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Viral Etiology of Acute Exacerbations of COPD in Hong Kong*

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Introduction: **Viral respiratory infections may precipitate acute exacerbations of COPD (AECOPD). However, little is known about viral etiology related to AECOPD in Asia. We aimed to study the viral etiology of AECOPD in Hong Kong.**

Methods: **Patients admitted to an acute hospital in Hong Kong with AECOPD were recruited prospectively from May 1, 2004, to April 30, 2005. Nasopharyngeal aspirate was collected and assessed by polymerase chain reaction (PCR) and viral culture. Spirometry was performed in the stable phase at 2 to 3 months after hospital discharge.**

Results: **There were 262 episodes of AECOPD among 196 patients (mean age, 75.7 ± 7.7 years [± SD]; 160 men). Mean FEV_1 was 39.6 ± 18.9% of predicted normal, and FEV_1/FVC ratio was 58.0 ± 15.2%. Fifty-eight episodes (22.1%) yielded positive viral PCR results. The viruses identified were influenza A (7.3%), coronavirus OC43 (4.6%), rhinovirus (3.1%), influenza B (2.7%), and respiratory syncytial virus (2.3%). The diagnostic yield of viral identification by PCR was 2.7 times higher than that based on conventional viral culture. The rates of identifying a positive viral etiology by PCR were similar among the subjects with $FEV_1 \geq 50\%$, ≥ 30 to 50%, and $< 30\%$ of predicted normal. Viral infection appeared to have no effect on subsequent readmissions or mortality rate over a study period of 1 year**

Conclusion: **Influenza A and two less-attended viruses, coronavirus OC43 and rhinovirus, were the common etiologic agents in patients hospitalized with AECOPD in Hong Kong. These should be considered in developing diagnostic and intervening strategies pertaining to AECOPD.**

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Key words: acute exacerbation; COPD; viruses

Abbreviations: AECOPD = acute exacerbations of COPD; CXR = chest radiograph; GOLD = Global Initiative for Chronic Obstructive Lung Disease; MDCK = Mardin Darby canine kidney; NPA = nasopharyngeal aspirate; NPPV = noninvasive positive pressure ventilation; PCR = polymerase chain reaction; RSV = respiratory syncytial virus

Acute exacerbations of COPD (AECOPD) lead to significant morbidity and mortality worldwide.[1] Previous studies[2,3] have shown that pulmonary function and quality of life were adversely affected by frequent exacerbations, particularly in active smokers. In Hong Kong, COPD was the fifth-leading cause of death, and accounted for at least 4% of all public hospital acute admissions in 2003. We have previously shown that in patients hospitalized with AECOPD, the 1-year readmission rate was 2.2 episodes, whereas the 1-year mortality rate was 14%.[4]

Common etiologic factors of AECOPD include infections,[5,6] air pollution,[7] withdrawal of medications,[8] or change in temperature.[9] Etiologic agents may vary in different geographic locations. We pre-

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viously conducted a 1-year prospective study[10] on the infectious etiology related to AECOPD in Hong Kong, with identification of viruses based on viral culture of the nasopharyngeal aspirate (NPA) and blood serology. However, these methods lack sensitivity in comparison to the newer technique of polymerase chain reaction (PCR) for virus identification.

The aim of this study was to assess prospectively the viral etiology of subjects hospitalized for AECOPD using the PCR method on the NPA specimen. In addition, we explored any relationship between viral identification and clinical parameters, such as the length of stay in hospital, need for noninvasive positive pressure ventilation (NPPV), readmissions, and mortality over the subsequent 12 months, lung function on recovery, and influenza vaccination in the preceding year.

Materials and Methods

Subject Recruitment

Patients who had been admitted to the Prince of Wales Hospital with AECOPD between May 1, 2004, and April 30, 2005, were recruited for this study on every alternate day. AECOPD was defined when a patient with background COPD[11] presented with at least two major symptoms (increased dyspnea, increased sputum purulence, increased sputum volume) or one major and one minor symptom (nasal discharge/congestion, wheeze, sore throat, cough) for at least 2 consecutive days.[2,12] Informed written consent was obtained from each subject, and the study was approved by the research ethics committee of the Chinese University of Hong Kong.

