Title

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

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Running title

Exclusion of bacterial co-infection in COVID-19

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Abstract

Background

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

Methods

Retrospective cohort study of CAP (lobar consolidation on chest radiograph) and COVID-19 (PCR detection of SARS-CoV-2) patients admitted to Royal Free Hospital (RFH) and Barnet Hospital (BH), serving as independent discovery and validation cohorts. All CAP and >90% COVID-19 patients received antibiotics on hospital admission.

Results

We identified 106 CAP and 619 COVID-19 patients at RFH. Compared to COVID-19, CAP was characterised by elevated baseline white cell count (WCC) (median 12.48 (IQR 8.2-15.3) versus 6.78 (IQR 5.2-9.5) $x10^6$ cells/mL, p<0.0001), C-reactive protein (CRP) (median 133.5 (IQR 65-221) versus 86.0 (IQR 42-160) mg/L, p<0.0001), and greater reduction in CRP 48-72 hours into admission (median ΔCRP -33 (IQR -112 to +3.5) versus +14 (IQR -15.5 to +70.5) mg/L, p<0.0001). These observations were recapitulated in the independent validation cohort at BH (169 CAP and 181 COVID-19 patients). A multivariate logistic regression model incorporating WCC and ΔCRP discriminated CAP from COVID-19 with AUC 0.88 (0.83-0.94). Baseline WCC >$8.2x10^6$ cells/mL or falling CRP identified 94% of CAP cases, and excluded bacterial co-infection in 46% of COVID-19 patients.

Conclusions

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

Abstract word count

249 words

Introduction

The COVID-19 pandemic caused by the novel beta coronavirus SARS-CoV-2 has caused >65 million infections and >1.5 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.[6–10] However, routine microbiological culture takes several days, lacks sensitivity[11] and does not readily distinguish bacterial colonisation from infection. Moreover, microbiological respiratory tract sampling is not performed routinely in patients admitted with COVID-19.[9] Therefore, despite guidance aimed at rationalising antibiotic use,[12] 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, hampering antimicrobial stewardship efforts and potentially increasing antimicrobial resistance.[13–16] Many studies have focused on clinical and laboratory features that risk stratify outcome in COVID-19,[17–20] 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.[21–24] Serial measurements of inflammatory markers may also assist in distinguishing bacterial from viral infections.[25,26] A small retrospective study comparing COVID-19 to community-acquired pneumonia (CAP) 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.[27]

In this study we aimed to identify features that discriminated viral COVID-19 infections from those complicated by bacterial co-infection. We used 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.[28] 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 (table 1), excluding patients with haematological malignancies. We defined COVID-19 by RT-PCR detection of SARS-CoV-2 from nasopharyngeal swabs, identifying patients between 1st March and 31st May 2020. These criteria yielded 619 and 181 COVID-19 patients from RFH and BH respectively. CAP was defined 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). We used RFH patients as a discovery cohort in our analyses to build a model and cut-off parameters to discriminate between CAP and COVID-19, and BH patients were as an independent cohort to validate the findings from RFH.

We identified 26 (4.2%) and 10 (5.5%) COVID-19 patients at RFH and BH respectively with microbiological evidence of bacterial co-infection. This was defined by the presence of a non-contaminant bacterial growth on blood culture, bacterial growth in sputum samples, detection of *Mycoplasma pneumoniae* by PCR from sputum or detection of *Streptococcus pneumoniae* antigen in urine (table 1 and table S1). In addition, at RFH, 4 (0.6%) COVID-19 patients had radiological evidence of lobar pneumonia on a chest radiograph within 72 hours of hospital admission. We collectively termed COVID-19 patients with microbiological or radiological evidence of bacterial co-infection “MR+ COVID-19”, as opposed to the remaining COVID-19 patients termed “MR- COVID-19”. Of the MR+ COVID-19 patients, 18 and 4 remained in hospital for ≥48 hours at RFH and BH respectively.

Statistical analysis

Baseline demographics were compared by Mann-Whitney test (age), Fisher's exact test (gender and microbiology) or Chi-square test (ethnicity and Charlson co-morbidities). Continuous variables were expressed as median and IQR, and patient groups were compared using non-parametric two-tailed Mann-Whitney U tests. A multivariate logistic regression model was used to determine factors that discriminated between CAP and MR- COVID-19. The model's categorical output variable was a diagnosis of CAP, and continuous dependent variables were baseline demographics and inflammatory markers. Variables were treated as interval data, with no true zero. In this way positive and negative values (i.e. the ones generated from Δ calculations) were treated equally by the model, with only the differences in their relative association between CAP and COVID-19 patients that contributed to their discriminatory capacity. This analysis generated Receiver Operating Characteristic (ROC) curves and AUC as a summary statistic. For pre-determined cut-offs, we also calculated sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios. All analyses were performed using Microsoft Excel and GraphPad Prism.

Results

Defining the discovery cohort

We identified 106 CAP and 619 COVID-19 patients at RFH. Male gender was overrepresented in COVID-19 (62% in COVID-19 versus 47% in CAP), whereas CAP patients were older (median age 72 in CAP and 68 in COVID-19). The proportion of Black, Asian, Mixed and Other (non-white) ethnicity patients was higher in COVID-19 compared to CAP and patients with CAP had more comorbidities and identified bacteria in routine microbiological investigations more commonly (table 1).

Distinguishing CAP from COVID-19

We tested the hypothesis that inflammatory markers could discriminate CAP from COVID-19 by comparing total WCC, its differential cell counts and CRP on the day of admission to hospital. We divided the COVID-19 population into 589 MR- and 30 MR+, highlighting that most COVID-19 patients did not show microbiological or radiological evidence of bacterial co-infection. Compared to CAP, COVID-19 was associated with significantly lower median WCC (12.48 versus 6.78 and 7.77×10^6/mL) and neutrophils (9.98 versus 5.36 and 6.51×10^6/mL) relative to both MR- and MR+ populations (fig 1). Lymphocyte counts were marginally lower in COVID-19 than CAP, and CRP was significantly higher in CAP than in both COVID-19 populations (median CRP 133.5, 86.0 and 89.5mg/L respectively) (fig 1). Notably, there were no differences in these markers between the COVID-19 subpopulations.

