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C-reactive protein level and microbial aetiology in patients hospitalised with acute exacerbation of COPD

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ABSTRACT Both viruses and bacteria are thought to cause exacerbations of chronic obstructive pulmonary disease (COPD); however, the relative importance of each remains uncertain. C-reactive protein (CRP) levels increase during exacerbations but the relationship with aetiology is not established. We aimed to explore the relationship between serum CRP and the rate of detection of viruses and bacteria.

This was a prospectively recruited, observational study of patients hospitalised with exacerbations of COPD. Nasopharyngeal swabs were tested for respiratory viruses by reverse transcriptase-PCR. Sputum and blood were collected for bacterial culture and urine tested for pneumococcal antigen. CRP levels were measured on sera. CRP and other factors associated with viral, bacterial or mixed detection were assessed using multiple logistic regression analysis.

264 patients with exacerbations of COPD were studied: 26% tested positive for respiratory viruses only, 13% had bacteria only, 12% had mixed viral/bacterial detection, and 49% had no pathogens detected. CRP level and temperature were strongly associated with viral detection rate ($p<0.001$ and $p=0.004$, respectively) and mixed viral/bacterial detection rate ($p=0.02$ and $p=0.03$, respectively) on multivariate analysis. Bacterial detection rate was not associated with CRP level or body temperature.

This study supports the role of viruses as important aetiological agents causing exacerbations of COPD.

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Introduction

An estimated 3 million people in the UK have chronic obstructive pulmonary disease (COPD) and the incidence is increasing worldwide [1]. Exacerbations of COPD are associated with increased medication use, prolonged hospital stays and significant mortality rates [2]. Most exacerbations are thought to be caused by respiratory infection, with both viruses and bacteria being implicated, although the relative importance of each is not well established. Historically, bacteria have been considered the most important pathogens causing exacerbations but their exact role remains uncertain as they are detected in only 30–50% of COPD exacerbations [3], and can be found in a similar proportion of patients and at equivalent concentrations in those who are clinically stable [3, 4]. Studies demonstrating the development of strain-specific antibodies to bacteria following exacerbation suggest that acquisition of a different strain of the same bacterial species may play a role [5–7], although new strain acquisition is not invariably associated with exacerbation [8]. Studies using modern sensitive molecular methods have demonstrated that viruses are commonly detected in patients with exacerbations of COPD [9–12]. In addition, the changes in inflammatory profile, less frequent detection in steady state and the seasonal changes in exacerbation rate suggest that viral acquisition is causally related to exacerbation [9–12]. Furthermore, recent challenge studies with human rhinovirus in patients with COPD have replicated the symptoms and inflammatory profile changes associated with naturally occurring exacerbations [13]. Studies examining bacterial and viral co-infection in COPD exacerbations have suggested a synergistic relationship between the two [12, 14], with a recent study demonstrating a quantitative change in bacteria in the lower respiratory tract of COPD patients following experimental rhinovirus challenge [15]. A longitudinal study of naturally occurring human rhinovirus-induced COPD exacerbations has demonstrated that secondary bacterial infection developed in 73% of patients by 14 days post onset of symptoms, suggesting that bacterial infection may become important later in the course of a viral-induced exacerbation [16].

C-reactive protein (CRP) is an acute phase protein that increases with infectious and inflammatory conditions and is measured routinely in clinical care. CRP levels increase during exacerbations of COPD but are not well correlated to severity or outcome [17, 18]. Few studies have examined the relationship between microbial aetiology and CRP levels in patients with exacerbations of COPD.

As exacerbations are thought to be caused by infectious agents and are accompanied by an inflammatory response, we hypothesised that the detection rate of viruses and bacteria would be associated with CRP levels if these agents are causally related to the exacerbation. Therefore, we aimed to compare CRP levels and other factors in patients with viruses only detected, bacteria only detected, mixed viral/bacteria detection and those with no pathogen detected using descriptive statistics, and to explore these relationships using multivariate analysis. Finally we aimed to examine individual viral and bacterial subtypes to evaluate their contribution to any relationship between pathogen detection rate and CRP levels.

Methods

Subjects

Patients were adults hospitalised with acute exacerbation of COPD as identified by discharge International Classification of Disease, 10th edition code classification [19] and confirmed by case note review. All patients had a diagnosis of COPD documented in hospital case notes and general practitioner records and 98% of patients were taking COPD medications (inhaled corticosteroids, bronchodilators and mucolytic agents) at the time of hospitalisation. Subjects were participants in a larger, prospective study of rapid, near patient testing for *Streptococcus pneumoniae* and influenza and met the following inclusion criteria; aged >18 years; able and willing to give written informed consent (or a relative or carer is able and willing to give informed assent); acute exacerbation of a chronic cardiorespiratory disease or new onset of acute cardiorespiratory illness of <7 days duration; and able to be recruited within 16 h of hospital admission. Exclusion criteria included: did not meet inclusion criteria; alternative diagnosis; participation in another study within 30 days; and diagnosis on admission of myocardial infarction or angina. Patients were recruited between the months of September and May from 2005 to 2008, across two hospital sites with acute medical admission units, within the University Hospitals of Leicester NHS Trust (Leicester, UK). Patients transferred directly from the emergency department to the critical care unit were not included although patients who were admitted to medical wards and subsequently deteriorated and required critical care admission were included. The study was approved by the Leicestershire, Northamptonshire and Rutland Ethics Committee (all UK) and all patients gave informed written consent.

