterial and transcutaneous

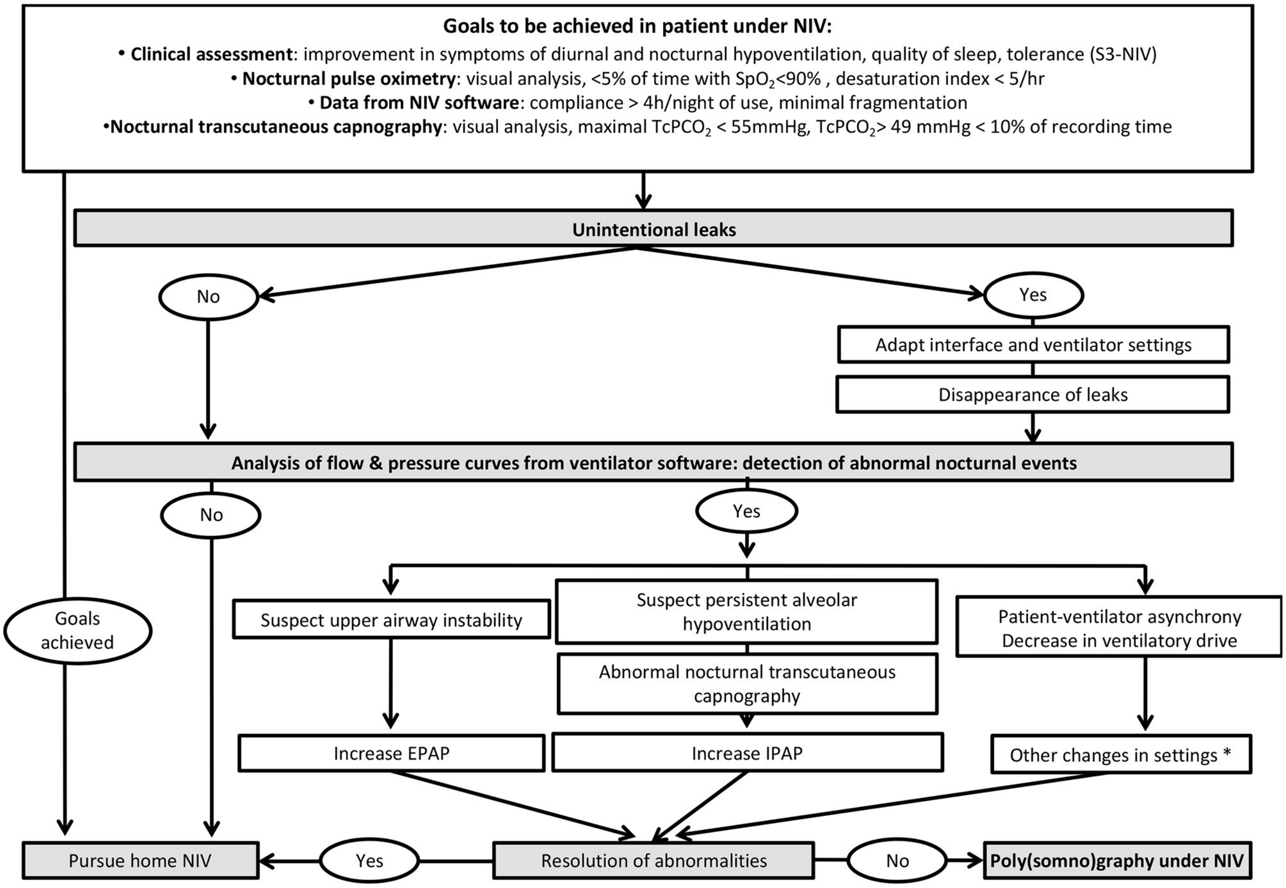

FIGURE 6 | Proposed algorithm for a systematic approach to monitoring of patients under LNIV and analysis of ventilator software. *: the reader is referred to references 4 and 8 for further details.

PCO_2 according to the device used, 2/the etiology of the underlying chronic respiratory failure and 3/$PaCO_2$ levels when NIV is started. Beyond this debate, capnography is currently a well-accepted surrogate procedure for diagnosing nocturnal residual hypoventilation in ventilated patients. Simultaneous recording of SpO_2 with the same ear probe improves the clinical contribution of nocturnal transcutaneous capnography. Indeed, sampling rate and averaging of SpO_2 and $TcPCO_2$ recordings are different. Therefore, SpO_2 traces can detect brief desaturations related to short ventilatory events while $TcPCO_2$ traces allow evaluating overnight trends because of a longer lag time (4).

Significant leaks and/or abnormal residual respiratory events (i.e., flow reduction or patient-ventilator asynchronism) are frequently detected in patients with normal nocturnal $TcPCO_2$ and SpO_2 (23, 59, 64).

In a study comparing different tools and their combination, Georges et al. demonstrated that combining the signals provided by $TcPCO_2$ and data from ventilator software provides the best noninvasive assessment of NIV efficacy (73). This approach allows monitoring of LTNIV at the hospital or at home without complex logistics. Interpretation of the results is simple and further analysis of detailed raw data provided by ventilator software can help clarify the underlying mechanism to optimize NIV settings, thus limiting the use of PSG to more complex cases.