All CAP patients were prescribed antibiotics on admission and in two independent surveys of COVID-19 patients from RFH, 95/100 (95%) and 104/118 (88%) were prescribed antibiotics to treat a presumptive pulmonary bacterial co-infection. We hypothesised that CAP and COVID-19 could be further discriminated by changes in inflammatory markers following initiation of antibiotics.[25] In the RFH cohort, 53 (50%) CAP, 313 (53%) MR- COVID-19 and 18 (60%) MR+ COVID-19 patients were admitted for >48 hours and had a blood sample collected as part of routine clinical care 48-72 hours into admission (table 1). Differences in inflammatory markers on admission within this subset mirrored that seen in the wider cohort (fig S1). At this later time point, CAP was still characterised by elevated median WCC (9.61, 7.28 and 7.41x10^6/mL respectively), but this difference was diminished compared to admission (fig S2). Moreover, the difference in CRP between CAP and either COVID-19 subpopulation was no longer evident (median CRP 113.0, 126.0 and 148 mg/L respectively) (fig S2). These changes were driven by a greater fall in WCC and CRP for CAP compared to MR- or MR+ COVID-19 (ΔWCC -2.32, -0.16, -0.94x10^6/mL and ΔCRP -33, +14, +26mg/L respectively) (fig 2). Similar to baseline samples, no differences were observed for changes in inflammatory markers between MR- and MR+ COVID-19 (fig 2).

Contribution of multiple variables to discriminate CAP from COVID-19

Our data suggested that elevated WCC and CRP, as well as a reduction in these parameters at 48-72 hours could discriminate between COVID-19 and CAP. To test this hypothesis, we applied a logistic regression model to the data collected from the CAP and MR- COVID-19 patient groups. We used the diagnosis of CAP as the binary outcome variable and explored how baseline and changes in inflammatory markers influenced the diagnostic accuracy (fig 3 & table 2). The maximal AUC obtained from using each variable alone was 0.78 (C.I. 0.70-0.85) with baseline WCC. We also tested whether combining variables would improve the diagnostic accuracy of the model, and observed some improvement in discriminating between CAP from COVID-19 using both baseline WCC and ΔCRP (AUC 0.81, C.I. 0.74-0.88) (fig 3 & table 2).

We performed sensitivity analyses to explore the role of possible confounders. Excluding baseline demographics from the model only minimally reduced AUCs (table S2), indicating that inflammatory markers were the predominant discriminatory variables. We also considered the role played by admission to intensive care unit (ICU). At RFH, 54/313 (17.2%) of MR- COVID-19 patients included in the logistic regression model were admitted to ICU within 72 hours of their admission (table 1). Excluding this subset of patients did not affect the maximal discriminatory power of the model (AUC 0.82, C.I. 0.75-0.89) (table S2). We also considered whether reclassification of MR+ COVID-19 patients as CAP may further improve discrimination from the MR- COVID-19 cohort. However, this approach moderately reduced the discriminatory power of the model, consistent with the differences observed between CAP and MR+ COVID-19 patient in figs 1&2. Overall, these analyses confirmed that WCC and ΔCRP are the variables that most discriminate between CAP and COVID-19

and are not confounded by patient demographics, ICU attendance or microbiological / radiological evidence of bacterial co-infection.

Decision making criteria to discriminate CAP and COVID-19

We sought to convert our observations into practical decision-making criteria for clinical practice, focusing on the larger MR- COVID-19 population. We generated a series of cut-offs between CAP and COVID-19 for variables with greatest discriminatory power, admission WCC and ΔCRP, as well as baseline CRP to permit assessment at the time of admission, and explored the trade off in sensitivity and specificity generated by these alone or in combination (table 3). For WCC cut-offs, we used the lower quartile value of the CAP cohort (>8.2x10^6/mL) and the upper quartile value of the COVID-19 cohort (>9.4x10^6/mL). We used the same criteria for baseline CRP, yielding cut-offs of 65 and 160 mg/L respectively. For cut-offs of ΔCRP we used the lower quartile of the COVID-19 cohort (<-15mg/L) and the upper quartile of the CAP cohort (<3.5mg/L), rounded to 0mg/L for simplicity. These analyses revealed that using CAP-derived quartile cut-offs for baseline WCC or CRP yielded greater sensitivity, at the expense of specificity. The lower prevalence of CAP in this cohort compared to COVID-19 offered a high negative predictive value (>90%). Requiring both a WCC>8.2x10^6/mL and ΔCRP<0 improved specificity, at the expense of sensitivity, but this strategy could result in many cases of CAP, and by extension pathological bacterial co-infection in COVID-19, being missed. Thus, we explored using either parameter to define CAP, yielding a sensitivity of >90%, and although the specificity of this approach was only 43%, it was still greater than if baseline CRP had replaced the ΔCRP cut-off (table 3). Therefore, the absence of both admission WCC>8.2x10^6/mL and ΔCRP<0 could exclude bacterial co-infection in 135 / 313 (43%) of the MR- COVID-19 cohort, in turn supporting antibiotic cessation in these patients (table 3).

Independent cohort validation

To demonstrate reproducibility of our findings, we used independent patient cohorts from a separate hospital, BH, consisting of 169 CAP and171 MR- COVID-19 and 10 MR+ COVID-19 patients. To ensure comparability to the RFH cohort, the patients were identified over the same time periods using identical criteria. Baseline demographic analyses were comparable to those in the RFH cohort (table 1), and 99 (59%), 56 (31%) and 4 (2%) of CAP, MR- and MR+ COVID-19 patients respectively were admitted for >48 hours and had a blood sample collected as part of routine clinical care 48-72 hours into admission (table 1).