Clinical data

Demographic and clinical data were collected at enrolment and outcome data was collected retrospectively from case notes and recorded on a standardised case report form. All patients had a chest radiograph

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performed within 24 h of admission and all radiographs were reported by a consultant radiologist. Patients with consolidation on chest radiographs (*i.e.* pneumonia) were excluded.

Samples

Blood, urine, sputum and nasopharyngeal swab samples were collected at enrolment by trained research staff. Blood cultures were incubated aerobically and anaerobically using the automated BacT/ALERT 3D blood culture system (bioMerieux, Durham, NC, USA). Sputum was examined by Gram stain microscopy according to local protocols and reported semi-quantitatively. Sputum was cultured on sheep blood agar and chocolate agar according to local protocols. Urine was tested for *S. pneumoniae* antigens using the NOW *S. pneumoniae* urinary antigen tests (Binax, Portland, ME, USA). Nasopharyngeal swabs were stored in viral transport medium and frozen at -70°C prior to testing. Respiratory virus detection was defined as the detection of any respiratory virus nucleic acid on nasopharyngeal swab samples by real-time reverse transcriptase (RT)-PCR. Bacterial detection was defined by a positive urinary antigen test for *S. pneumonia*e, or positive cultures of blood or sputum with an organism deemed to be significant.

Real-time multiplex RT-PCR for respiratory viruses

Four quadruplex real-time (TaqMan; Applied Biosystems (Life Technologies S.A.), Madrid, Spain) RT-PCR assays including an internal control (bacteriophage MS2) were used to detect respiratory virus nucleic acid on nasopharyngeal swab samples, including: influenza A and B; respiratory syncytial virus (RSV) A and B; parainfluenza types 1–4; adenovirus; enterovirus; rhinovirus; human metapneumovirus (hMPV); group 1 coronaviruses (HCoV-229E and HCoV-NL63); and group 2 coronaviruses (HCoV-OC43 and HCoV-HKU1). Full primer and probe sequences and details of the molecular methods are given in Appendix A [20–23].

Statistical analysis

Data were analysed using PRISM Version 6 (Graphpad Software, La Jolla, CA, USA) and Stata version 12 (Stata Corporation, College Station, TX, USA). Categorical data are presented as frequencies and percentage, and continuous data as median (interquartile range). Medians were compared using the Mann–Whitney U-test and proportions compared using the Chi-squared test and Fisher's exact test as appropriate. Continuous data were categorised for logistic regression analysis, which was undertaken separately for virus only detection, bacteria only detection and mixed viral/bacterial detection, using a two-step approach. The main exposures of interest were CRP and temperature on admission. Age was considered a forced variable *a priori*. Antibiotic use prior to hospitalisation, which could influence the detection of pathogens, was considered an important confounder and therefore included in all multivariable models. In the first step of the analysis, odds ratios adjusted for age were calculated for each variable in turn with corresponding 95% confidence intervals and p-values from the likelihood ratio test. Variables associated with pathogen detection with a p-value <0.1 were shortlisted for the multivariable analysis. In the multivariable analysis shortlisted variables were added to a model with CRP on admission, temperature on admission, antibiotic use prior to hospitalisation and age. Adjusted odds ratios were calculated, with 95% confidence intervals and p-values derived from the likelihood ratio test.

Results

264 patients were studied. Figure 1 shows the flowchart for patients included in the study. In total, 100 (38%) out of 264 patients tested positive for respiratory viruses, 69 (26%) as virus only detected and 31 (12%) as mixed viral/bacterial detection. The detected viruses were: picornaviruses (rhinoviruses n=43 and enteroviruses n=3), influenza A and B (n=18), coronaviruses (n=13), parainfluenza viruses (n=11), RSV (n=9), hMPV (n=9) and adenovirus (n=1). In total, 66 (25%) patients had bacteria detected: 35 (13%) as a bacteria only detection and 31 (12%) as a mixed viral/bacterial detection. The detected bacteria were: *Haemophilus influenza* (n=34), *S. pneumoniae* (urine antigen positive n=16, sputum culture positive n=7 and positive in both n=2), *Pseudomonas aeruginosa* (n=9), *Moraxella catarrhalis* (n=5), and *Staphylococcus aureus* (n=3).