Demographic Data and Management in Hospital

Demographic data and length of hospital stay of patients with AECOPD were recorded. Comorbid conditions were noted and scored by the Charlson index.[13] Scoring of the Charlson index ranged from 0 to 33, with a higher score indicating more in number and severity of the coexisting illnesses. In addition, chest radiographs (CXRs) were assessed by the investigators (respiratory physicians). Only those without pneumonic changes on their CXRs were included for analysis in this study. The use of NPPV, invasive mechanical ventilation, and ICU admissions were recorded.

Microbiological Examination

NPA was obtained by catheter aspiration from the posterior nasal pharyngeal space via the nostril with the patient in the sitting position, as described on our previous study.[10]

Viral Culture

NPA was collected in 3 mL of viral transport medium containing gentamycin (4 mg/mL), penicillin/streptomycin (50,000 IU/50,000 μg/mL), and fungizone (1 mg/mL), and was processed (usually within 12 h) for viral culture and PCR assay. Briefly, the aspirate was centrifuged at 2,000/min for 5 min, and the cell pellet washed with phosphate-buffered saline solution and coated onto glass slides. The supernatant was inoculated onto rhesus monkey kidney (LLC-MK2), human laryngeal carcinoma (HEp-2), Mardin Darby canine kidney (MDCK), and human embryonic lung fibroblast cell monolayers. All cell cultures were incubated at 37°C, except for MDCK, which was incubated at 33°C. Cell monolayers were examined daily for cytopathic effect. After 14 days of incubation, a hemadsorption test for LLC-MK2 and MDCK monolayers was performed. When suspicious cytopathic effect was observed or when the hemadsorption test result was positive, the presence of virus growth was confirmed by immunofluorescence staining using specific monoclonal antibodies.

PCR

Five groups of nested multiplex PCR assays targeting 17 respiratory viruses and three bacteria were applied. The five groups of viruses and the outer and inner sets of primer sequences, and the amplicon sizes are listed in Table 1. Primers were designed based on modifying published primer sequences[14–22] or constructed from the consensus regions, as obtained from GenBank.

A 200-μL aliquot of the cell-free supernatant of spun nasopharyngeal aspirate was used for extraction of RNA and DNA (QIAamp MinElute Virus Spin Kit; Qiagen; Hong Kong). PCRs were performed using a "fast" thermal cycler (Applied Biosystems; Foster City, CA) with DNA polymerase (Fast PCR Master Mix, GeneAmp; Applied Biosystems). Both first-round and second-round PCRs were conducted in a 20-μL reaction with 2 μL of complementary DNA as template, and 0.2-μL aliquot of the first-round PCR product in the first-round and second-round PCRs, respectively. The cycling conditions for both rounds of PCRs were an initial denaturation at 95°C for 10 s, then 30 cycles of denaturation at 95°C for 1 s and annealing/extension at 64°C for 40 s, followed by a final extension at 72°C for 10 s. For adenovirus detection, 35 cycles of denaturation at 95°C for 5 s were used instead. Cultured stocks of the target pathogens were used as positive controls; and when cultured stocks are not available, clinical specimens known to contain the target agents were used.

Follow-up of Patients After Discharge

Spirometry before and after bronchodilation (Vitalograph; Buckingham, UK) was performed at 2 to 3 months after discharge from the hospital (*ie*, stable COPD) according to the American Thoracic Society standard.[23] Updated predicted spirometry values for Hong Kong Chinese were adopted.[24] The patients were contacted by telephone, and their medical records were reviewed 12 months later to check for any deaths or hospital readmissions.

Statistical Analysis

Data were analyzed using statistical software (SPSS, version 11.5; SPSS; Chicago, IL). Associations between the identification of a virus in the NPA during an episode of AECOPD and the lung function and clinical outcomes of patients (such as mortality, NPPV, use and length of hospital stay) were assessed by Mann Whitney *U* test, χ^2 test, and Fisher exact test as appropriate. Data are presented as mean ± SD, and $p < 0.05$ was considered significant.

