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BMJ Open: Original Research Article

Title:

False-negative RT-PCR for COVID-19 and a diagnostic risk score: a retrospective cohort study among patients admitted to hospital

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ABSTRACT

Objective: To describe the characteristics and outcomes of patients with a clinical diagnosis of COVID-19 and false negative SARS-CoV-2 RT-PCR, and develop and internally validate a diagnostic risk score to predict risk of COVID-19 (including RT-PCR negative COVID-19) amongst medical admissions

Design: Retrospective cohort study

Setting: Two hospitals within an acute NHS trust in London, UK

Participants: All patients admitted to medical wards between 2nd March and 3rd May 2020.

Outcomes: Main outcomes were diagnosis of COVID-19, SARS-CoV-2 RT-PCR results, sensitivity of SARS-CoV-2 RT-PCR and mortality during hospital admission. For the diagnostic risk score, we report discrimination, calibration and diagnostic accuracy of the model and simplified risk score, and internal validation.

Results: 4008 patients were admitted between 2nd March and 3rd May 2020. 1792 patients (44.8%) were diagnosed with COVID-19, of whom 1391 were SARS-CoV-2 RT-PCR positive, and 283 had only negative RT-PCRs. Compared to a clinical reference standard, sensitivity of RT-PCR in hospital patients was 83.1% (95% CI 81.2-84.8%). Broadly, patients with false-negative RT-PCR COVID-19 and those confirmed by positive PCR had similar demographic and clinical characteristics, but lower risk of ITU admission and lower in-hospital mortality (adjusted odds ratio 0.41, 95% CI 0.27-0.61). A simple diagnostic risk score comprising of age,

sex, ethnicity, cough, fever or shortness of breath, National Early Warning Score (NEWS2), C-Reactive Protein, and chest radiograph appearance had moderate discrimination (area under the receiver-operator-curve 0.83, 95% CI 0.82-0.85), good calibration and was internally validated.

Conclusion: RT-PCR negative COVID-19 is common and is associated with lower mortality despite similar presentation. Diagnostic risk scores could potentially help triage patients requiring admission, but need external validation.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Large cohort of consecutive acute medical admissions in two hospitals covering a diverse population in London, UK, during first COVID-19 'peak'
- Assessment of 'real world' performance of SARS CoV-2 RT-PCR from nasopharyngeal swabs for diagnosis of COVID-19
- Inherent limitations of retrospective cohort study design, including some missing data
- Not all patients had SARS-CoV-2 RT-PCR testing

INTRODUCTION

The coronavirus disease 2019 (COVID-19) global pandemic, caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to unprecedented numbers of unwell and infectious patients requiring admission to hospital. The symptoms of COVID-19 can be non-specific, so diagnostic confirmation in hospital is often sought by detection of SARS-CoV-2 ribonucleic acid (RNA) sequences by reverse transcription-polymerase chain reaction (RT-PCR) from a clinical specimen.

Since the beginning of the pandemic, the standard sample for PCR testing has been a nasopharyngeal swab (NPS) or aspirate, but there are concerns that a significant proportion of cases test negative on initial RT-PCR of an NPS sample, with many patients having repeated sampling to confirm the diagnosis.[1] A systematic review of real-world diagnostic sensitivity of SARS-CoV-2 RT-PCR reports that up to 33% of patients with COVID-19 may have an initial false negative NPS result despite a compatible clinical illness, consistent thoracic imaging and/or subsequent positive antibodies to COVID-19.[2–5] False negative RT-PCR may result from inadequate nasopharyngeal sampling technique, delayed time to analysis, ineffective sample storage, variable gene targets in RT-PCR assays leading to imperfect analytic sensitivity, or if a patient is tested at a point when viral throat carriage is absent or below the detectable threshold (either too early or too late).[6,7] This high false negative rate complicates both hospital infection control and clinical decision making. Being able to identify patients with a high probability of COVID-19 despite a negative RT-PCR is crucial for effective clinical care.