Differences in inflammatory markers within the BH cohort reflected those observed at RFH, with admission WCC and CRP levels being higher in CAP compared to either COVID-19 population and accompanied by a reduction following 48-72 hours of admission not observed in COVID-19 (fig S3). As for RFH, we also observed no differences in the levels of these variables between MR- and MR+ COVID-19 patients (fig S3). Applying these inflammatory marker parameters into a logistic regression model demonstrated similar findings to the RFH cohort, with optimal AUC (0.88, C.I. 0.83-0.94) to discriminate CAP from MR- COVID-19 being derived

from inclusion of both admission WCC and ΔCRP as variables in the model (table 2). Sensitivity analyses again demonstrated that demographic differences did not play a significant role, ICU admission within 72 hours of hospital admission (seen in 19/56, 34.0% COVID-19 patients) did not significantly confound the model and reassigning MR+ COVID-19 patients to the CAP cohort did not improve AUC scores (table S3).

Next, we applied the cut-offs independently derived from the RFH cohort on BH patient data, and observed a similar trade-off between sensitivity and specificity (table 4). High sensitivity (91.9%) with very low specificity (26.8%) was achieved when utilising either baseline criteria (WCC>8.2x10^6/mL or CRP>65mg/L) to diagnose CAP. In contrast using either admission WCC>8.2x10^6/mL or ΔCRP<0mg/L yielded a sensitivity for CAP approaching 95% but with an improved specificity (46.4%), permitting exclusion of bacterial co-infection in 26/56 (46.4%) MR- COVID-19 patients that had been admitted for >48hours (table 4).

Finally, we attempted to estimate the impact that the diagnostic criteria could have had on antibiotic prescribing in the MR- COVID-19 cohort at BH. We assumed antibiotic courses of 5-day duration, and thus estimated up to 855 antibiotic days in total for the 171 patients admitted in this cohort. Using absence of both baseline WCC>8.2x10^6/mL and CRP>65 to exclude bacterial co-infection could have prevented 229 antibiotic days, a 27% reduction. Instead, the 56 MR- COVID-19 patients admitted for >48 hours would have received up to 280 antibiotic days in total. Excluding bacterial co-infection by the absence of both WCC>8.2 and ΔCRP<0 has greater specificity (46.4%) but could only be applied to the final 3 days of antibiotic prescriptions. Therefore, within this cohort admitted for >48 hours, we extrapolated a total saving of 78 antibiotic days, a 28% reduction in total antibiotic prescriptions.

Discussion

Elevated inflammatory responses, high case fatality and bacterial co-infections observed in influenza contribute to frequent antibiotic prescriptions in COVID-19.[2,4,9,29] However, radiological findings in COVID-19 are heterogenous [30] and microbiological investigations rarely identify pathogenic bacteria,[6–10] precluding reliable identification of co-infection. Therefore, novel approaches to exclude bacterial co-infection in COVID-19 are a research priority to facilitate antimicrobial stewardship efforts.[15,31–33]

We used patients admitted with CAP prior to the COVID-19 pandemic as a benchmark to define processes that occur in bacterial pulmonary infections, including co-infection in COVID-19. This demonstrated that, at a population level, admission WCC (predominantly neutrophils) and CRP can discriminate CAP from COVID-19, and that WCC and CRP decreased following antibiotic therapy in pneumonia, but not in COVID-19. We used these observations to construct a model and decision-making criteria to assist with excluding bacterial co-infection in many cases of COVID-19. To overcome variability in individual patient responses (e.g. 26% and 35% of CAP patients at RFH and BH respectively showed a rise in CRP), we show that using two inflammatory

markers (WCC and ΔCRP) yielded the greatest sensitivity and specificity. We propose that absence of both admission WCC >$8.2x10^6$/mL and decreasing CRP could support stopping antibiotics in almost 50% of hospitalised COVID-19 patients, reducing total antibiotic prescriptions in this population by up to 25%. This approach would exceed most antimicrobial stewardship achievements,[31,32] and reduce selection for antibiotic resistant bacteria during this pandemic. [13,15]

The combination of selected cut-offs yielded the greatest sensitivity for CAP, ensuring ongoing antibiotic treatment where needed, but came at the expense of specificity. This was further illustrated by an alternative strategy, the use of baseline WCC and CRP, which yielded comparable sensitivity but lower specificity than assessments including the later timepoint. The absolute number of antibiotic days saved by each approach will depend on each centre's antibiotic prescribing rate, but notably both strategies could have yielded comparable proportions of antibiotic prescribing reduction. However, we caution that predicted savings in antibiotic prescriptions dependent solely on baseline inflammatory markers are likely to be significantly over-estimated. On hospital attendance, confirmation of COVID-19 diagnosis, formal chest radiograph reporting and clinical improvement trajectory would all be lacking, supporting precautionary antibiotic prescribing in many cases. Nevertheless, for either scenario, criteria relying solely on WCC and CRP measurements remain permissive to excessive antibiotic prescribing in COVID-19, and additional markers may improve sensitivity and specificity.PCT can discriminate bacterial from some viral respiratory tract infections,[23,24] but has not been systematically compared between bacterial pneumonia and COVID-19. Unfortunately, we could not investigate PCT as it was not measured routinely in our CAP cohorts. Future studies should also assess the discriminatory capacity of PCT, D-dimers,[9,24,34] and other novel biomarkers, such as transcriptional signatures that quantify inflammatory cytokine activity[35] or those that discriminate bacterial from viral infections.[36]

The large, standardised populations studied, use of routinely available clinical investigations, and the reproducibility of our findings in an independent validation cohort population are key strengths of our study. We were also able to convert population level findings into practical diagnostic criteria that can be used in generalised clinical settings. The cut-offs used were derived from a separate population in which they were tested, adding scientific validity to our conclusions, but does not negate individual care providers determining institution-specific cut-offs. Moreover, all data were collected before the beneficial effects of dexamethasone were published,[37] and WCC cut-offs may need revising in this context. In addition, our criteria should not be considered in isolation from clinical decision making. Clinical improvement, reduced supplemental oxygen requirement, and the absence of consolidation on chest radiograph in COVID-19 patients may all contribute to excluding bacterial co-infection and could be used alongside the WCC- and CRP-based criteria to support cessation of antibiotics.