Table 1 shows the demographic, clinical and outcome data for patients with viruses only detected, bacteria only detected, mixed viral/bacterial detection and no pathogen detected. Comparison across patient groups showed no difference in age, sex, smoking status, influenza vaccination status, comorbidity, duration of illness prior to hospitalisation, antibiotic use prior to hospitalisation, antibiotic or oral corticosteroid use during hospitalisation, duration of hospital stay or mortality. Median CRP levels and body temperature were significantly different across the groups ($p<0.001$ and $p<0.001$, respectively). CRP was higher in the virus only detection group compared to the no pathogens detected group ($p<0.001$) and the virus only detection group compared to the bacteria only detection group ($p=0.08$), although this did not reach statistical significance. There was no difference in CRP level between the bacteria only detection group and

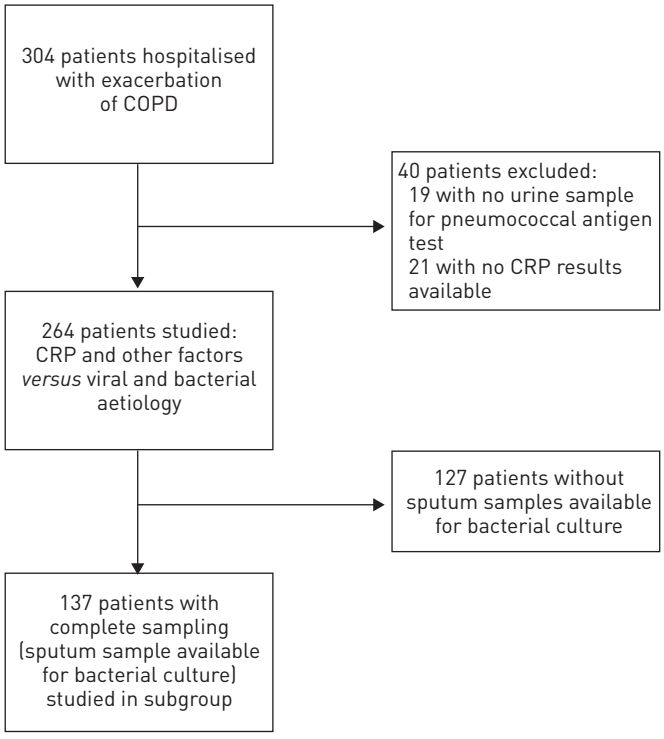

FIGURE 1 Flowchart for patients included in the study. Results of the subgroup analysis for only those patients with sputum samples available for culture (n=137) are detailed in Appendix B. COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein.

the no pathogens detected group (p=0.18) or between the mixed viral/bacterial detection and the virus only detection group (p=0.31). Temperature was higher in the virus only detection group compared to the no pathogens detected group (p<0.001) and the virus only detection group compared to the bacteria only detection group (p=0.003). There was no difference in temperature between the bacteria only detection group and the no pathogens detected group (p=0.62), or between the mixed viral/bacterial detection group and the virus only detection group (p=0.75). Figure 2 shows the comparison of CRP levels and temperature between the four groups.

Antibiotic use was more common in patients with viruses only (88%) and mixed virus/bacterial detection (88%) compared to patients with bacteria only (78%) and no pathogens detected (75%), although this did not reach statistical significance (p=0.08). Subgroup analysis of only those patients with sputum samples available for bacterial culture (n=137) showed similar results to the main cohort with regards to temperature and CRP (table S1).

Table 2 shows the adjusted odds ratios for the detection of viruses only by univariate and multivariate analysis. On multivariate analysis, CRP levels were strongly associated with viral detection rate (p<0.001). Body temperature was also strongly associated with viral detection rate (p=0.004, test for linear trend p<0.001). On univariate analysis there was an association between virus detection rate and duration of illness prior to hospitalisation (p=0.03); however, this association disappeared on multivariate analysis (p=0.16).

Table 3 shows the adjusted odds ratio for the detection of bacteria only by univariate and multivariate analysis. On multivariate analysis, CRP level and temperature were not associated with bacterial detection rate (p=0.47 and p=0.71, respectively). On multivariate analysis, antibiotic use prior to hospitalisation was not associated with a difference in bacterial detection rate (p=0.37).

Table 4 shows the adjusted odds ratio for the detection of mixed viral/bacterial infection by univariate and multivariate analysis. On multivariate analysis, CRP levels and body temperature were associated with mixed viral/bacterial detection rate (p=0.02 and p=0.03, respectively). On multivariate analysis antibiotic use prior to hospitalisation was not associated with a difference in mixed viral/bacterial detection rate (p=0.46). There was an association between age and mixed viral/bacteria detection with detection less likely in those aged >80 years (p=0.07), although this did not reach statistical significance. Figure 3 shows the

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TABLE 1 Demographic, clinical and outcome data for all patients and those with viruses only, bacteria only, mixed virus and bacterial, and no pathogens detected