Results

Altogether 350 episodes of AECOPD were screened for this study. Cases (n = 76) with pneu-

Table 1—*Primers Used in Nested Multiplex PCR

Virus	Forward and Reverse Primers, Sequence (5′ → 3′)	Product Length, Base-Pair
Primers used in the first round of multiplex nested PCR		
Group 1		
Influenza A	TYGAGGCTCTCATGGARTGGCTAAAG	412
	GCTGGCCARMACCATTCTGTTYTCAT	
Influenza A H1	CCCAGGRTATTTCKCCGAYTATGAGG	760
	TACCATTCCAGTCCACCCCCCTTCA	
Influenza A H3	ATGGGACCTTTTTRTYGAACGCAGCA	519
	CCCCKAGGAGCAATTAGATTCCCTGT	
Influenza A H5	ATCAAACAGATTAGTCCTTGCG	265
	GGCCTCAAACTGAGTGTTCATT	
Influenza B	AGGAAGRGCAATGGCAGAYAGAGG	883
	TGCTGTGTCCCTCCCAAAGAAGAAA	
Group 2		
Parainfluenza 1	TCTGGATCCACCACAATTTCAG	848
	WACCAGTTGCAGTCTKGGTTTC	
Parainfluenza 2	CTTGCAGCATTTTCTGGGGAACTCC	716
	GCATCATCATCCTGGGAGCCTCTGT	
Parainfluenza 3	GATTTTTGGAGATGCACGTCTG	1118
	GAGAGTGTTYTGTTTCGGATGG	
Parainfluenza 4	AYGGATGCATTCGAATTCCATCATTC	432
	TCCRTRAGRCCYCCATACAARGG	
Group 3		
Human RCV A	CAGCTCCGTTATCACATCTCTAGGAGCC	576
	TGGGTTGTCTATGAGCAGATAKKAAACCA	
Human RSV B	CGGGCCAGAAGAGAAGCACCACAGTA	673
	TGATCCTTCTTTGATGTTGGTGGTGCA	
Human rhinovirus	CACTTCTGTTTCCCCGGAGCGAG	388
	GAAACACGGACACCCAAAGTAGTCGGT	
Human enterovirus	CTGCGYTGGCGGCCYMCC	281
	CCGGATGGCCAATCCAATAACTATATGGT	
Group 4		
Human coronavirus OC43	CGGTTACACTGTTCAGCCAATYGCA	793
	CCAACCCAAAAATGCTTGTGGTYG	
Human SARS-CoV	CACCGTTTCTACAGGTTAGCTAACGA	310
	AAATGTTTACGCAGGTAAGCGTAAAA	
Human coronavirus 229E	TCACCCATTTGAAGAATTGGAATTTTGG	566
	TCGTACGTAGAAAACCCAGCCTGTGC	
Human metapneumovirus	CAATATGGTTCCCTTTGTTTCAGGCCA	462
	TGGTCTGCTTCACTGCTTATWGCAGCTT	
Group 5		
Mycoplasma pneumonia	GACCATTCCACCCAGCCCCAGC	343
	GTTCAGCGAGTGGGGTGCGTACAATA	
Chlamydia pneumoniae	TGCGCTACTTGGTGCGACGCTA	571
	CGCCTTTATAGCCCTTGGGTTTRTTT	
Legionella	CGCTCAATTGGCTTTAACCGAACAG	425
	CGCTRCGTGGRCCATATGCARGAC	
Adenovirus	TACATGCACATCKCSGGVCAGGA	983
	CCRGCCARHACHCCCATRTTDCCHGT	
Primers used in second round of multiplex nested PCR		
Group 1		
Influenza A	AAGACCAATCCTGTCACCTCTGA	73
	CAAAGCGTCTACGCTGCAGTCC	
Influenza A H1	TCGCCGACTATGAGGAACTGAGGGA	431
	TTGTATCCCCGGGTTCCAGCAGAGT	
Influenza A H3	CCCTTATGATGTGCCGGATTATGCC	259
	GGTGGTGAACCCCCCAAATGTACAA	
Influenza A H5	TGCGACTGGRCTCAGAAATA	172
	GGATTCTTTGTCTGCAGCGT	
Influenza B	AAAACAARTGCTCTGCRCCYCAAC	516
	CRTCTCCACCTACTTCRTTYCCCCC	

