1 Title

2 Exclusion of bacterial co-infection in COVID-19 using baseline inflammatory markers and their response to

3 antibiotics

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Abstract

Background

COVID-19 is infrequently complicated by secondary bacterial infection, but nevertheless antibiotic prescriptions are common. We used community-acquired pneumonia (CAP) as a benchmark to define the processes that occur in a bacterial pulmonary infection, and tested the hypothesis that baseline inflammatory markers and their response to antibiotic therapy could distinguish CAP from COVID-19.

Methods

In patients admitted to Royal Free Hospital (RFH) and Barnet Hospital (BH) we defined CAP by lobar consolidation on chest radiograph, and COVID-19 by SARS-CoV-2 detection by PCR. Data were derived from routine laboratory investigations.

Results

On admission all CAP and >90% COVID-19 patients received antibiotics. We identified 106 CAP and 619 COVID-19 patients at RFH. CAP was characterised by elevated white cell count (WCC) and C-reactive protein (CRP) compared to COVID-19 (median WCC 12.48 (IQR 8.2-15.3) vs 6.78 (IQR 5.2-9.5) x10^6 cells/ml and median CRP CRP 133.5 (IQR 65-221) vs 86 (IQR 42-160) mg/L). Blood samples collected 48-72 hours into admission revealed decreasing CRP in CAP but not COVID-19 (CRP difference -33 (IQR -112 to +3.5) vs +15 (IQR -15 to +70) mg/L respectively). In the independent validation cohort (BH) consisting of 169 CAP and 181 COVID-19 patients, admission WCC >8.2x10^6 cells/ml or falling CRP during admission identified 95% of CAP cases, and predicted the absence of bacterial co-infection in 45% of COVID-19 patients.

Conclusions

We propose that in COVID-19 the absence of both elevated baseline WCC and antibiotic-related decrease in CRP can exclude bacterial co-infection and facilitate antibiotic stewardship efforts.

Introduction

The COVID-19 pandemic caused by the novel beta coronavirus SARS-CoV-2 has caused over 34 million infections and over 1 million deaths worldwide [1]. The drivers of pathology remain to be elucidated, but a hyperinflammatory response is associated with worse case fatality [2]. Other viral respiratory tract infections, best characterised by influenza, can be complicated by bacterial co-infections that also raise inflammatory markers and are associated with high mortality [3,4], but distinguishing severe viral pneumonia from bacterial co-infection is challenging [5]. In COVID-19, several studies have found bacterial co-infection to be rare, as determined by identification of causative pathogens [6–9]. However, routine microbiological culture takes several days, lacks sensitivity [10] and does not readily distinguish bacterial colonisation from infection. Moreover, microbiological respiratory tract sampling is not routinely performed in patients admitted with COVID-19 [9]. Therefore, despite guidance aimed at rationalising antibiotic use [11], it is unsurprising that diverse and elevated rates of antibiotic prescriptions have been reported in patients admitted for COVID-19 infection [8,9].

It is likely that many COVID-19 associated antibiotic prescriptions are given in the absence of bacterial co-infection, thus hampering antimicrobial stewardship efforts and potentially increasing antimicrobial resistance [12–14]. Many studies have focused on clinical and laboratory features that risk stratify outcome in COVID-19 [15–18], but currently infections caused by virus alone cannot be readily distinguished from those with a bacterial component. C-reactive protein (CRP), white cell count (WCC) and procalcitonin (PCT) have been used to distinguish between influenza and bacterial pneumonia, allowing antibiotic treatment to be omitted or stopped [19–22]. Serial measurements of inflammatory markers may also assist in distinguishing bacterial from viral infections [23,24]. A small retrospective study comparing COVID-19 to community-acquired pneumonia patients identified differences in admission neutrophil counts, D-dimers and CRP, but did not provide a rigorous definition for the pneumonia cases, nor explored changes in these markers over time [25].

In this study we aimed to identify features that discriminated viral COVID-19 infections from those complicated by bacterial co-infection. We used community-acquired pneumonia (CAP) as a benchmark to define the processes that occur in bacterial pulmonary infections, and tested the hypothesis that baseline inflammatory markers and their response to antibiotics could distinguish CAP from most COVID-19 infections. To address this research question, we performed a retrospective, cohort study from a large split-site academic hospital in the UK. We used the independent nature of the two sites to discover and validate our findings, extending their generalisability.

Methods

Data extraction and ethics

Anonymised demographics, antimicrobial prescriptions, haematological and biochemical investigations were extracted from the Clinical Practice Group analysis team, Cerner Electronic Patient Records and the electronic Clinical Infection Database (elCID), and microbiological investigations from WinPath at Royal Free London (RFL) NHS Trust [26]. The study was approved by the Research and Innovation Group at RFL NHS Trust, which stated that confidential patient information could be used under COVID-19 COPI notice made by Department of Health and Social Care, and that as this was a retrospective review of routine clinical data, formal ethical approval was not required.

Patient selection

We identified patients from 2 hospital sites of RFL NHS Trust in London, UK: Royal Free Hospital (RFH) and Barnet Hospital (BH). These hospitals are separated by 11 kilometres and patient care is delivered by non-overlapping clinical staff, using non-identical clinical care bundles and antibiotic policies. We included patients aged >18 years old admitted to hospital, of which a subset was admitted for >48 hours (tables 1 & 4), and excluded patients with haematological malignancies. We defined COVID-19 and influenza patients by RT-PCR detection of SARS-CoV-2 and influenza A or B viruses respectively from nasopharyngeal swabs. COVID-19 patients were identified between 1st March and 31st May 2020, and influenza patients between 1st January and 31st May 2019. These criteria yielded 619 and 181 COVID-19 patients from RFH and BH respectively, and 188 and 162 influenza patients from RFH and BH respectively. There were no patients co-infected with SARS-CoV-2 and influenza viruses. CAP patients were identified by a clinical diagnosis of CAP made between 1st January and 31st May 2019 with focal consolidation on chest radiograph reported by consultant radiologists (106 patients at RFH and 169 at BH).