Consistent with previous observations, we found microbiological or radiological evidence of bacterial co-infection to be rare in COVID-19.[6–10] Notwithstanding the low numbers, levels of inflammatory markers in this subset, both at baseline and during admission, were strikingly almost indistinguishable from those seen in the wider COVID-19 population, and remained distinct from those in CAP patients. Furthermore, reclassifying MR+ COVID-19 patients as individuals with CAP did not improve the discriminatory capacity of our model, indicating these to be distinct populations. The vast majority of patients classified as MR+ COVID-19 showed microbiological evidence of infection and we infer that in most instances, bacterial identification, particularly from the respiratory tract, lacks sensitivity and specificity to establish causal involvement in a bacterial pulmonary infection.[11,38] This is well illustrated by the low rate of microbiological identification observed in the CAP group, supporting the syndromic approach taken in our study to define populations with bacterial and viral pulmonary infections.

Our study has some notable limitations. First, the populations were identified at non-overlapping times, due to the disproportionate prevalence of COVID-19 cases in 2020. We attempted to mitigate for the enforced use of historical pneumonia comparator groups by identifying these patients over the same months of 2019 as COVID-19 cases in 2020. We also did not collect clinical severity or outcome data for the patients, and thus we cannot measure a direct impact on prognosis. Second, we used a radiological, but not microbiological, definition of pneumonia, and although standardised, it is possible that some pneumonia cases had non-bacterial aetiology. Third, we inferred that true bacterial co-infection in COVID-19 shares pathophysiology and inflammatory marker responses with CAP in the absence of COVID-19. This hypothesis remains untested and the divergence in responses between CAP and MR+ COVID-19 illustrates the difficulty of positively diagnosing COVID-19 patients with bacterial co-infection that requires antibiotic therapy. Fourth, we did not include suspected COVID-19 patients with negative SARS-CoV-2 results, therefore our findings may not be applicable to this cohort. Finally, we focused on patient assessments made within 72 hours of admission, and thus our decision-making tools are not applicable to patients with prolonged hospital admissions.

In conclusion, we demonstrate that routine clinical parameters, admission WCC and changes in CRP following antibiotic administration, can be translated into a set of diagnostic criteria that can exclude bacterial co-infection in up to half of COVID-19 patients. The routine nature of the investigations required mean that, even in the context of a pandemic, this approach can form the basis of protocols to assist reductions in unnecessary antibiotic prescriptions for viral infections, minimising drug-associated adverse effects and reducing development of antimicrobial resistance.

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Transparency declaration

The authors have no conflict of interests to declare in relation to this manuscript.

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		Royal Free Hospital			Barnet Hospital		
Diagnosis		**CAP**	**COVID-19**	**P value**	**CAP**	**COVID-19**	**p value**
Numbers		106	619	-	169	181	-
Chest radiograph, n (%)		Lobar consolidation 106 (100)	Lobar consolidation 4 (0.6) CVXC0: 62 (10.0) CVCX1: 281 (45.3) CVCX2: 136 (22.0) CVCX3: 17 (2.7) Ungraded: 123 (19.9)	-	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 (%)		50 (47)	386 (62)	0.0037	81 (47.9)	104 (57.5)	0.0865
Age, median (range)		72 (19-99)	68 (18-100)	0.1401	74 (18-98)	71 (29-98)	0.0633
Ethnicity, n (%)	White	60 (57)	250 (40)	0.0007	128 (76)	115 (64)	0.0335
	Asian	23 (22)	83 (13)		11 (7)	26 (14)	
	Black	6 (6)	69 (11)		1 (1)	11 (6)	
	Mixed	2 (2)	7 (1)		0 (0)	2 (1)	
	Other/ unknown	15 (14)	210 (34)		29 (17)	27 (15)	
Charlson index co-morbidities n (%)	0	18 (17)	243 (39)	<0.0001	51 (30)	81 (45)	0.0206
	1	33 (31)	190 (31)		51 (30)	58 (32)	
	2	28 (26)	109 (18)		35 (21)	23 (13)	
	3+	27 (25)	77 (13)		32 (19)	19 (10)	
Patients with microbiological identification of bacteria, n (%)		15 (14.2)	26 (4.2)	0.0003	15 (8.9)	10 (5.5)	0.2992
Microbiology results, n (%)	Sputum	6 (5.7)	8 (1.3)		2 (1.2)	5 (2.8)	
	Blood	2 (1.9)	12 (1.9)		6 (3.6)	5 (2.8)	
	Urine Ag	4 (3.8)	3 (0.5)		8 (4.7)	0 (0.0)	
	Mycoplasma PCR	5 (4.7)	3 (0.5)		0 (0.0)	0 (0.0)	
Blood samples collected 48-72hr into admission, n (%)		53 (50.0)	331 (53.5)		99 (58.6)	60 (33.1)	

Table 1. Patients identified in each diagnostic group at RFH and 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 and each hospital site. Comparisons between the cohorts at each hospital site were performed by Mann-Whitney test for age, by Fisher's exact test for gender, and by Chi-square test for ethnicity, Charlson co-morbidities and microbiological results. p values represent comparisons between CAP and COVID-19 and each hospital site.