A suggested strategy for nocturnal NIV monitoring and parameter optimization is summarized in **Figure 6**.

Patient-Ventilator Asynchrony and Its' Relevance

Several studies have reported the—sometimes frequent—occurrence of patient ventilator asynchrony (PVA) in patients under LTNIV (23, 57–59, 63, 64, 76) (**Figures 4**, **5**). Their specific semiology and a suggested framework for their analysis has been proposed by the SomnoNIV group (49). Briefly, after correction of leaks and obstructions, which are the major contributors to PVA, a visual analysis of tracings is necessary to detect the presence of rate asynchrony and/or intracycle asynchrony. Rate asynchrony is further subdivided into multiple triggering, uncoupling and ineffective efforts, while intracycle asynchrony can reveal either flow asynchrony (under-assistance) or phase asynchrony (premature or delayed cycling). Clinical relevance of PVA is still a matter of debate, especially if not associated with discomfort and hypoventilation and/or nocturnal desaturation. Scoring of PVA is not yet standardized in the medical literature, and prevalence can vary considerably according to working definitions (23). Seeking for PVA seems however appropriate after correction of leaks in the presence of symptoms and/or unexplained desaturations.

Tele-Monitoring

Although the use of tele-monitoring has become in many countries standard practice for following patients treated by CPAP and Sleep-related breathing disorders (SRBD), and, in some countries, is even required for its reimbursement, this is not the case for LTNIV. Many devices used for LTNIV have the necessary technology built-in, but each manufacturer has its software, and way of reporting certain parameters, representing a challenge for clinicians. Reliability of data remains an issue for some data, as previously discussed (77). In spite of these limitations, home titration and follow-up of LTNIV are emerging strategies. Use of tele-monitoring as an adjunct to home initiation of LTNIV is also an option, and has been shown to be cost-effective in selected indications such as CHRF related to neuromuscular disorders or other restrictive lung diseases (78, 79). Several studies suggest a benefit on healthcare utilization (emergency visits, hospital admissions) and cost-effectiveness of tele-monitoring in ALS (80–82). Preliminary studies have explored the possibility of early detection of exacerbations of COPD under LTNIV (47). For instance, changes in number of hours of NIV use may be a sign of imminent exacerbation, of worsening of underlying disease, or of discomfort, and can be a useful signal for early home intervention (7, 47). On-going trials (reviewed by Borel et al.) (77) will help clarify the modalities of a cost-effective use of tele-monitoring in NIV, and importantly, its benefit (or absence of) on relevant outcomes (such as HRQL, survival, hospital admissions). Points of potential interest for telemonitoring are: follow-up after initiation of NIV following an acute episode of respiratory failure (effective daily use, leaks etc.), progressive titration of NIV after home initiation of LTNIV, and, once specific algorithms will have been validated, early detection of exacerbations or deterioration of disorder causing CRF. The present tools for tele-monitoring already provide a limited "traffic light system" (red, yellow and green icons) usually based on leaks, AHI and compliance. A more elaborate signaling system, using limits for tidal volume, minute ventilation, respiratory rate etc. has yet to be validated in clinical practice, the risk being an unwarranted increase of "red lights." The possibility of adapting certain parameters at a distance (pressure support, expiratory positive airway pressure, or back-up respiratory rate) allows progressive changes in these settings for increased patient comfort and tolerance, and reduces the requirement for in-hospital titration (83).

The Contribution of "Big Data"

The concept of "Big data" refers to access to a massive quantity of data, its speed of acquisition, and the diversity and heterogeneity of data sources, with potential uncertainties regarding data quality (84). The widespread use of telemonitoring for CPAP has opened the way to a new approach for exploring patient pathways, identifying potentially relevant phenotypes, and elements impacting on adherence and efficacy. For instance, Liu et al. provided very useful data for a better understanding of trajectories of patients with central sleep apnea (emergent, transient, persistent) after initiation of CPAP (85). Technical information such as the impact of changes of interface can be easily assessed through daily longitudinal follow-up data at a large scale. Detailed analysis of day by day use of devices reveals behavioral patterns which improve our understanding of how patients adhere and accept their treatment (86, 87). Recently, it has been possible through telemonitoring to evaluate the impact of the lockdown during the COVID pandemic on adherence to CPAP or NIV (88, 89). Undoubtedly, in a near future, this type of information will become increasingly available and provide a useful adjunct to clinical trials.

CONCLUSIONS

The relevance and necessity of a regular monitoring of patients under LTNIV is clearly established, and has an impact on prognosis and HRQL. The progressive shift from hospital-based to home-based assessment over the past years is important for patient comfort and cost-effectiveness of LTNIV. Strategies for monitoring LTNIV include clinical assessment, symptom scores, simple tools such as $TcPCO_2$ or oximetry, ventilator software, and in specific situations, respiratory polygraphy or polysomnography. Although telemonitoring of patients treated by CPAP for SRBD is widely accepted, its use in LTNIV still has to be explored and shown to be cost-effective. Access to big data may provide additional information to better clarify phenotypes of responders and non-responders to LTNIV.

AUTHOR CONTRIBUTIONS

J-PJ, CC, PP, MG, and CR have contributed to the writing, designing, and revision of the final version of this manuscript and are accountable for the content of this manuscript. All authors contributed to the article and approved the submitted version.

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