For some analyses, we excluded COVID-19 patients with radiological or microbiological evidence of ongoing bacterial co-infection. This was defined by presence of lobar pneumonia on a chest radiograph within 72 hours of hospital admission, or by a non-contaminant bacterial growth on blood culture, growth in sputum samples of *Streptococcus pneumoniae*, *Staphylococcus aureus* or *Moraxella catarrhalis,* detection of *Mycoplasma pneumoniae* by PCR from sputum or detection of *Streptococcus pneumoniae* antigen in urine (tables 1 & 3 and table S1).

Statistical analysis

Baseline demographics were compared by Mann-Whitney test (age), Fisher's exact test (gender) or Chi-square test (ethnicity, Charlson co-morbidities and microbiological results). Continuous variables from blood tests were expressed as median and interquartile range (IQR), and patient groups were compared using the non-

105 parametric two-tailed Mann-Whitney U test. A multivariate logistic regression model was used to determine

106 factors that discriminated between CAP and COVID-19. The diagnosis of CAP was the categorical output

107 variable, and blood tests were used as continuous dependent variables in the model, which generated

108 Receiver Operating Characteristic curves (ROC) and areas under the curve (AUC) as a summary statistic. For

109 pre-determined cut-offs, we also calculated sensitivity, specificity, positive and negative predictive values, and

110 positive and negative likelihood ratios. All analyses were performed using Microsoft Excel and GraphPad Prism

111 version 8.

112

Results

Defining the discovery cohort

115 We identified 107 CAP, 620 COVID-19 and 188 influenza patients at RFH. 2 patients were excluded due to

116 haematological malignancy. Male gender was overrepresented in COVID-19 (62% in COVID-19 vs 47% and 52%

117 in CAP and influenza respectively), whereas CAP patients were older (median age 72, 68 and 56 in CAP, COVID-

118 19 and influenza respectively). The proportion of Black, Asian, Mixed and Other (non-white) ethnicity patients

119 was higher in COVID-19 compared to CAP and patients with CAP had more comorbidities and identified

120 bacteria in routine microbiological investigations more commonly (table 1).

Distinguishing pneumonia from COVID-19

122 We tested the hypothesis that inflammatory markers could discriminate CAP from COVID-19 or influenza by

123 comparing total WCC, its differential cell counts and CRP levels on the day of admission to hospital. Compared

124 to CAP, COVID-19 was associated with significantly lower median WCC (12.48 vs 6.78×10^6/ml) and neutrophils

125 (9.98 vs 5.37×10^6/ml (fig 1)). Influenza was also characterised by significantly lower median WCC (7.28×10^6/ml)

126 and neutrophils (median 5.10×10^6/ml) than pneumonia (fig 1). Lymphocyte counts did not differ between the

127 groups. CRP was also significantly higher in CAP than in COVID-19, and lower in influenza (median CRP 133.5,

128 86.0 and 31mg/L respectively) (fig 1).

129 All CAP patients were prescribed antibiotics on admission and in two independent surveys of COVID-19

130 patients from RFH, 95/100 (95%) and 104/118 (88%) were prescribed antibiotics to treat a presumptive

131 superadded pulmonary bacterial infection. We hypothesised that CAP and viral infections could be further

132 discriminated by changes in inflammatory markers following initiation of antibiotics [23]. In the RFH cohort,

133 53 (50%) CAP, 331 (53%) COVID-19 and 53 (28%) influenza patients were admitted for >48 hours and had a

134 blood sample collected as part of routine clinical care 48-72 hours into admission (table 1). Differences in

135 inflammatory markers on admission within this subset mirrored that seen in the wider cohort (fig S1). At this

136 later time point, CAP was still characterised by elevated median WCC (9.5 vs 7.01×10^6/ml), but this difference

137 was diminished compared to admission (fig S2). Moreover, the difference in CRP between CAP and COVID-19

138 was no longer evident (median CRP 107.5 vs 127.0mg/L) (fig S2). These changes were driven by a greater fall

139 in WCC and CRP for CAP compared to COVID-19 (ΔWCC -2.32 vs -0.17x10^6/ml and ΔCRP -33 vs +15mg/L

140 respectively) (fig 2).

Contribution of multiple variables to discriminate pneumonia from COVID-19

142 Our data suggested that elevated WCC and CRP, as well as a reduction in these parameters at 48-72 hours

143 could discriminate between COVID-19 and CAP. To test this hypothesis, we applied a logistic regression model

144 to the data collected from these two patient groups. We used the diagnosis of CAP as the binary outcome

145 variable and observed that WCC or ΔCRP measurements alone yielded the greatest diagnostic accuracy (fig 3

146 & table 2). The maximal AUC obtained from this analysis was 0.75 (C.I. 0.68-0.83), demonstrating the trade off

147 in sensitivity and specificity when using these variables alone. We also tested whether their combined use

148 would improve the diagnostic accuracy of the model, and observed some improvement in discriminating

149 between CAP from COVID-19 (AUC 0.80, C.I. 0.73-0.87), with no added benefit to also including ΔWCC

150 measurements (fig 3 & table 2).

151 We considered whether the ability of WCC and ΔCRP to discriminate between COVID-19 and CAP was

152 confounded by admission to intensive care unit (ICU). At RFH, 79/331 (23.9%) of COVID-19 patients included

153 in the logistic regression model were admitted to ICU within 72 hours of their admission (table 1). Excluding

154 this subset of patients did not affect the discriminatory power of the model (AUC 0.81, C.I. 0.74-0.88) (table

155 2). We also considered whether COVID-19 patients with microbiological or radiological evidence of a bacterial

156 infection could have confounded discrimination from pneumonia. We identified 16 (4.8%) COVID-19 patients

157 included in the logistic regression model with microbiological results consistent with an active bacterial

158 process (sputum culture positivity with a respiratory pathogen or a non-contaminant bacterial growth in blood

159 cultures (table 1)), and 2 (0.1%) further COVID-19 patients with lobar pneumonia on chest radiograph (table

160 1). Excluding these 18 patients did not alter the differences observed in admission WCC or ΔCRP between CAP

161 and COVID-19 (data not shown), and did not affect the classification accuracy of the remaining patients in the

162 logistic regression model (table 2).