	All patients	Virus only detected	Bacteria only detected	Mixed detection[#]	No pathogen detected	p-value[¶]
Subjects n	264	69 (26)	35 (13)	31(12)	129 (49)	
Age years	70 (62–77)	72 (62–79)	69 (62–76)	67 (61–72)	71 (63–78)	0.43
Male	140 (53)	34 (49)	21 (60)	17 (55)	68 (53)	0.77
Current smoker[+]	91 (35)	22 (32)	11 (31)	7 (32)	51 (40)	0.17
Influenza vaccination[§]	192 (74)	50 (72)	22 (63)	25 (81)	94 (73)	0.43
Cardiovascular disease[f]	135 (52)	41 (59)	13 (37)	14 (45)	66 (51)	0.12
Diabetes[##]	33 (13)	13 (19)	3 (9)	3 (10)	14 (11)	0.32
Duration of illness h	96 (48–168)	96 (72–168)	96 (48–168)	120 (72–144)	96 (48–168)	0.82
Temperature °C[¶¶]	36.6 (36.2–37.2)	37.0 (36.5–37.7)	36.5 (36.2–37.0)	37.0 (36.4–37.7)	36.5 (36.0–37.0)	**<0.001**
C-reactive protein mg·L^{-1}	17 (5–46)	31 (14–57)	20 (3–39)	39 (15–97)	8 (3–28)	**<0.001**
Antibiotics prior to admission	76 (29)	25 (36)	12 (34)	6 (19)	33 (26)	0.22
Antibiotics during admission[##]	211 (81)	61 (88)	26 (78)	28 (90)	96 (76)	0.08
Steroids during admission[++]	213 (86)	56 (86)	27 (90)	21 (81)	109 (87)	0.78
Duration of stay days	3 (1–6)	4 (1–6)	2 (1–8)	3 (1–6)	2 (1–6)	0.67
In-hospital mortality	6 (2)	3 (4)	0 (0)	0 (0)	3 (2)	0.41

Data are presented as median (interquartile range) or n (%), unless otherwise stated. Bold indicates statistical significance. [#]: concurrent detection of viruses and bacteria; [¶]: Chi-squared across all groups; [+]: n=263; [§]: n=258; [f]: n=259; [##]: n=260; [¶¶]: n=257; [++]: n=247.

detection rates of viruses only, bacteria only, mixed viral/bacterial detection and no pathogens detected by CRP level and temperature.

Examining viral and bacterial subtypes for patients with virus only detection and bacteria only detection demonstrated a significant association between the detection rates of influenza, rhino-enterovirus and "other" viruses (coronavirus, parainfluenza, RSV, hMPV and adenovirus), CRP levels and temperature. Detection of all virus types was less likely with CRP levels <10 mg·L^{-1} ($p<0.001$) and a temperature <37°C ($p<0.001$). Rhino-enterovirus was most commonly detected in patients with CRP levels of 10–50 mg·L^{-1} (OR 4.0, 95% CI 1.8–9.0; $p<0.001$) whereas influenza was most commonly detected in patients with CRP levels of 50–100 mg·L^{-1} (OR 7.6, 95% CI 2.7–21.3; $p=0.004$). There was no association between the detection rate of bacterial subtypes and CRP level or temperature. Figure 4 shows viral and bacterial subtype detection rate by CRP and temperature.

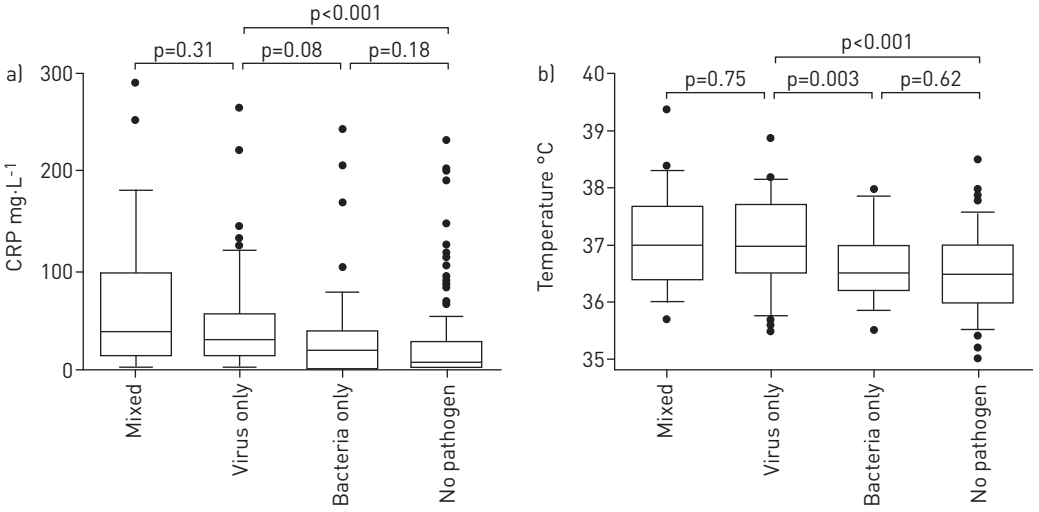

FIGURE 2 Box and whisker plots showing a) serum C-reactive protein (CRP) levels and b) body temperature in patients with mixed viral and bacterial detection, virus only detected, bacteria only detected, and no pathogen detected. Data are presented as median (interquartile range).