The clinical characteristics and outcomes of hospitalised patients with COVID-19 have been well described globally, but these studies are limited to patients with RT-PCR confirmed

COVID-19.[8–10] The pattern of disease and outcomes of patients with false negative COVID-19 tests has not been well reported to date, nor has the diagnostic accuracy of RT-PCR assays in secondary care settings in the United Kingdom (UK). Several studies have derived and validated risk scores to assess severity and prognosis amongst patients with COVID-19. However few risk scores focus on identifying patients with COVID-19 amongst those needing hospital admission and those that do are from outside the UK, do not consider all hospital admissions, rely on high-resolution computerised tomography (CT) scanning of the lungs, and exclude patients without RT-PCR-confirmed disease.[11]

We therefore aim to describe the characteristics and outcomes of patients with a clinical diagnosis of COVID-19 but with negative RT-PCR from NPS, and the real-world sensitivity of RT-PCR for COVID-19. Secondly, we describe predictors of COVID-19 amongst general medical admissions, including assessing whether a simple diagnostic risk score could be derived, internally validated, and used to predict which patients admitted to medical wards will have COVID-19.

METHODS

Study design

This is a retrospective observational cohort study of consecutive admissions in London North West University Healthcare NHS Trust, comprising two hospitals, Northwick Park and Ealing. Patients were included in this study if they were admitted via the acute medical team between 2nd March and 3rd May 2020 inclusive.

Data collection

Cases were identified retrospectively through electronic medical admission lists. De-identified data on patient demographics, co-morbidities, clinical characteristics, vital signs, routine biochemical, haematological and microbiological tests, diagnosis and clinical outcomes were extracted from routinely collected clinical data using electronic patient record systems, and other NHS Trust health information systems. Physiological observations were those first recorded on admission to the emergency department. All biochemical and haematological data were from the first samples taken within 48 hours of admission. Thoracic imaging (chest radiographs and CT) were reported by consultant radiologists and coded based upon COVID-19 guidelines from the British Society of Thoracic Imaging (BSTI).[12]

RT-PCR of a clinical specimen from NPS was the only SARS-CoV-2 testing available during the study period. The decision to test was based on a clinical suspicion of COVID-19. Testing was performed at the point of admission or as soon as possible afterwards. Due to high demand and limited capacity, some patients with high clinical suspicion did not undergo SARS-CoV-2 testing. Routine testing for all admissions was introduced after the study period. Most SARS-CoV-2 testing was done using Panther Fusion™ (Hologic; ORF1ab Region 1 / 2 target) or Abbott RealTime™ (RNA-dependent RNA polymerase, Nucleocapsid target) assays on NPS.

Approval for this study was provided by London North West University Healthcare NHS Trust research and governance department, and the NHS Health Regulatory Authority (IRAS ID 285815). Written informed consent from participants was not obtained in compliance with Secretary of State for Health and Social Care 'Notice' under Regulation 3(4) of the Health Service Control of Patient Information Regulations 20021 (COPI) requiring health providers to

process confidential patient and Control of Patient Information Regulations due to the COVID-19 pandemic.

Definitions

Patients were assigned as having RT-PCR confirmed COVID-19 if they had a positive SARS-CoV-2 RT-PCR within 7 days before or after the date of admission, and had a discharge diagnosis of COVID-19 recorded by the clinical team. False-negative RT-PCR COVID-19 was defined as patients with a discharge diagnosis of COVID-19 made by the clinical team and one or more negative SARS-CoV-2 RT-PCR within 48 hours of admission in the absence of any positive SARS-CoV-2 RT-PCR results. Patients with evidence of alternative diagnoses (i.e. not COVID-19) made by the clinical team and no positive SARS-CoV-2 RT-PCR results were defined as not having COVID-19. Medical records for patients with positive SARS-CoV-2 tests greater than 7 days after admission but before discharge, and a diagnosis of COVID-19 were reviewed as to whether the admission was likely to represent a missing or delayed SARS-CoV-2 RT-PCR result (i.e. patients with community-acquired COVID-19) or nosocomial COVID-19 transmission. Mortality was assessed at discharge from hospital.