Decision making criteria to discriminate bacterial pneumonia and COVID-19

164 We sought to convert our observations into practical decision-making criteria for clinical practice. We

165 generated a series of cut-offs in the variables with greatest discriminatory power between CAP and COVID-19

166 (admission WCC and ΔCRP), and explored the trade off in sensitivity and specificity generated by these alone

167 or in combination (table 3). For cut offs of WCC we used the lower quartile value of the CAP cohort

168 (>8.2x10^6/ml) and the upper quartile value of the COVID-19 cohort (>9.5x10^6/ml). For cut offs of ΔCRP we used

169 the lower quartile of the COVID-19 cohort (<-15mg/l) and the upper quartile of the CAP cohort (<3.5mg/l),

170 rounded to 0mg/L for simplicity This revealed that using CAP-derived quartile cut-offs yielded greater

171 sensitivity, at the expense of specificity. The lower prevalence of CAP in this cohort compared to COVID-19

172 offered a high negative predictive value (>90%). Requiring both a WCC>8.2x10^6/ml and ΔCRP<0 improved

173 specificity, at the expense of sensitivity. However, as such a strategy would result in many cases of CAP being

174 missed, we also explored using the presence of either parameter to define CAP, yielding a sensitivity of >90%.

175 Although the specificity of this approach was only 43%, the absence of both admission WCC>8.2x10^6/ml and

176 ΔCRP<0 could still exclude CAP, and by extension bacterial co-infection alongside COVID-19, promoting

177 antibiotic cessation in 142 / 331 (43%) COVID-19 patients from this cohort (table 3).

178 ## Independent cohort validation

179 To demonstrate reproducibility of our findings, we used independent patient cohorts from a separate hospital,

180 BH, consisting of 169 CAP, 181 COVID-19 and 162 influenza A/B patients. To ensure comparability to the RFH

181 cohort, the patients were identified over the same time periods using identical criteria. Baseline demographic

182 analyses were comparable to those in the RFH cohort (table 4), and 99 (59%), 60 (33%) and 47 (29%) of CAP,

183 COVID-19 and influenza patients respectively were admitted for >48 hours and had a blood sample collected

184 as part of routine clinical care 48-72 hours into admission (table 4).

185 Differences in inflammatory markers within the BH cohort reflected those observed at RFH, with admission

186 WCC and CRP levels being higher in CAP compared to COVID-19 and accompanied by a reduction following 48-

187 72 hours of admission not observed in COVID-19 (fig S3). Applying these parameters into a logistic regression

188 model demonstrated similar discriminatory power of each variable to that seen in RFH patients, and once

189 more an optimal AUC (0.84, C.I. 0.78-0.91) was derived when both admission WCC and ΔCRP were included

190 as variables in the model (table 5). We explored the exclusion of COVID-19 patients included in the logistic

191 regression model that either attended ICU within 72 hours of hospital admission (21/60, 35.0%) or

192 demonstrated microbiological or radiological evidence pneumonia (4/60, 6.7%) (table 5). However, as seen in

193 the RFH cohort, excluding either subset of patients did not significantly affect the discriminatory ability of the

194 logistic regression model (table 5).

195 Finally, we applied the cut-offs independently derived from the RFH cohort on BH patient data, and observed

196 a similar trade-off between sensitivity and specificity. Using either admission WCC>8.2x10^6/ml or ΔCRP<0

197 yielded a CAP sensitivity approaching 95% and negative predictive value of 84% (table 6). Despite the low

198 specificity resulting from these cut-offs, this would still have excluded CAP in 26/56 (46.4%) of COVID-19

199 patients with no microbiological or radiological evidence of pneumonia and admitted for >48hours. 55 (98%)

200 of these patients were prescribed a 5-day course of antibiotics on admission (203 antibiotic days in total).

201 Stopping antibiotic prescriptions 48 hours into admission in the 26 patients that did not meet either diagnostic

202 criterium for CAP would have saved 51 antibiotic days overall, a 25% reduction in total antibiotic prescriptions

203 in the BH COVID-19 cohort.

Discussion

205 Elevated inflammatory responses, high case fatality and bacterial co-infections observed in influenza

206 contribute to frequent antibiotic prescriptions in COVID-19 [2,4,9,27]. However, radiological findings in COVID-

207 19 are heterogenous [28] and microbiological investigations rarely identify pathogenic bacteria [6–9],

208 precluding reliable identification of co-infection. Therefore, novel approaches to exclude bacterial co-infection

209 in COVID-19 are a research priority to facilitate antimicrobial stewardship efforts [14,29,30].

210 We used patients admitted with CAP prior to the COVID-19 pandemic as a benchmark to define the processes

211 that occur in bacterial pulmonary infections, including bacterial co-infection in COVID-19. This demonstrated

212 that admission WCC (predominantly neutrophils), and CRP can discriminate CAP from both COVID-19 and

213 influenza A/B at a population level. Moreover, WCC and CRP decreased following antibiotic therapy in

214 pneumonia, but not in viral infections. We used these observations to construct a model and decision-making

215 criteria to assist with excluding bacterial co-infection in many cases of COVID-19. We propose that the absence

216 of both admission WCC >8.2×10^6/ml and a fall in CRP would support stopping antibiotics in almost 50% of

217 COVID-19 patients during their admission, reducing total antibiotic prescriptions in this population by up to

218 25%. This approach would exceed most antimicrobial stewardship achievements [29,30], and reduce selection

219 for antibiotic resistant bacteria during this pandemic [12,14].

220 The combination of selected cut-offs yielded the greatest sensitivity for CAP, ensuring ongoing antibiotic

221 treatment where needed, but came at the expense of specificity. Therefore, these criteria remain permissive

222 to excessive antibiotic prescribing in COVID-19, particularly on admission to hospital. PCT can discriminate

223 bacterial from some viral respiratory tract infections [21,22], but has not been systematically compared

224 between bacterial pneumonia and COVID-19. Unfortunately, we could not investigate PCT as it was not

225 measured routinely in our CAP cohorts. Future studies should also assess, alongside admission WCC and ΔCRP,

226 the discriminatory capacity of PCT, D-dimers [9,22,31], and other novel biomarkers, such as transcriptional

227 signatures that quantify inflammatory cytokine activity [32] or those that discriminate bacterial from viral

228 infections [33].