Table 1—*Continued

Virus	Forward and Reverse Primers, Sequence (5′ → 3′)	Product Length, Base-Pair
Group 2		
Parainfluenza 1	AATTGGTGATGCAATATATGCKTATTC	600
	TCGACAACAATYTTTGGCCTATC	
Parainfluenza 2	AGGACAGCAGAGGACCTCGGCATG	343
	ACCTGATGTTCTTTGCGGTATGGGG	
Parainfluenza 3	CAACTGTGTTCRACTCCCAAAG	717
	TGGGTTYACTCTCGATTTTTGY	
Parainfluenza 4	GACGGATGYYTRCKGWATTGTGT	231
	CCRTRAGRCCYCCATACAARGGAA	
Group 3		
Human RSV A	TGACCCATTAGTGTTCCCCTCTGATGAAT	228
	CTTCTGGCCTTRCAGTATARGAGCAGT	
Human RSV B	GTCGCATCTCCAACA TTGRAAC	336
	TGGTGCATAGAGGTG ATGTGTG	
Human rhinovirus	CACTTCTGTTTCCCCGGAGCGAGG	283
	CCGCATTCAGGGGCCGGAG	
Human enterovirus	CCTCCGGCCCCTGAATGCG	106
	CCAAAGTAGTCGGTTCCGCYRCRGA	
Group 4		
Human coronavirus OC43	CKGTGCCCTCTCCATTAAATTGGG	635
	GACCCGAACAGTGCTCACCTATGCC	
Human SARS-CoV	AGTGAGATGGTCATGTGTGG	210
	CACTCATAGAGCCTGTGTTG	
Human coronavirus 229E	TTGGGATTCTAATTGGGCCTTTGTTGC	361
	GCTCGGCACGGCAACTGTCATGTAT	
Human metapneumovirus	CCCTTTGTTTCAGGCCAAYACACCACC	431
	GCAGCTTCAACAGTRGCTGATTCACTCTC	
Group 5		
M pneumonia	AGGGGGTTCTTCAGGCTCAGGTCAA	160
	CCCCACCACATCATTCCCCGTATTA	
C pneumoniae	RCCTACWGGATCCGCTRCTGCRAA	317
	GCRCCTACGCTCCAAGMRAAAGWRG	
Legionella	TGAAAACAAAAACAAGCCAGGCGTTG	232
	TGGCATCAATTGYAAAGCYTCTGTCC	
Adenovirus	TGGCYWSCACNTWCTTTGACATYMG	463
	GCRWAWGAHCCRTARCAKGGYTDCAT	

*SARS-CoV = severe acute respiratory syndrome coronavirus. Degenerate primer abbreviations: M:A/C, R:A/G, W:A/T, S:C/G, Y:C/T, K:G/T, V:A/C/G, H:A/C/T, D:A/G/T, N:A/C/G/T.

monic changes on CXR or predominantly congestive heart failure were excluded. Among those cases that fitted the inclusion criteria, 12 patients refused to consent for the study. Finally, 262 episodes of AECOPD were included for analysis among 196 patients. Only 165 subjects had returned for lung function tests at 117.88 ± 92.6 days after discharge from the hospital for AECOPD. Thirteen subjects died (either as inpatients or after discharge) before lung function test could be arranged, whereas 18 subjects defaulted follow-up. Among those who died or defaulted follow-up, lung function data obtained at stable state within 1 year prior to the admission for AECOPD were taken for statistical analysis (data available for 11 deceased subjects and 8 defaulters).

The majority of our subjects were men (81.6%), and the length of stay in the acute care hospital was 5.8 ± 3.0 days. After the first episode of hospitalization for AECOPD, 25 patients died, whereas 121 patients were readmitted to the hospital with another episode of AECOPD within 12 months. Among all the episodes of AECOPD, 23 episodes (8.8%) required NPPV support. Neither ICU admission nor invasive mechanical ventilation was required by our subjects. Demographic data of the subjects are presented in Table 2.