TABLE 2 Factors associated with virus only detection compared to no pathogens detected by univariate and multivariate analysis

Variable	Virus only detected/ virus only detected + no pathogen detected	Baseline model[#]	p-value	Multivariate model[¶]	p-value
C-reactive protein mg·L^{-1}					
Total	69/198				
<10	13/85 (15)	1	**<0.001**	1	**<0.001**
10–50	36/71 (51)	**5.6 (2.7–12.0)**		**4.6 (2.0–10.4)**	
51–100	13/24 (54)	**6.7 (2.5–18.3)**		**3.5 (1.1–11.4)**	
>100	7/18 (39)	**3.7 (1.2–11.7)**		2.7 (0.7–10.3)	
Temperature °C[+]					
Total	66/192				
<37.0	31/123 (25)	1	**<0.001**	1	**0.004**
37.0–37.5	16/43 (37)	1.7 (0.8–3.7)		1.2 (0.5–2.7)	
>37.5	19/26 (73)	**8.0 (3.1–20.9)**		**5.3 (1.8–15.6)**	
Age years					
Total	69/198				
⩽65	25/66 (38)	1	0.75	1	0.98
66–80	34/103 (33)	0.8 (0.4–1.5)		1.0 (0.5–2.2)	
>80	10/29 (34)	0.9 (0.3–2.2)		1.0 (0.3–3.0)	
Sex					
Total	69/198				
Male	35/96 (35)	1	0.65	NA	
Female	34/102 (34)	1.1 (0.6–2.1)		NA	
Antibiotics prior to admission					
Total	69/198				
No	44/140 (31)	1	0.14	1	0.11
Yes	25/58 (43)	1.6 (0.9–3.0)		1.8 (0.8–3.8)	
Duration of illness days					
Total	69/198				
>5	22/69 (32)	1	**0.03**	1	0.16
3–5	34/74 (46)	1.8 (0.9–3.6)		1.7 (0.8–3.9)	
⩽2	13/55 (24)	0.7 (0.3–1.5)		0.8 (0.3–2.0)	
Diabetes mellitus					
Total	67/195				
No	54/168 (32)	1	0.1	NA	
Yes	13/27 (48)	2.0 (0.9–4.9)		NA	
Cardiovascular disease					
Total	67/195				
No	26/87 (30)	1	0.18	NA	
Yes	41/108 (38)	1.5 (0.8–2.9)		NA	
Current smoker					
Total	69/197				
No	47/124 (38)	1	0.23	NA	
Yes	22/73 (30)	0.7 (0.4–1.3)		NA	
Influenza vaccination					
Total	67/193				
Yes	50/145 (35)	1	0.97	NA	
No	17/48 (35)	1.0 (0.5–2.0)		NA	

Data are presented as n/N (%) or adjusted OR (95% CI), unless otherwise stated. Bold indicates statistical significance. NA: not included. [#]: adjusted for age; [¶]: adjusted for C-reactive protein and temperature on admission, age, antibiotic use prior to admission and duration of illness prior to admission; [+]: also associated with viral detection rate as a linear trend ($p<0.001$).

Discussion

This is the largest study examining CRP and aetiology in hospitalised patients with exacerbations of COPD and, to our knowledge, the only study to examine the association between CRP and viral and bacterial subtypes. It demonstrates that the detection rate of respiratory viruses was strongly associated with CRP level and body temperature on admission to hospital, whereas the detection rate of bacteria was not. Increasingly frequent detection of viruses with increasing levels of CRP and temperature in our multivariate model supports other evidence for the causal nature of the association between detected viruses and

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TABLE 3 Factors associated with bacteria only detection compared to no pathogens detected by univariate and multivariate analysis

Variable	Bacteria only detected/ bacteria only detected + no pathogen detected	Baseline model[#]	p-value	Multivariate model[¶]	p-value
C-reactive protein mg·L^{-1}					
Total	35/164				
<10	15/87 (17)	1	0.39	1	0.47
10–50	13/48 (27)	1.8 (0.8–4.2)		1.6 (0.7–3.9)	
51–100	2/13 (15)	0.9 (0.2–4.4)		0.8 (0.2–4.0)	
>100	5/16 (31)	2.4 (0.7–8.3)		2.4 (0.7–8.9)	
Temperature °C					
Total	34/160				
<37.0	25/117 (21)	1	0.7	1	0.71
37.0–37.5	6/33 (18)	0.8 (0.3–2.1)		0.7 (0.3–2.0)	
>37.5	3/10 (30)	1.6 (0.4–6.7)		1.4 (0.3–6.4)	
Age years					
Total	35/164				
⩽65	14/55 (25)	1	0.64	1	0.69
66–80	16/85 (19)	0.7 (0.3–1.5)		0.7 (0.3–1.7)	
>80	5/24 (21)	0.8 (0.2–2.5)		0.7 (0.2–2.3)	
Sex					
Total	35/164				
Male	21/89 (24)	1	0.48	NA	
Female	14/75 (19)	0.8 (0.4–1.6)		NA	
Antibiotics prior to admission					
Total	35/164				
No	23/119 (19)	1	0.37	1	0.37
Yes	12/45 (27)	1.5 (0.6–3.4)		1.5 (0.6–3.6)	
Duration of illness days					
Total	35/164				
>5	12/59 (20)	1	0.67	NA	
3–5	14/54 (25)	1.3 (0.6–3.2)		NA	
⩽2	9/51 (18)	0.9 (0.3–3.3)		NA	
Diabetes mellitus					
Total	35/163				
No	32/146 (22)	1	0.72	NA	
Yes	3/17 (18)	0.8 (0.2–2.9)		NA	
Cardiovascular disease					
Total	35/163				
No	22/83 (27)	1	0.17	NA	
Yes	13/80 (16)	0.6 (0.3–1.3)		NA	
Current smoker					
Total	35/164				
No	24/101 (24)	1	0.32	NA	
Yes	11/62 (18)	0.7 (0.3–1.5)		NA	
Influenza vaccination					
Total	34/160				
Yes	22/117 (19)	1	0.24	NA	
No	12/43 (28)	1.6 (0.7–3.7)		NA	