229 The large, standardised populations studied, use of routinely available clinical investigations, and the

230 reproducibility of our findings in an independent validation cohort population are key strengths of our study.

231 We were also able to convert population level findings into practical diagnostic criteria that can be used in

232 generalised clinical settings. The cut-offs used were derived from a separate population in which they were

233 tested, adding scientific validity to our conclusions, but does not negate individual care providers determining

234   the distribution of admission WCC in their own cases of CAP to define institution-specific IQR cut-offs. In

235   addition, our criteria should not be considered in isolation from clinical decision making. Clinical improvement,

236   reduced supplemental oxygen requirement, and the absence of consolidation on chest radiograph in COVID-

237   19 patients may all contribute to excluding bacterial co-infection and could be used alongside the WCC and

238   ΔCRP criteria to support cessation of antibiotics.

239   Our study has some notable limitations. First, the populations were identified at non-overlapping times, due

240   to the disproportionate prevalence of COVID-19 cases in 2020. We attempted to mitigate for the enforced use

241   of historical pneumonia and influenza comparator groups by identifying these patients over the same months

242   of 2019 as COVID-19 cases in 2020. We also did not collect clinical severity or outcome data for the patients,

243   and thus we cannot measure a direct impact on prognosis. Second, we used a radiological, but not

244   microbiological, definition of pneumonia, and although standardised, it is possible that some pneumonia cases

245   had non-bacterial aetiology. Third, we inferred that bacterial co-infection in COVID-19 shares pathophysiology

246   and inflammatory marker responses with CAP in the absence of COVID-19. This hypothesis remains untested

247   but is supported by the divergent inflammatory marker responses observed between most COVID-19 and CAP

248   patients. Fourth, we did not include suspected COVID-19 patients with negative SARS-CoV-2 results, therefore

249   our findings may not be applicable to this cohort. Finally, we focused on patient assessments made within 72

250   hours of admission, and thus our decision-making tools are not applicable to patients discharged before this

251   time or those with prolonged hospital admissions.

252   In conclusion, we demonstrate that routine clinical parameters, admission WCC and changes in CRP following

253   antibiotic administration, can be translated into a set of diagnostic criteria that can exclude bacterial co-

254   infection in up to half of COVID-19 patients. The routine nature of the investigations required mean that, even

255   in the context of a pandemic, this approach can form the basis of protocols to assist reductions in unnecessary

256   antibiotic prescriptions for viral infections, minimising drug-associated adverse effects and reducing

257   development of antimicrobial resistance.

Acknowledgements

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Figure legends

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369   **Figure 1** Admission blood samples for all patients admitted to RFH. Violin plots represent distribution of values

370   for CAP (n=106), COVID-19 (n=619) and influenza A/B (n=188) patients. Bold lines represent median values.

371   Dotted lines represent IQR values. **** indicates $p<0.0001$ by Mann-Whitney test.

372   **Figure 2** Change in values between admission blood samples and those collected 48-72 hours into admission

373   at RFH. Violin plots represent distribution of difference (◌) in investigation results between those collected on

374   hospital admission and 48-72 hours into admission in CAP (n=53), COVID-19 (n=331) and influenza A/B (n=53)

375   patients. Bold lines represent median values. Dotted lines represent IQR values. **** indicates $p<0.0001$ by

376   Mann-Whitney test.

377   **Figure 3** Accuracy of blood parameters to diagnose CAP in RFH patients. Receiver operating curves generated

378   from logistic regression models that incorporate combinations of WCC on admission and difference (Δ) in CRP

379   between samples on admission and 48-72 hours into admission in order to discriminate RFH patients

380   diagnosed with CAP from those diagnosed with COVID-19.