Among those with NPA specimens collected, 58 subjects (22.1%) had positive viral PCR results. The most common virus identified was influenza A (19 of 58 patients, 7.3%), followed by coronavirus OC43 (12 of 58 patients, 4.6%), rhinovirus (8 of 58 patients, 3.1%), influenza B (7 of 58 patients, 2.7%), and respiratory syncytial virus (RSV) [6 of 58 patients, 2.3%]. Results of the viral etiology based on PCR assay are shown in Figure 1. All the influenza A belonged to the H3 subtype, with neither H1 nor

Table 2—*Demographic Characteristics of the Patients (n = 196) Admitted to the Hospital With AECOPD Who Had NPA Saved for Assessment

Characteristics	Data
Age, yr	75.7 ± 7.7
Male gender	160 (81.6)
Body mass index, kg/m^2	21.2 ± 4.34
Prebronchodilator FEV_1, L†	0.71 ± 0.36
Prebronchodilator FVC, L†	1.23 ± 0.55
Postbronchodilator FEV_1, L†	0.76 ± 0.37
Postbronchodilator FVC, L†	1.36 ± 0.61
Postbronchodilator FEV_1/FVC ratio†	0.58 ± 0.15
Postbronchodilator FEV_1 % predicted normal, %†	39.58 ± 18.92
Postbronchodilator FVC % predicted normal, %†	50.19 ± 20.26
Premorbid status	
Chair bound	7 (3.6)
Home bound	20 (10.2)
Could walk on level ground	52 (26.5)
Could walk up at least one flight of stairs	117 (59.7)
Smoking status	
Nonsmoker	2 (1.0)
Ex-smoker	161 (82.1)
Current smoker	33 (16.8)
Home oxygen use	33 (16.8)
Inhaled corticosteroids	102 (52.0)
Oral theophylline	72 (36.7)
Influenza vaccination over the past 12 mo	79 (40.3)
Charlson index	1.50 ± 0.93

*Data are presented as mean ± SD or No. (%).

†Score of Charlson index ranged from 0 to 33 with a higher score indicating having more in number and seriousness of the coexisting illnesses.[13]

H5 subtypes identified. There were four cases of parainfluenza viruses detected, with one case for each of the following strains identified: parainfluenza type 1, 2, 3, and 4. Table 3 shows PCR results of the NPA in relation to the lung function of the subjects. The rates of identifying a positive viral etiology by PCR were similar among the subjects with $FEV_1 \geq 50\%$, ≥ 30 to 50%, and $< 30\%$ of predicted normal.

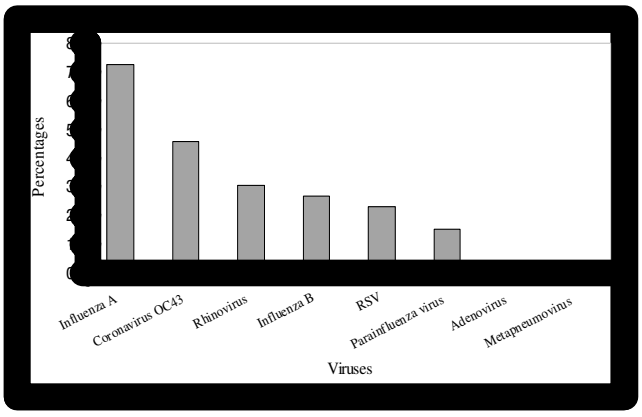

FIGURE 1. Viruses as detected by PCR method in the nasopharyngeal aspirates of patients (n = 262).

The seasonal pattern of the viruses is illustrated in Figure 2. The rates of positive virus identification by PCR method in the NPA peaked in July to September of the year. Among our subjects admitted with AECOPD, 41.8% had received influenza vaccination within the past 12 months, but there was no difference in the detection rates of influenza A or B in the NPA when compared to those without influenza vaccination (11.1% vs 9.0%, p = 0.58). The relationship between influenza vaccination and the detection rates of influenza viruses in NPA by PCR method is shown in Table 4.