Data are presented as n/N (%) or adjusted OR (95% CI), unless otherwise stated. NA: not included. [#]: adjusted for age; [¶]: adjusted for C-reactive protein and temperature on admission, age, and antibiotic use prior to admission.

exacerbation of COPD. In addition, the association between increasing CRP and viral detection rate was maintained across all virus types including influenza, rhinovirus and other viruses. The levels of CRP associated with specific virus types in our study are consistent with a previous study examining the natural kinetics of CRP levels over time in patients with acute respiratory illness [24]. As with asthma exacerbations, it seems likely that respiratory viruses are the principal initiating infectious agent in exacerbations of COPD, with new bacterial infections being of lesser importance. The reasons as to the relatively low rate of viral detection in exacerbations of COPD compared to asthma are unclear, although the overall detection rate of 38% in this study is highly consistent with other studies [9–11].

TABLE 4 Factors associated with mixed viral and bacterial detection compared to no pathogens detected by univariate and multivariate analysis

Variable	Mixed viral and bacterial detection/mixed viral and bacterial detection + no pathogen detected	Baseline model[#]	p-value	Multivariate model[¶]	p-value
C-reactive protein mg·L^{-1}					
Total	31/160				
<10	6/78 (8)	1	**<0.001**	1	**0.02**
10–50	11/46 (24)	**3.8 (1.3–11.3)**		**3.0 (1.0–9.2)**	
51–100	8/19 (42)	**8.9 (2.5–31.0)**		**5.1 (1.3–19.8)**	
>100	6/17 (35)	**8.8 (2.2–34.6)**		**6.5 (1.5–28.0)**	
Temperature °C[+]					
Total	31/157				
<37.0	15/107 (14)	1	**0.002**	1	**0.03**
37.0–37.5	8/35 (23)	1.7 (0.6–4.4)		1.2(0.4–3.6)	
>37.5	8/15 (53)	**8.5 (2.6–30.0)**		**4.8(1.3–18.0)**	
Age years					
Total	31/160				
⩽65	13/54 (24)	1	0.32	1	0.07
66–80	16/85 (19)	0.7 (0.3–1.7)		0.6 (0.2–1.5)	
>80	2/21 (10)	0.3 (0.1–1.6)		**0.2 (0.1–1.0)**	
Sex					
Total	31/160				
Male	17/85 (20)	1	0.87	NA	
Female	14/75 (19)	0.9 (0.6–2.1)		NA	
Antibiotics prior to admission					
Total	31/160				
No	25/121 (21)	1	0.27	1	0.46
Yes	6/39 (15)	0.6 (0.2–1.6)		0.7 (0.2–2.0)	
Duration of illness days					
Total	31/160				
>5	8/55 (15)	1	0.07	1	0.1
3–5	17/57 (30)	2.4 (0.9–6.3)		1.9 (0.7–5.6)	
⩽2	6/48 (13)	0.9 (0.3–2.8)		0.5 (0.1–2.0)	
Diabetes mellitus					
Total	30/158				
No	27/141 (19)	1	0.84	NA	
Yes	3/17 (18)	0.9 (0.3–3.3)		NA	
Cardiovascular disease					
Total	29/157				
No	15/76 (20)	1	0.91	NA	
Yes	14/81 (17)	1.0 (0.4–2.2)		NA	
Current smoker					
Total	31/159				
No	24/101 (24)	1	0.03[§]	NA	
Yes	7/58 (12)	0.4 (0.4–1.3)		NA	
Influenza vaccination					
Total	31/157				
Yes	25/120 (21)	1	0.49	NA	
No	6/37 (16)	0.7 (0.3–1.9)		NA	

Data are presented as n/N (%) or adjusted OR (95% CI), unless otherwise stated. Bold indicates statistical significance. NA: not included. [#]: adjusted for age; [¶]: adjusted for C-reactive protein and temperature on admission, age, and antibiotic use prior to admission; [+]: also associated with mixed viral or bacterial detection rate as a linear trend ($p<0.001$); [§]: despite a p-value of <0.1 on univariate analysis, this variable could not be added to the multivariate models due to the small number of outcomes (*i.e.* detection of mixed viruses/bacteria, n=31).