Figure 1

Figure 2

Figure 3

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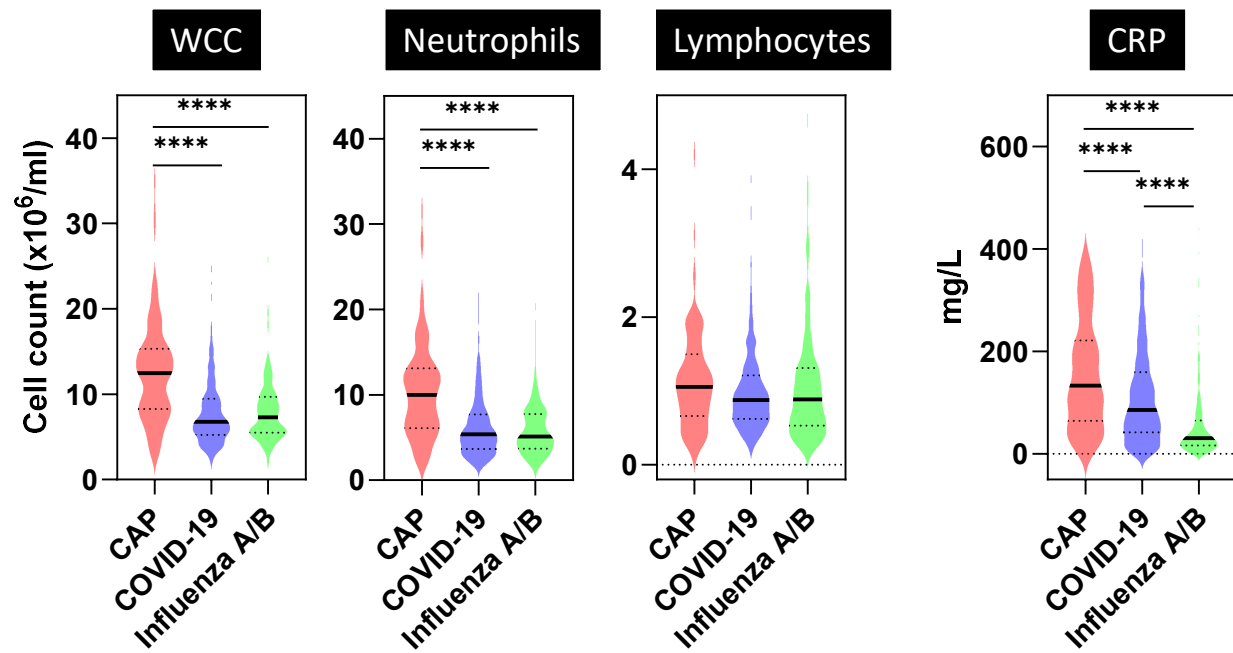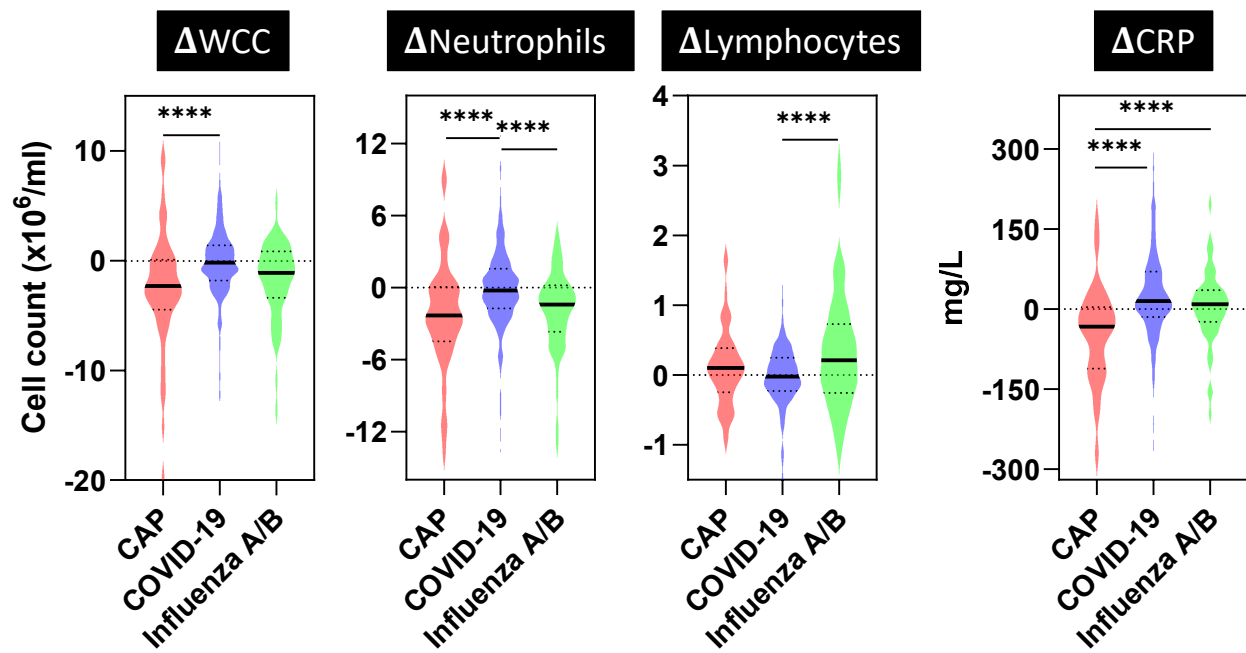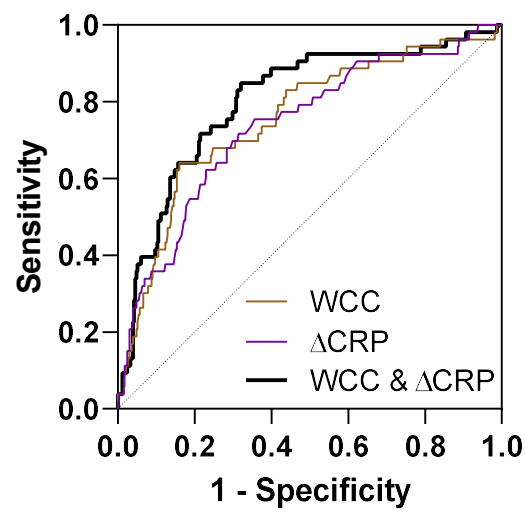
Diagnosis		CAP	COVID-19	Influenza	p value
Numbers		106	619	188	-
Chest radiograph		Lobar consolidation 106 (100%)	Lobar consolidation 5 (0.8) CVXC0: 62 (10.0) CVCX1: 281 (45.3) CVCX2: 136 (22.0) CVCX3: 17 (2.7) Ungraded: 123 (19.9)	-	-
Male, n (%)		50 (47)	386 (62)	98 (52)	0.0037
Age, median (range)		72 (19-99)	68 (18-100)	56 (18-95)	0.1401
Ethnicity, n (%)	White	60 (57)	250 (40)	87 (46)	0.0007
	Asian	23 (22)	83 (13)	31 (16)	
	Black	6 (6)	69 (11)	13 (7)	
	Mixed	2 (2)	7 (1)	3 (2)	
	Other/ unknown	15 (14)	210 (34)	54 (29)	
Charlson index co-morbidities n (%)	0	18 (17)	243 (39)	86 (46)	<0.0001
	1	33 (31)	190 (31)	58 (31)	
	2	28 (26)	109 (18)	26 (14)	
	3+	27 (25)	77 (13)	18 (10)	
Positive microbiology results, n (%)	Sputum	5 (4.7)	3 (0.004)	4 (2.1)	<0.0001
	Blood	2 (1.9)	12 (1.9)	6 (3.2)	
	Urine Ag	4 (3.8)	3 (0.004)	2 (1.1)	
	Mycoplasma PCR	5 (4.7)	2 (0.003)	0 (0.0)	
Blood samples collected 48-72hr into admission, n (%)		53 (50.0)	331 (53.5)	53 (28.2)	

Table 1. Patients identified in each diagnostic group at RFH. Chest radiograph codes for COVID-19
patients based on British Society of Thoracic Imaging guidelines, CVCX0 = Normal, CVCX1 = Classic for COVID-
19, CVCX2 = Indeterminate for COVID-19, CVCX3 = Non-COVID-19. p values represent comparisons between
CAP and COVID-19.