Altogether, 244 episodes of AECOPD had both PCR and conventional viral culture performed for viral identification in the NPA, whereas viral culture was not performed in 18 patients. In comparison to conventional viral culture, the overall diagnostic yield of PCR was approximately three times higher. Comparisons of PCR and conventional viral culture in the NPA specimen are shown in Table 5. The NPA PCR-positive and viral culture-positive cases (n = 15) had a longer length of hospital stay than the cases (n = 40) with positive PCR and negative viral culture results (7.73 ± 5.16 days vs 4.93 ± 1.64 days, p = 0.03). However, lung function (FEV_1 percentage of predicted value) between the two groups did not show any significant difference (34.5 ± 15.1% vs 40.8 ± 19.5%, p = 0.269).

Episodes of AECOPD with positive viral identification were compared to those without, but the length of hospital stay was similar (median, 5 days; interquartile range, 4 to 6 days; and 5 days; interquartile range, 4 to 7 days) for virus-positive and virus-negative groups, respectively (p = 0.39). There were no significant differences in the rates of hospital readmissions between the virus-positive and virus-negative groups on their initial admission (virus-positive group, 56.0%; virus negative group, 63.7%; p = 0.33). One-year mortality rates in the virus-positive vs virus-negative groups were also similar (virus-positive group, 8.0%; virus-negative group, 14.4%; p = 0.24). Furthermore, there was no difference in the rates of NPPV usage between the virus-positive and virus-negative groups (6.9% vs 9.3%, respectively; p = 0.57).

DISCUSSION

To the best of our knowledge, this was the first large-scale prospective study conducted in Asia on the viral etiology of patients hospitalized with AECOPD using the sensitive technique of PCR on respiratory specimens. Influenza A, coronavirus OC43, and rhinovirus were the common viruses identified in our subjects, whereas the PCR technique for viral identification based on NPA speci-

Table 3—*Summary of the NPA PCR Results in Relation to COPD GOLD Stages**

NPA Findings	All Cases (n = 245)	GOLD Stage: Stage 1–2, $FEV_1 \geq 50\%$ of Predicted (n = 49)	Stage 3, $FEV_1 \geq 30$ to 50% of Predicted (n = 101)	Stage 4, $FEV_1 < 30\%$ of Predicted (n = 95)
Negative	191 (77.9)	37 (75.5)	80 (79.2)	74 (77.8)
Influenza A	18 (7.3)	3 (6.1)	8 (7.9)	7 (7.3)
Coronavirus OC43	12 (4.9)	2 (4.1)	4 (4.0)	6 (6.3)
Rhinovirus	8 (3.3)	4 (8.2)	2 (2.0)	2 (2.1)
Influenza B	6 (2.4)	2 (4.1)	1 (1.0)	3 (3.2)
RSV	6 (2.4)	0 (0)	3 (3.0)	3 (3.2)
Adenovirus	1 (0.4)	0 (0)	1 (1.0)	0 (0)
Metapneumovirus	1 (0.4)	0 (0)	1 (1.0)	0 (0)
Parainfluenza type 1	1 (0.4)	0 (0)	0 (0)	0 (0)
Parainfluenza type 2	1 (0.4)	0 (0)	1 (1.0)	0 (0)
Parainfluenza type 3	1 (0.4)	0 (0)	0 (0)	1 (1.1)
Parainfluenza type 4	1 (0.4)	0 (0)	0 (0)	1 (1.1)

*Data are presented as No. (%).

mens had a diagnostic yield 2.7 times higher than that of conventional viral culture.

In comparisons to other studies,[25,26] our diagnostic yield of viruses using the same PCR technique was relatively lower, and this might be due to geographic differences in the viral etiology of AECOPD and the different sites where the respiratory specimens were collected. Respiratory viruses were identified in the nasal samples in 39% of the East London COPD cohort with AECOPD, which was managed in the outpatient setting.[25] In a study[26] of German patients with more severe AECOPD requiring hospitalizations, respiratory viruses were detected in the nasal samples in 31%, whereas the diagnostic yield from induced sputum was even higher at 47%. In a recent study[27] of patients with very severe exacerbations that necessitated intubation and mechanical ventilation, viral agents were identified in 43% from respiratory specimens obtained via the endotracheal tube. In a study[28] of the viral etiology among 17 patients hospitalized with near-fatal asthma (from endotracheal aspiration), 29 patients with acute asthma (from induced sputum), and 14 patients with COPD (from induced sputum) in Singapore, influenza virus was identified in 36% of the subjects hospitalized with AECOPD. Further studies using sputum PCR will improve our knowledge of the viral etiology of AECOPD in our locality. Ambient air pollution[29,30] may also lead to AECOPD, but this was not assessed in this study.