	Royal Free Hospital		Barnet Hospital	
Characteristic	**AUC**	**95% CI**	**AUC**	**95% CI**
WCC on admission	0.78	0.70-0.85	0.84	(0.78-0.90)
CRP on admission	0.71	0.64-0.80	0.79	(0.71-0.86)
WCC and CRP	0.78	0.71-0.85	0.86	(0.80-0.92)
ΔWCC	0.72	0.64-0.80	0.79	(0.72-0.87)
ΔCRP	0.77	0.70-0.84	0.82	(0.76-0.89)
WCC and ΔWCC	0.78	0.70-0.85	0.84	(0.77-0.90)
WCC and ΔCRP	0.81	0.74-0.88	0.88	(0.83-0.94)

Table 2. Discriminatory accuracy of WCC, CRP, ΔWCC and ΔCRP for diagnosis of CAP compared to MR- COVID-19 at Royal Free Hospital (RFH) and Barnet Hospital (BH). Populations included were all patients admitted >48 hour: n=53 for CAP and n=313 for MR- COVID 19 at RFH and n=99 for CAP and n=56 for MR- COVID-19 at BH. 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	79.2	58.8	24.6	94.4	1.92	0.35
WCC>9.4	69.8	70.0	28.2	93.2	2.32	0.43
CRP>65	84.9	33.2	17.7	92.9	1.27	0.45
CRP>160	49.1	71.6	22.6	89.2	1.73	0.71
WCC>8.2 AND CRP>65	69.8	70.3	28.5	93.2	2.35	0.43
WCC>8.2 OR CRP>65	94.3	21.7	16.9	95.8	1.21	0.26
ΔCRP<3.5	75.5	61.3	24.8	93.7	1.95	0.40
ΔCRP<-15	62.3	75.0	29.7	92.2	2.50	0.50
ΔCRP<0	73.6	65.2	26.4	93.6	2.11	0.41
WCC>8.2 AND ΔCRP<0	62.3	80.8	35.4	92.6	3.25	0.47
WCC>8.2 OR ΔCRP<0	90.6	43.1	21.2	96.4	1.59	0.22

Table 3. Discriminatory performance of WCC and ΔCRP cut-offs for diagnosis of CAP in RFH patients. Populations included were all patients admitted >48 hour for CAP (n=53) and MR- COVID-19 (n=313). WCC values represent cell numbers $x10^6$/mL. CRP values represent concentrations in mg/L.

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>9.4	79.8	69.6	82.3	66.1	2.63	0.29
CRP>65	80.8	32.1	67.8	48.6	1.19	0.6
CRP>160	54.5	73.2	78.3	47.7	2.04	0.62
WCC>8.2 AND CRP>65	68.7	0.75	82.9	57.5	2.75	0.42
WCC>8.2 OR CRP>65	91.9	26.8	68.9	65.2	1.26	0.30
ΔCRP<3.5	66.3	73.2	79.7	57.7	2.47	0.46
ΔCRP<-15	56.2	80.4	82.0	53.6	2.86	0.55
ΔCRP<0	65.1	75.0	80.6	57.5	2.61	0.46
WCC>8.2 AND ΔCRP<0	55.1	85.7	85.9	54.5	3.85	0.52
WCC>8.2 OR ΔCRP<0	94.4	46.4	73.6	83.9	1.76	0.12

403 **Table 4.** Discriminatory performance of WCC and ΔCRP cut-offs for diagnosis of CAP in BH patients.
404 Populations included were all patients admitted >48 hour for CAP (n=99) and MR- COVID-19 (n=56). WCC
405 values represent cell numbers $x10^6$/mL. CRP values represent concentrations in mg/L.

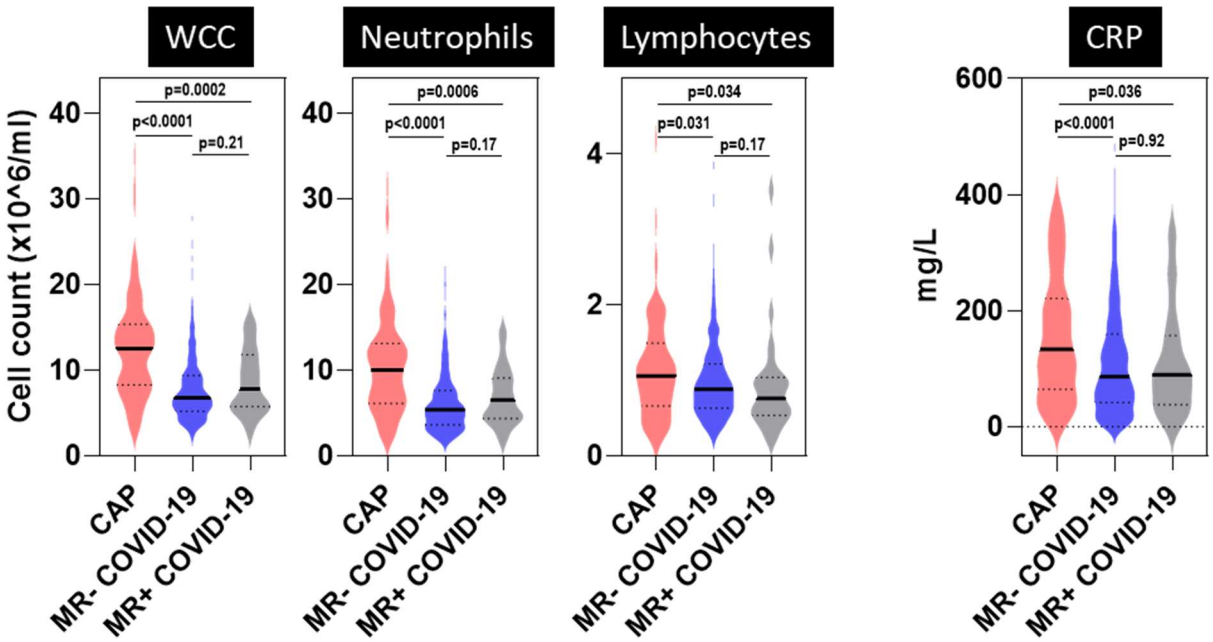

Figure 1 Admission blood samples for all patients admitted to RFH. Violin plots represent distribution of values for CAP (n=106), MR- COVID-19 (n=589) and MR+ COVID-19 (n=30) patients. Bold lines represent median values. Dotted lines represent IQR values. p values derived from two-tailed Mann-Whitney test.