In contrast, the lack of association between bacterial detection rate and CRP levels or body temperature suggests that bacterial detection during exacerbation may often represent airway colonisation [25]. In our study, patients with mixed detection of viruses and bacteria behaved similarly to the patients with viruses only detected suggesting that the viral component was responsible for the associations between mixed

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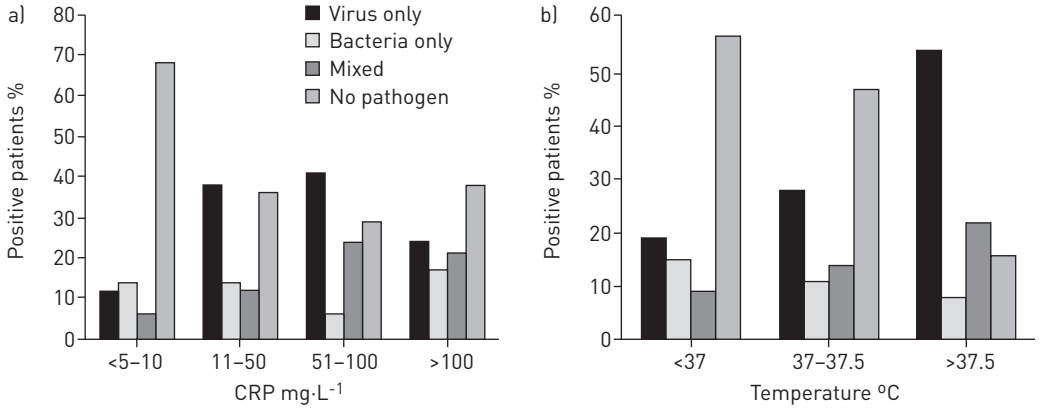

FIGURE 3 The proportion of patients with viruses only detected, bacteria only detected, mixed viral and bacterial detection, and no pathogens detected by a) serum C-reactive protein (CRP) and b) temperature.

detection, CRP and temperature. Unlike some previous studies, markers of severity including duration of hospital stay were not higher in patients with mixed infection compared to other groups [11].

Although previous studies have demonstrated more severe respiratory symptoms, prolonged recovery times and higher levels of serum interleukin-6 levels in patients with virus-induced exacerbation compared to non-virus-induced exacerbations [9, 26], our study is the first to show that CRP levels are strongly associated with viral rather than bacterial detection. Several other studies have examined CRP levels in viral