Although rhinovirus was detected in only 3.1% of episodes of hospitalization in this study, it is the most common viral pathogen identified in other studies of AECOPD. Rhinovirus is a major cause of common cold in the community.[25,31] As there is increasing evidence that rhinovirus may directly infect the

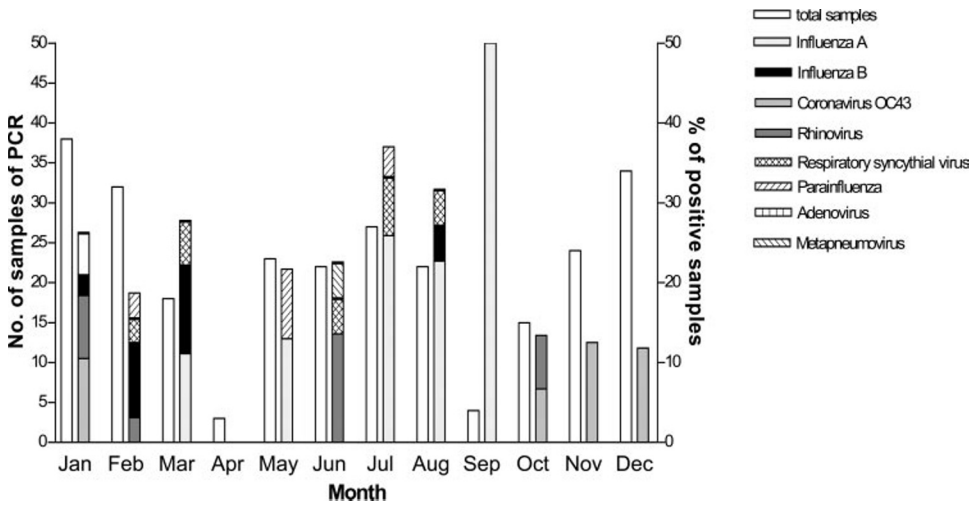

FIGURE 2. Seasonal pattern of viruses for patients admitted with acute exacerbation of COPD.

Table 4—*Effect on Influenza Vaccination of Influenza A and B NPA PCR Results*

Influenza Vaccination	Total Cases, No.	Identification of Influenza A or B, No. (%)	
		Yes	No
Yes	117	13 (11.1)	104 (88.9)
No	144	13 (9.0)	131 (91.0)

lower airway in patients with COPD,[32–34] sampling lower airway secretions in addition to the upper airway may lead to a higher diagnostic yield of viruses. In our study, the most common virus identified was influenza virus. This might be due to the relatively low influenza vaccination rate of 40.3% in this study as compared to 74% in the East London cohort.[25] In Hong Kong, H1N1 has been the predominant circulating strain of influenza in recent years,[35,36] but all our patients with AECOPD and proven influenza A infection in this study had H3 strain identified. Previous studies[37,38] in the United States have reported that H3N2 was more virulent than H1N1, and hospital admissions for chest infection and related deaths were significantly higher when H3N2 influenza was prevalent.

Similar to our study, other studies of AECOPD have also identified rhinovirus, coronavirus, influenza B virus, parainfluenza virus, RSV, and adenovirus in their subjects, although the percentages varied in different studies.[25,39,40] Human metapneumovirus was identified in only one episode (0.4%) of AECOPD in this study, whereas other studies have reported low detection rates of 2.3% and 6% from induced sputum[41] and NPA samples,[42] respectively. We have found no evidence of *M pneumoniae* and *C pneumoniae* infection, and this observation was consistent with previous studies.[25,43]

It appeared that patients with positive NPA PCR and positive viral culture results had more severe exacerbations, as evidenced by a longer length of stay in hospital, than those with positive NPA PCR and negative viral culture results. The results might suggest that the viruses in the NPA of patients with positive NPA PCR and viral culture results were "active" and "replicating," and thus resulted in more severe AECOPD and a longer hospital stay.