Characteristic	Total population		Excluding ICU attendance ≤72hr of hospital admission		Excluding microbiological or radiological pneumonia	
	AUC	95% CI	AUC	95% CI	AUC	95%CI
WCC on admission	0.75	0.68-0.83	0.77	0.70-0.84	0.76	0.68-0.83
CRP on admission	0.66	0.58-0.74	0.71	0.63-0.79	0.65	0.57-0.73
ΔWCC	0.67	0.59-0.76	0.66	0.57-0.75	0.68	0.59-0.76
ΔCRP	0.74	0.66-0.81	0.73	0.65-0.80	0.74	0.66-0.81
WCC and ΔWCC	0.75	0.68-0.83	0.77	0.70-0.84	0.76	0.68-0.83
WCC and ΔCRP	0.80	0.73-0.87	0.81	0.74-0.88	0.80	0.73-0.87
ΔWCC and ΔCRP	0.74	0.67-0.82	0.73	0.65-0.81	0.74	0.67-0.82
WCC, ΔWCC and ΔCRP	0.80	0.74-0.87	0.81	0.74-0.88	0.80	0.73-0.87

Table 2. Discriminatory accuracy of WCC, CRP, ΔWCC and ΔCRP for diagnosis of CAP compared to COVID-19 in RFH patients. Populations included were all patients (n=384) admitted >48 hours (n=53 for CAP and n=331 for COVID 19) or excluding 79 COVID-19 patients that attended ICU within 72 hours of hospital admission, or excluding 18 COVID-19 patients with microbiological or radiological evidence of pneumonia. AUC, area under the curve; CI, confidence interval.

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Cut-off	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %	Positive likelihood ratio	Negative likelihood ratio
WCC>8.2	79.2	58.8	24.6	94.4	1.92	0.35
WCC>9.5	67.9	70.6	28.1	92.9	2.31	0.45
∆CRP<3.5	75.5	61.3	24.8	93.7	1.95	0.40
∆CRP<-15	62.3	75.0	29.7	92.2	2.50	0.50
∆CRP<0	73.6	65.2	26.4	93.6	2.11	0.41
WCC>8.2 AND ∆CRP<0	62.3	80.8	35.4	92.6	3.25	0.47
WCC>8.2 OR ∆CRP<0	90.6	43.1	21.2	96.4	1.59	0.22

Table 3. Discriminatory performance of WCC and ∆CRP cut-offs for diagnosis of bacterial pneumonia. Populations included were 366 patients admitted to hospital >48hours, including 52 pneumonia cases and 331 COVID-19 cases with no microbiological or radiological evidence of pneumonia.

Diagnosis		CAP	COVID-19	Influenza	p values
Numbers		169	181	162	-
Chest radiograph		Lobar consolidation 169 (100%)	Lobar consolidation 0 (0%) CVCX0: 22 (12.2) CVCX1: 71 (39.2) CVCX2: 36 (19.9) CVCX3: 5 (2.8) Ungraded: 47 (26.0)		-
Male, n (%)		81 (47.9)	104 (57.5)	60 (37.0)	0.0865
Age, median (range)		74 (18-98)	71 (29-98)	63 (19-98)	0.0633
Ethnicity, n (%)	White	128 (76)	115 (64)	108 (67)	0.0335
	Asian	11 (7)	26 (14)	18 (11)	
	Black	1 (1)	11 (6)	7 (4)	
	Mixed	0 (0)	2 (1)	2 (1)	
	Other/ unknown	29 (17)	27 (15)	27 (17)	
Charlson index co-morbidities n (%)	0	51 (30)	81 (45)	78 (48)	0.0206
	1	51 (30)	58 (32)	40 (25)	
	2	35 (21)	23 (13)	30 (19)	
	3+	32 (19)	19 (10)	14 (8)	
Positive microbiology results, n (%)	Sputum	2 (1.2)	2 (1.1)	3	0.0393
	Blood	6 (3.6)	5 (2.8)	1	
	Urine Ag	8 (4.7)	0 (0.0)	2	
	Mycoplasma PCR	0 (0.0)	0 (0.0)	2	
Blood samples collected 48-72hr into admission, n (%)		99 (58.6)	60 (33.1)	47 (29.0)	-

Table 4. Patients identified in each diagnostic group at BH. Chest radiograph codes for COVID-19 patients based on British Society of Thoracic Imaging guidelines, CVCX0 = Normal, CVCX1 = Classic for COVID-19, CVCX2 = Indeterminate for COVID-19, CVCX3 = Non-COVID-19. p values represent comparisons between CAP and COVID-19.

Characteristic	Total population		Excluding ICU attendance ≤72hr of hospital admission		Excluding microbiological or radiological pneumonia	
	AUC	95% CI	AUC	95% CI	AUC	95%CI
WCC on admission	0.79	0.72-0.87	0.83	0.76-0.90	0.80	0.73-0.88
CRP on admission	0.65	0.56-0.73	0.68	0.59-0.76	0.66	0.57-0.74
∆WCC	0.77	0.70-0.84	0.78	0.70-0.85	0.77	0.69-0.84
∆CRP	0.76	0.68-0.83	0.76	0.68-0.84	0.75	0.67-0.83
WCC and ∆WCC	0.81	0.74-0.88	0.83	0.77-0.90	0.81	0.74-0.88
WCC and ∆CRP	0.84	0.78-0.91	0.87	0.81-0.93	0.84	0.78-0.91
∆WCC and ∆CRP	0.80	0.73-0.87	0.81	0.73-0.88	0.79	0.72-0.86
WCC, ∆WCC and ∆CRP	0.84	0.78-0.91	0.87	0.80-0.93	0.84	0.77-0.91

Table 5. Discriminatory accuracy of WCC, CRP, ∆WCC and ∆CRP for detection of bacterial pneumonia in BH patients. Populations included were all patients (n=159) admitted >48 hours (n=99 for CAP and n=60 for COVID 19) or excluding 21 COVID-19 patients that attended ICU within 72 hours of hospital admission, or 4 COVID-19 patients with microbiological or radiological evidence of pneumonia. AUC, area under the curve; CI, confidence interval.