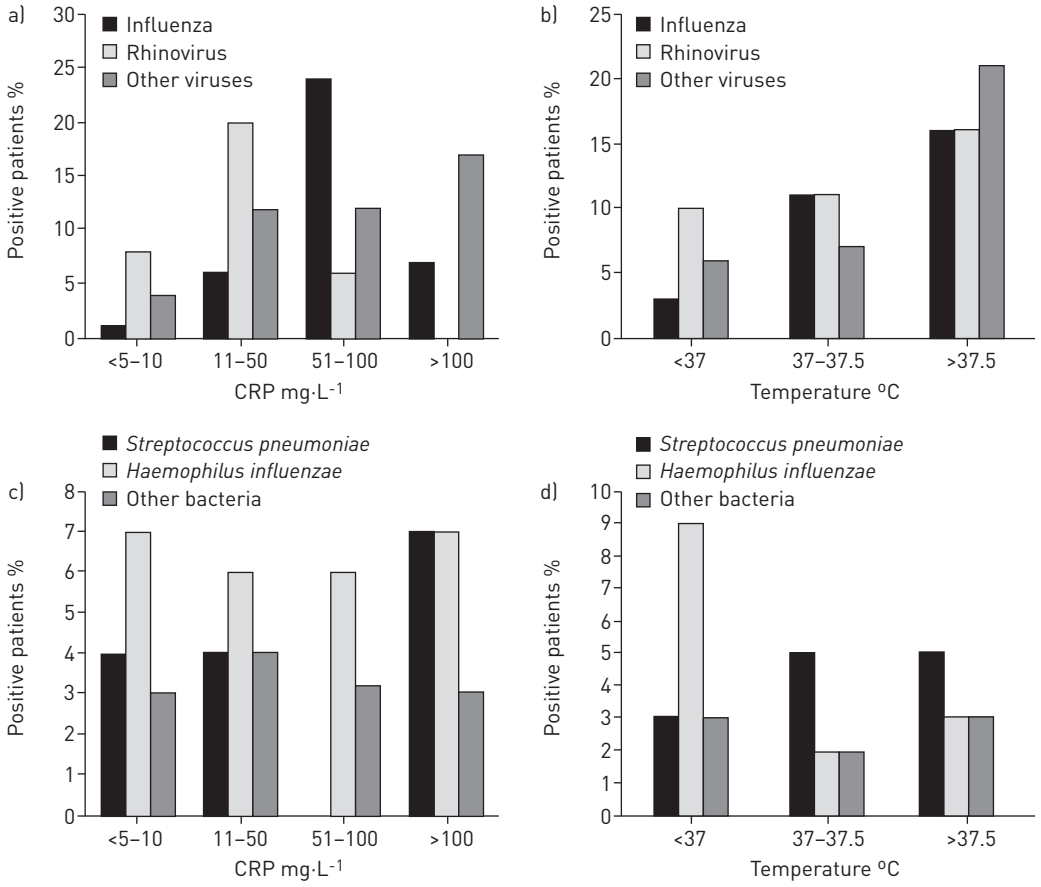

FIGURE 4 The proportion of patients positive for a, b) viral subtypes in patients with viruses only and c, d) bacterial subtypes in patients with bacteria only detected by a, c) serum C-reactive protein (CRP) and b, d) temperature. Other viruses include respiratory syncytial virus, parainfluenza, human metapneumovirus, coronavirus and adenovirus. Other bacteria include *Moraxella catarrhalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

and bacterial exacerbation and have generally not shown a difference in CRP levels between the two; however, these studies have been limited by smaller numbers of patients or have compared viral *versus* non-viral exacerbations so that many patients with viral detection have also had bacteria detected [11, 27, 28]. Furthermore, none of these studies have used multivariate analysis to examine the relationships between CRP levels and detection rate of viruses or bacteria in detail.

As bacterial detection occurs in patients across all levels of CRP and temperature, the implications of this study for antibiotic use in exacerbations of COPD are not clear. Antibiotics are frequently used in exacerbations of COPD based on clinical criteria and have been shown to be beneficial in more severe cases [29]. Although viruses may be the main infectious agent initiating COPD exacerbations this does not preclude clinical benefit from antibiotics as the interaction between newly acquired viruses and chronically colonising bacteria may subsequently lead to an increase in bacterial load or a phenotypic change [14–16]. Furthermore, as the patients in this study had been unwell for <7 days it is possible that bacteria become more prevalent and important later in the course of a virally triggered exacerbation, as suggested by a recent study [16].

Whilst this is a ''real world'' clinical study it does have several limitations. As it is a study of hospitalised patients conducted outside of the summer months, findings cannot be generalised to patients treated for exacerbations in the community or during the summer months when the incidence of respiratory virus infection may be lower. As we excluded patients with exacerbations of COPD and radiological pneumonia it must be stressed that these findings apply only to patients with non-pneumonic exacerbations of COPD and cannot be extrapolated to patients with pneumonia or in those in whom pneumonia has not yet been excluded. As we excluded patients who had been unwell for <7 days the results of this study cannot be applied to those patients who have been unwell for longer periods. As it is an observational study and the numbers of patients with individual virus and bacterial types were low in some cases, we would suggest that the findings in this study are validated in a larger study.

Sputum bacterial cultures may underestimate the frequency of bacterial detection compared to newer molecular methods [30]. In our study we did not use molecular methods to identify or quantify bacteria and so cannot exclude the possibility that true bacterial detection rate was higher or that there was a relationship between CRP levels and quantitative bacterial load. Another possible weakness is that almost one-third of patients were already on antibiotics at enrolment which could have led to a reduction in the ability to culture bacteria in sputum and underestimated the proportion of patients with bacterial detection. However, the prior use of antibiotics was not associated with reduced detection rates of bacteria compared to those who had not received antibiotics in our multivariate model, giving reassurance that this did not affect our results. The rate of bacterial detection in our study is low compared to other studies of COPD exacerbation examining bacteria in sputum by conventional culture [12, 14, 31], although similar to others [29]. Our subgroup analysis of only those patients who were able to produce a sputum sample demonstrated a higher rate of bacterial detection overall (44%), suggesting that this was the explanation for the lower rates in the main analysis. The associations between viral detection, bacterial detection, mixed viral/bacterial detection, CRP and temperature were not significantly different in this subgroup suggesting that the low detection rate in the main analysis has not altered these associations.

As CRP levels can be elevated in patients with COPD independently of exacerbation [32–34], this is a potential confounding factor in our study. Although studies have shown that baseline CRP levels can are elevated in patients with severe COPD this is using a highly sensitive CRP assay and corresponds to levels of ~4–7 $mg \cdot L^{-1}$ in more severe COPD compared to lower levels in less severe COPD or healthy controls [34]. In this study levels of CRP <10 $mg \cdot L^{-1}$ were not associated with an increase in viral detection rate and, in fact, levels of <10 $mg \cdot L^{-1}$ were independently associated with a lack of viral detection compared to higher levels. Therefore, small elevations in CRP below this level will not have influenced our results.

In conclusion, this work demonstrates that the detection rate of respiratory viruses in patients hospitalised with exacerbations of COPD is strongly associated with host inflammatory response as measured by CRP level and body temperature. This relationship reinforces evidence suggesting that respiratory viruses initiate a large proportion of exacerbations of COPD, and could be used in clinical practice to guide viral testing and directed antiviral therapy where available.

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