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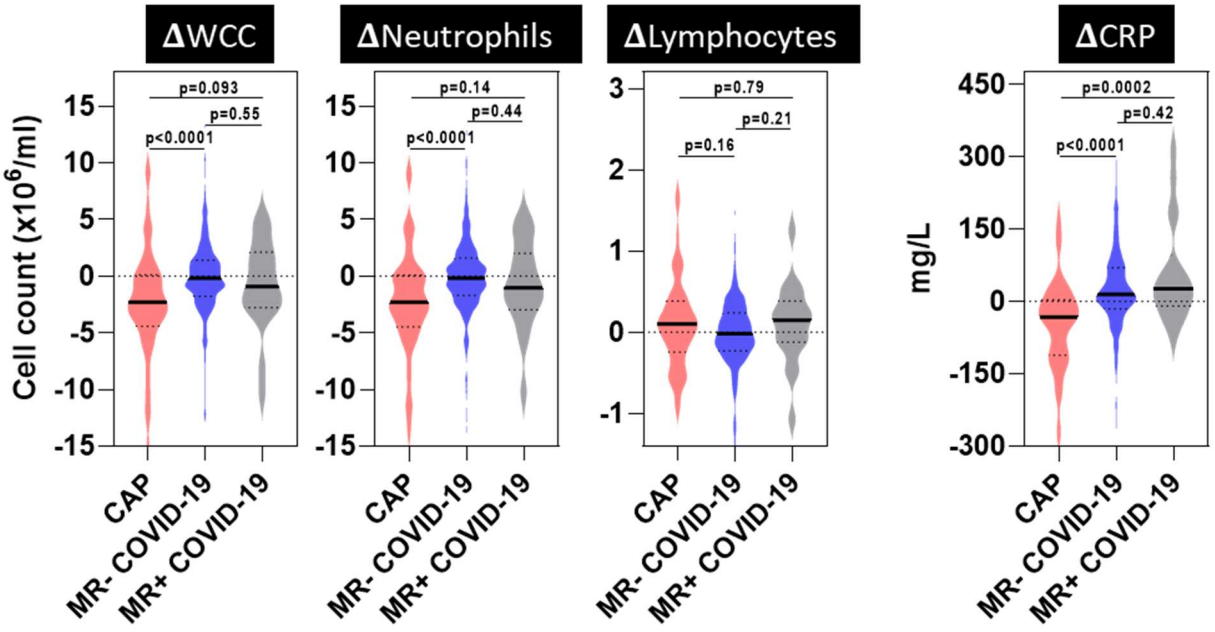

411

412 **Figure 2** Change in values between admission blood samples and those collected 48-72 hours into admission
413 at RFH. Violin plots represent distribution of difference (Δ) in investigation results between those collected on
414 hospital admission and 48-72 hours into admission in CAP (n=53), MR- COVID-19 (n=313) and MR+ COVID-19
415 (n=18) patients. Bold lines represent median values. Dotted lines represent IQR values. p values derived from
416 two-tailed Mann-Whitney test.

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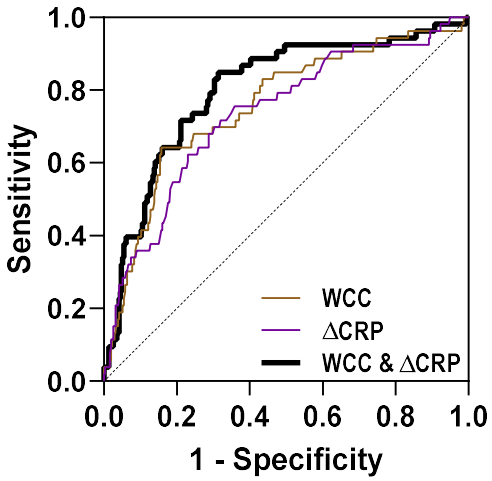

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419 **Figure 3** Accuracy of blood parameters to diagnose CAP in RFH cohorts of CAP and MR- COVID-19 patients.
420 ROC curves generated from logistic regression models that incorporate combinations of WCC on admission
421 and difference (Δ) in CRP between samples on admission and 48-72 hours into admission in order to
422 discriminate RFH patients diagnosed with CAP from those diagnosed with COVID-19.

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

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

	Excluding demographics		**Excluding ICU attendance ≤72hr of hospital admission**		**Assigning MR+ COVID-19 to CAP**	
Characteristic	**AUC**	**95% CI**	**AUC**	**95% CI**	**AUC**	**95% CI**
WCC on admission	0.76	(0.68-0.83)	0.80	(0.72-0.87)	0.73	(0.66-0.80)
CRP on admission	0.65	(0.57-0.73)	0.74	(0.66-0.82)	0.67	(0.59-0.75)
WCC and CRP	0.76	(0.69-0.83)	0.80	(0.73-0.87)	0.73	(0.66-0.80)
ΔWCC	0.68	(0.59-0.76)	0.71	(0.63-0.80)	0.68	(0.60-0.76)
ΔCRP	0.74	(0.66-0.81)	0.76	(0.69-0.84)	0.70	(0.62-0.77)
WCC and ΔWCC	0.76	(0.68-0.83)	0.80	(0.72-0.87)	0.70	(0.63-0.78)
WCC and ΔCRP	0.80	(0.73-0.87)	0.82	(0.75-0.89)	0.74	(0.67-0.82)

Table S2. Discriminatory accuracy of WCC, CRP, ΔWCC and ΔCRP for diagnosis of CAP compared to MR-COVID-19 at Royal Free Hospital (RFH). Sensitivity analyses excluded from the logistic regression model patient demographics from all patients or MR- COVID-19 patients admitted to ICU within 72 hours of hospital admission (n=54). Alternatively, MR+ COVID-19 patients (n=18) were added to the model and assigned a diagnosis of CAP. AUC, area under the curve; CI, confidence interval.