We noted two peaks of respiratory viral (Fig 2) infections in our COPD patients over a study period of 1 year. The observation of influenza season in the spring/winter period was consistent with other overseas studies[9,40] and another local study.[44] A second peak of influenza-like illness in summer in this current study of AECOPD patients requiring hospitalization was also in line with the seasonality of influenza in the general community in Hong Kong.[36]

Table 5—*Comparison of PCR Results Against Conventional Viral Culture**

NPA Viral Identification by PCR	NPA Viral Identification by Viral Culture	
	Positive	Negative
Positive		
Any (n = 55)	15 (27.3)	40 (73.7)
Influenza A	10	9
Coronavirus OC43	0	12
Rhinovirus	0	7
Influenza B	1	5
RSV	4	2
Adenovirus	0	1
Metapneumovirus	0	1
Parainfluenza type 1	0	1
Parainfluenza type 2	0	1
Parainfluenza type 3	0	1
Parainfluenza type 4	1	0
Negative (n = 189)	4 (2.1)	185 (97.9)
Influenza A	2	
Coronavirus OC43	0	
Rhinovirus	0	
Influenza B	1	
RSV	0	
Adenovirus	0	
Metapneumovirus	0	
Parainfluenza type 1	0	
Parainfluenza type 2	0	
Parainfluenza type 3	1	
Parainfluenza type 4	0	

*Data are presented as No. (%) or No.

There was no significant difference in the rates of positive PCR results from NPA between COPD patients who had received influenza vaccination over the past 12 months and those who had not. Wongsurakiat et al[45] previously showed that AECOPD (defined by symptoms in their study) and influenza-related acute respiratory illness (confirmed by serology test or viral culture from respiratory secretions) requiring hospitalization could not be decreased by influenza vaccination. It is currently recommended by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guideline that annual influenza vaccination be offered to COPD patients.[1] In elderly subjects in the general community (not specific for COPD subjects), some studies have found that influenza vaccine could halve the incidence of serologic and clinical influenza[46] and reduce utilization of hospital resources.[47] However, not all subjects who have received influenza vaccination could have a protective antibody response.[45] In the study by Wongsurakiat et al,[45] approximately 80% and 50% of the subjects had an antibody response to influenza A and influenza B, respectively. Further studies are needed to assess the efficacy of annual

influenza vaccination and any role for a booster dose for COPD patients.

The limitation of this study was that this was a single-center study, although our hospital served a large population of 0.6 million. Due to limited resources, patients were recruited every alternate day, although the demographics of these patients were representative of the whole group when compared to our previous study.[10] In addition, no patients with a stable state of COPD were recruited as control subjects, and it was thus not sure whether the viruses identified in the NPA were true pathogens or represented "colonization" or "chronic infection." Previous studies[25,26,48] using the PCR technique could identify some viruses (including piconavirus, influenza A and B virus, parainfluenaza 3, and RSV) in the respiratory specimens of stable COPD patients. As the rate of viruses identification in patients with stable-state COPD was much lower than that during AECOPD and most RSV infections in patients with COPD were associated with symptomatic respiratory illness together with measurable immune responses,[48] it is highly likely that viral infections play an important role in triggering AECOPD.

In conclusion, we have identified viruses with the PCR technique on NPA specimens in 22.1% of AECOPD episodes requiring hospitalization. The common viruses were influenza A, coronavirus OC43, and rhinovirus. Viral infections appeared to have no effect on subsequent readmission rates or mortality rate over a study period of 1 year, and there was no difference in the rates of viral infections among COPD patients in different GOLD stages. In recent years, there is growing epidemiologic evidence linking viruses to AECOPD.[49] It is worth noting that coronavirus OC43 and rhinovirus are both fastidious to growth, and are not covered by the routine diagnostic spectrum in most service laboratories. Our data have shown that coronavirus OC43 and rhinovirus could also exacerbate COPD. Further studies on the diagnostics, therapeutics, and vaccines for these "trivial" viruses are needed in addition to exploring the mechanisms of how viruses may induce AECOPD.

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