Cut-off	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %	Positive likelihood ratio	Negative likelihood ratio
WCC>8.2	84.3	57.1	75.8	69.6	1.97	0.28
WCC>9.5	76.4	71.4	81.0	65.6	2.67	0.33
∆CRP<3.5	66.3	73.2	79.7	57.7	2.47	0.46
∆CRP<-15	56.2	80.4	82.0	53.6	2.86	0.55
∆CRP<0	65.1	75.0	80.6	57.5	2.61	0.46
WCC>8.2 AND ∆CRP<0	55.1	85.7	85.9	54.5	3.85	0.52
WCC>8.2 OR ∆CRP<0	94.4	46.4	73.6	83.9	1.76	0.12

Table 6. Discriminatory performance of WCC and ∆CRP cut-offs for diagnosis of bacterial pneumonia in BH patients. Populations included were 155 patients admitted to hospital >48hours, including 99 pneumonia cases and 56 COVID-19 cases with no microbiological or radiological evidence of pneumonia.
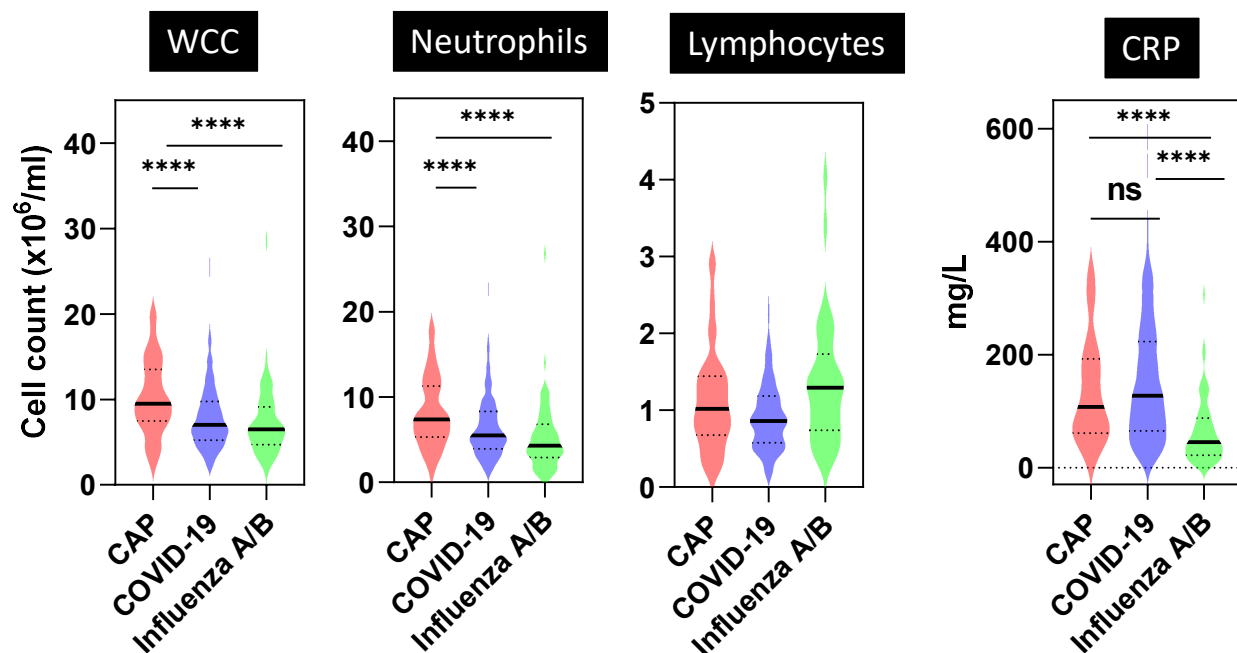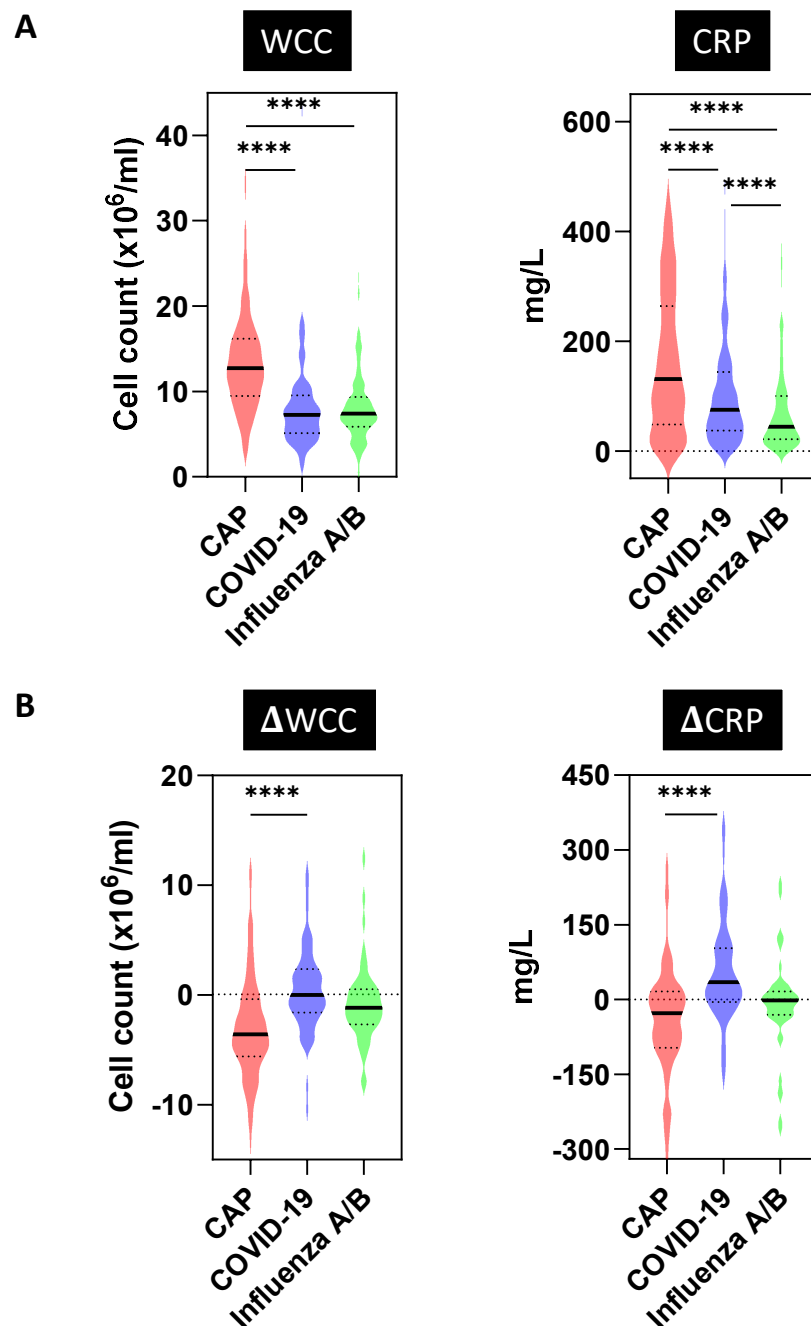
Figure S1
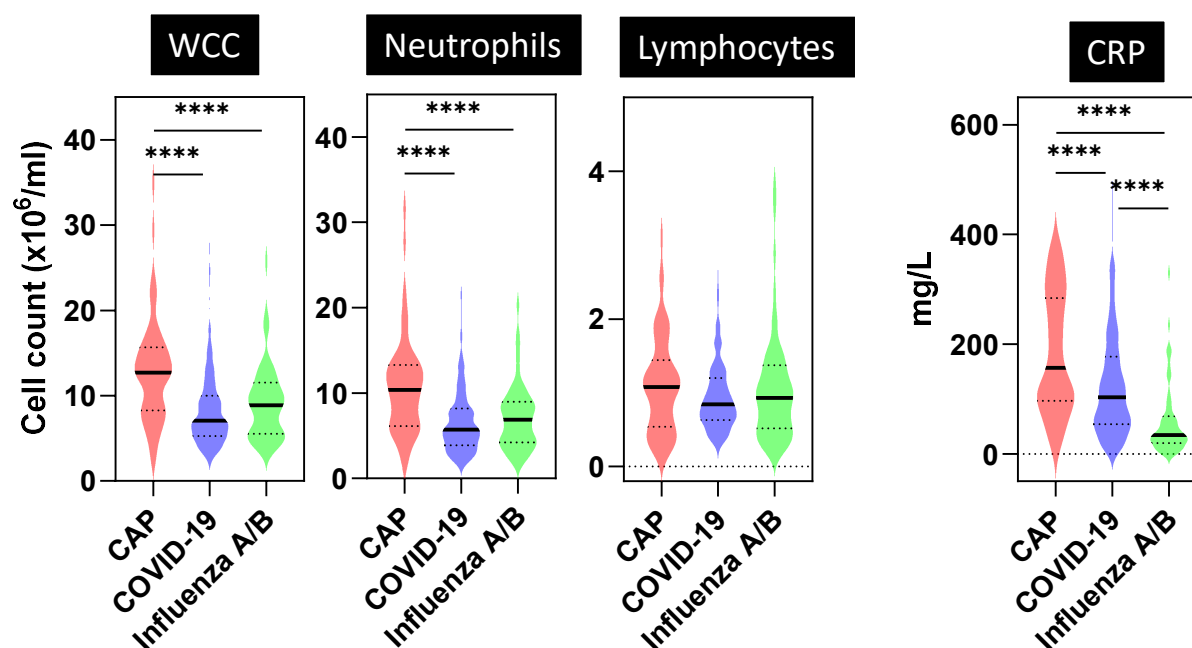
Figure S1. Admission blood samples for RFH patients admitted >48hours. Violin plots represent distribution of values for CAP (n=53), COVID-19 (n=331) and influenza A/B (n=53) patients. Bold lines represent median values. Dotted lines represent IQR values. **** indicates p<0.0001 by Mann-Whitney test.