	Excluding demographics		**Excluding ICU attendance ≤72hr of hospital admission**		**Assigning MR+ COVID-19 to CAP**	
Characteristic	**AUC**	**95% CI**	**AUC**	**95% CI**	**AUC**	**95% CI**
WCC on admission	0.81	(0.73-0.88)	0.84	0.77-0.91	0.83	(0.77-0.90)
CRP on admission	0.66	(0.57-0.74)	0.77	(0.69-0.85)	0.78	(0.71-0.85)
WCC and CRP	0.81	(0.74-0.89)	0.87	(0.80-0.93)	0.86	(0.80-0.92)
ΔWCC	0.77	(0.69-0.84)	0.76	(0.67-0.85)	0.77	(0.69-0.85)
ΔCRP	0.75	(0.67-0.83)	0.80	(0.72-0.88)	0.81	(0.74-0.87)
WCC and ΔWCC	0.81	(0.74-0.88)	0.84	(0.77-0.91)	0.84	(0.77-0.90)
WCC and ΔCRP	0.84	(0.78-0.91)	0.88	(0.81-0.94)	0.87	(0.82-0.93)

Table S3. Discriminatory accuracy of WCC, CRP, ΔWCC and ΔCRP for diagnosis of CAP compared to MR-COVID-19 at Barnet Hospital (BH). Sensitivity analyses excluded from the logistic regression model patient demographics from all patients or MR- COVID-19 patients admitted to ICU within 72 hours of hospital admission (n=19). Alternatively, MR+ COVID-19 patients (n=4) were added to the model and assigned a diagnosis of CAP. AUC, area under the curve; CI, confidence interval.

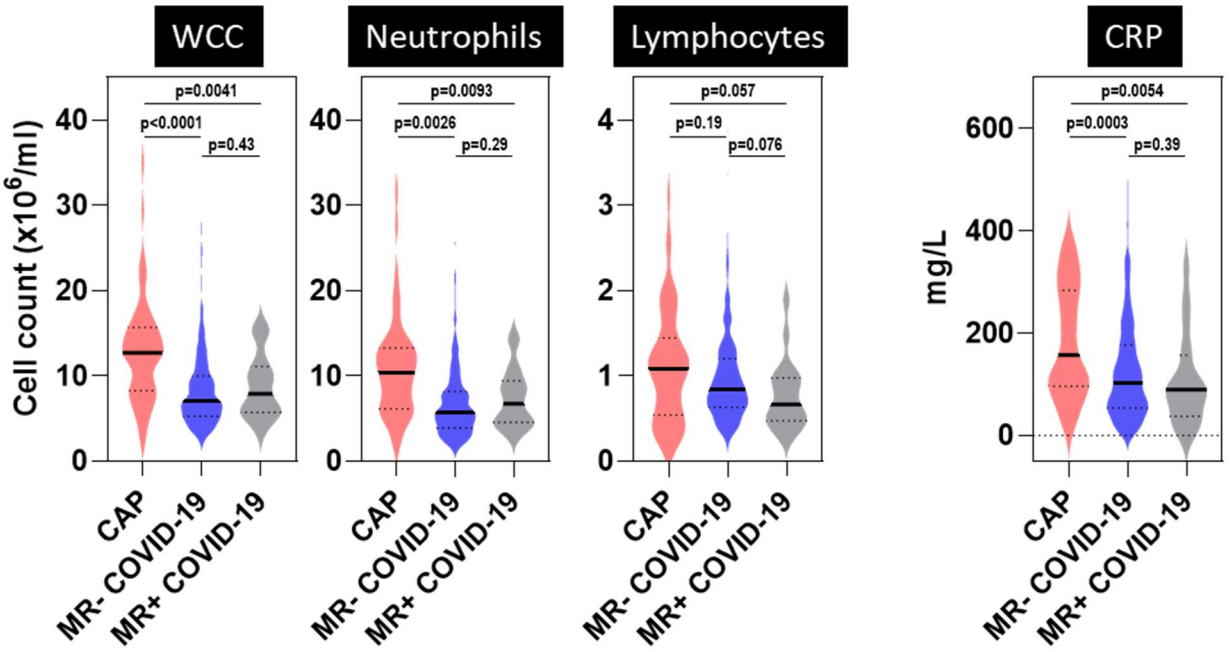

Figure S1. Admission blood samples for RFH patients admitted >48hours. Violin plots represent distribution of values for CAP (n=53), COVID-19 MR- (n=313) and COVID-19 MR+ (n=18) patients. Bold lines represent median values. Dotted lines represent IQR values. p values derived from two-tailed Mann-Whitney test.