Figure S2

Figure S2. Blood samples collected from RFH patients 48-72 hours into admission. Violin plots represent distribution of values for CAP (n=53), COVID-19 (n=331) and influenza A/B (n=53) patients. Bold lines represent median values. Dotted lines represent IQR values. **** indicates p<0.0001 and ns indicates non-significant by Mann-Whitney test.

Figure S3

Figure S3. (A) Blood samples collected from BH patients on admission for CAP (n=169), COVID-19 (n=181) and influenza A/B (n=162) patients. (B) Difference (Δ) in blood parameters between samples collected on admission and samples collected 48-72 hours into admission for CAP (n= 99), COVID-19 (n=60) and influenza A/B (n=47) patients. Violin plots represent distribution of values. Bold lines represent median values. Dotted lines represent IQR values. **** indicates p<0.0001 by Mann-Whitney test.

	Hospital	RFH			BH		
	Disease	**CAP**	**COVID-19**	**Influenza**	**CAP**	**COVID-19**	**Influenza**
SPUTUM	*S pneumoniae*	2	0	2	0	0	0
	H influenzae	2	0	1	1	0	2
	M catarrhalis	1	1	0	1	0	0
	S aureus	0	2	1	0	6	2
	M pneumoniae (PCR)	5	2	0	0	0	0
BLOOD	*S pneumoniae*	1	0	1	6	0	1
	Non-pneumococcal streptococci	0	2	0	0	1	0
	S aureus	0	5	1	0	1	0
	Gram negative organisms	1	1	2	0	2	0
	Other	0	4	2	0	1	0
URINE	*S pneumoniae* antigen	4	3	2	8	0	0

Table S1. Bacteria identified by conventional microbiology investigations in RFH and BH patients. Numbers
quantity routine clinical samples that yielded specified bacteria from patients described in tables 1 & 4.