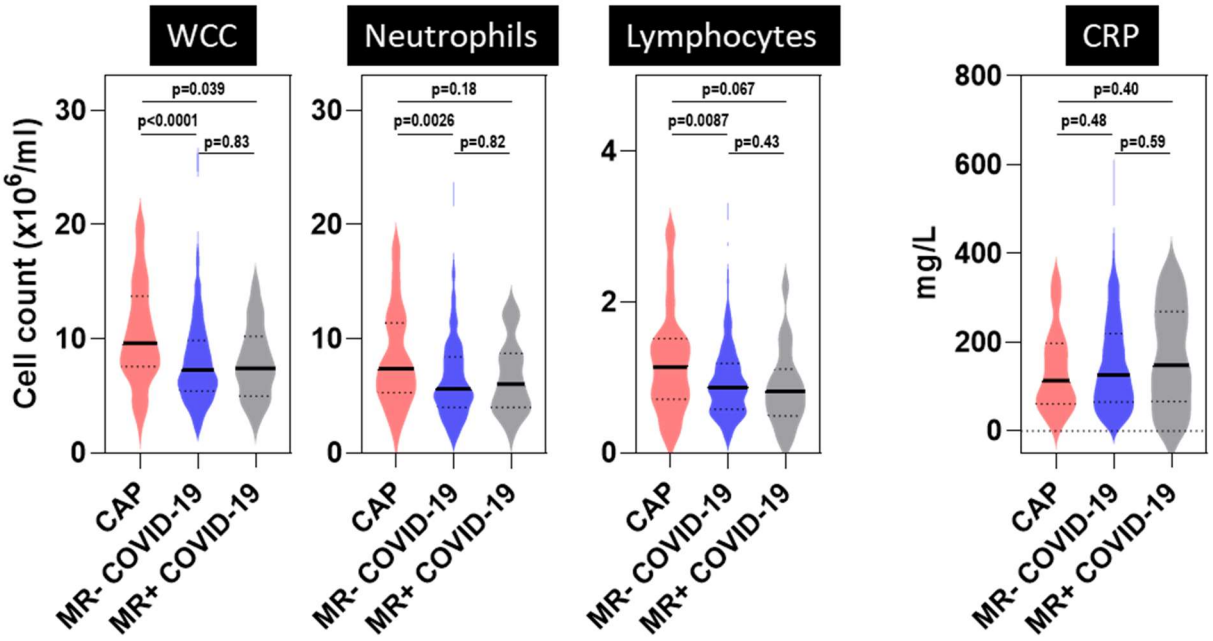

Figure S2. Blood samples collected from RFH patients 48-72 hours into admission. Violin plots represent distribution of values for CAP (n=53), COVID-19 MR- (n=313) and COVID-19 MR+ (n=18) patients. Bold lines represent median values. Dotted lines represent IQR values. p values derived from two-tailed Mann-Whitney test.

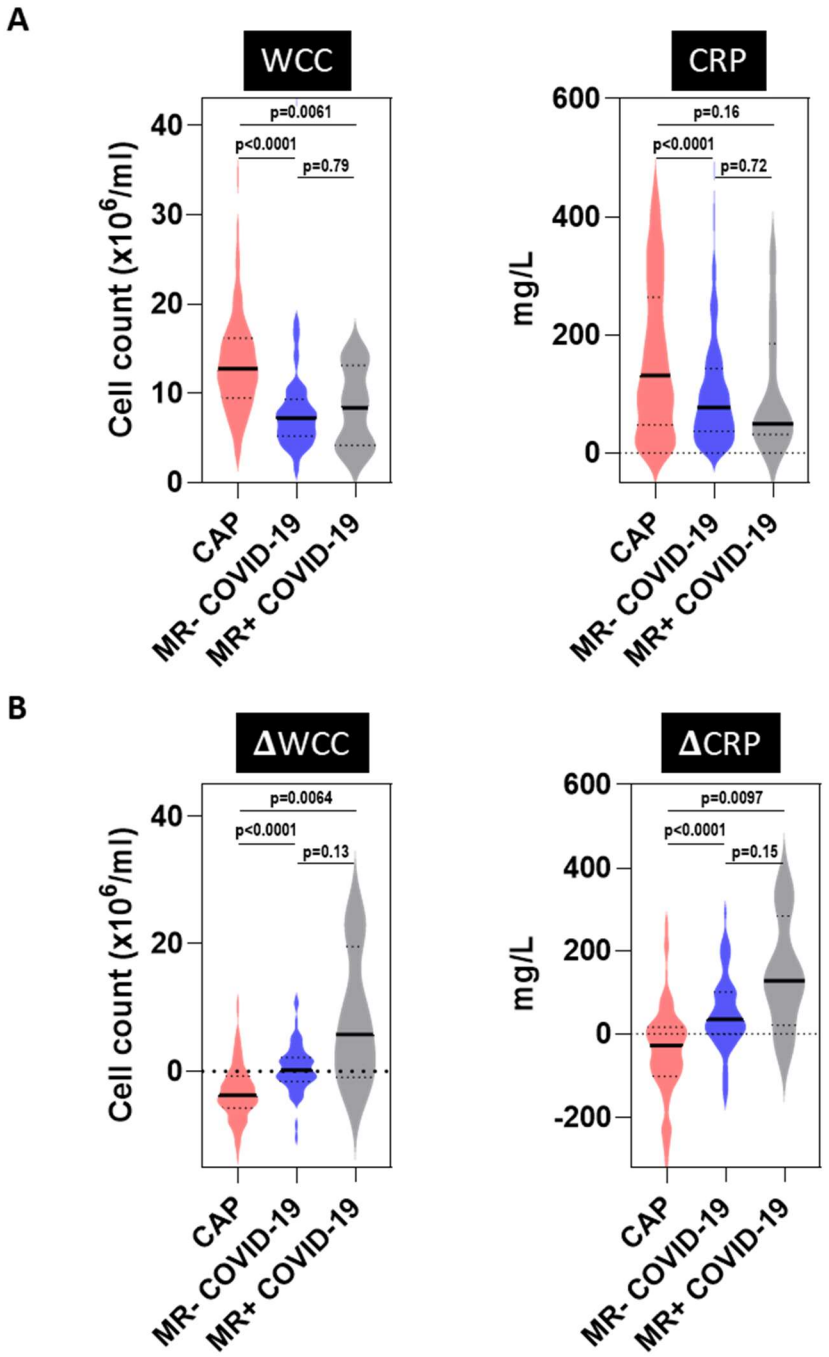

Figure S3. (A) Blood samples collected from BH patients on admission for CAP (n=169), COVID-19 MR- (n=171) and COVID-19 MR+ (n=10) patients. (B) Difference (Δ) in blood parameters between samples collected on admission and samples collected 48-72 hours into admission for CAP (n= 99), COVID-19 MR- (n=56) and COVID-19 MR+ (n=4) patients. Violin plots represent distribution of values. Bold lines represent median values. Dotted lines represent IQR values. p values derived from two-tailed Mann-Whitney test.