Statistical methods

Basic descriptive statistics were performed, with continuous data presented as median (interquartile range) and categorical data as frequency (%). Comparisons were made using chi-squared tests for proportions, t-tests for means and Wilcoxon rank sum for medians. Logistic regression was used to assess associations between variables and diagnosis of COVID-19. In exploratory analyses to assess association between RT-PCR negative COVID-19 and

mortality, a multivariable logistic regression model was used adjusting for other variable associated with poor outcomes in COVID-19.[13]

Sensitivity and false-negative RT-PCR

The real-world sensitivity of SARS-CoV-2 RT-PCR from NPS against a reference standard of a clinical diagnosis of COVID-19 was estimated as the proportion of patients positive from any RT-PCR, excluding those without any valid RT-PCR results. Sensitivity was also calculated by restricting analyses to patients with two or more RT-PCR results from NPS taken in a 24- and 48-hour period. The reference standard was patients with at least one positive RT-PCR in the time period. Incremental yield of a second RT-PCR following an initial negative result in patients was also calculated. Specificity of SARS-CoV-2 RT-PCR was assumed to be 100%.

Diagnostic Risk Score

In development of a score to predict COVID-19 among medical admissions, candidate predictor variables were selected based on *a priori* knowledge, published literature, clinical reasoning and the need for variables to be objective, reproducible, available in the emergency department soon after presentation. We considered demographic characteristics (age, sex, ethnicity), clinical symptoms associated with COVID-19 (cough, fever or shortness of breath), vital signs (including National Early Warning [NEWS] Score 2), and laboratory bloods (including C-reactive protein (CRP) and arterial/venous blood gas) at the time of presentation to hospital.

Continuous variables were assessed for non-linearity using fractional polynomials, and categorised based on established cut-off values and/or fractional polynomials. Complete case analysis was chosen for derivation and internal validation of the score, given most key variables

had fewer than 10% missing data. To derive a prediction model, we undertook univariable logistic regression analysis assessing associations between candidate variables and COVID-19 diagnosis (including all COVID-19 irrespective of RT-PCT status). We then used a backward elimination approach to create a multivariable predictive model, with stepwise elimination of variables, using likelihood ratio tests and Akaike information criterion to compare models. Interaction in the model were also assessed using likelihood ratio testing.

Points were assigned to each variable by identifying clusters of regression coefficients from the final model, then taking the median of those clustered coefficients and scaling so the lowest point score is at least one, and then rounding to the nearest integer.[14] A COVID-19 diagnostic risk score was then derived by combining the points based on patient characteristics. Performance of both the full predictive model and risk score was assessed using the area under the receiver-operator curve (AUROC, also known as concordance-statistic) for discrimination, and plots of predicted probability of COVID-19 against observed risk of COVID-19 for calibration (calibration plots). Decision curve analysis was also conducted to help weigh benefits of using the model, compared to assuming all or no patients were diagnosed with COVID-19, and comparison with other single variables with strong associations with COVID-19.

Internal model validation was done using the bootstrap procedure, with final model applied to each bootstrap sample (n=200), and an optimism corrected AUROC calculated.[15] A prediction model was also generated using bootstrap samples and tested on the original dataset. Cut-off thresholds were defined to identify patients at high- and low-risk of COVID-19 after plotting risk score against observed COVID risk such that the high-risk group accounted for as many COVID-19 cases as the low-risk as few as possible. Sensitivity, specificity, positive predictive

value (PPV) and negative predictive value (NPV) were calculated for each threshold, and NPV and PPV calculated for varying prevalence of COVID-19 amongst medical admissions. Sensitivity analysis used multivariate multiple imputation with chained equations for missing data, assuming they were missing at random. Imputation was done for missing candidate predictor variables using 20 imputations, and model generation and performance repeated. All analyses were done using Stata version 16 (StataCorp 2019). Predictive modelling elements are presented in accordance with TRIPOD guidance.[16]

RESULTS

Patient characteristics

Between 2nd March and 3rd May 2020, 4008 patients were admitted (2536 at Northwick Park Hospital, and 1472 at Ealing Hospital), with 1792 (44.7%) diagnosed with COVID-19 (figure 1). There were a median of 65 (IQR 57-76) admissions daily, including median daily admission of 47 (IQR 28-56) patients diagnosed with COVID-19 (supplementary figure 1). 1391 (77.6%) COVID-19 diagnoses had at least one positive SARS-CoV-2 RT-PCR. 283 (15.8%) had at least one negative and no positive RT-PCR, and 119 (6.6%) did not have a RT-PCR result.

There were several differences between patients with and without a COVID-19 diagnosis at discharge (including those with false negative RT-PCR results, table 1 and supplementary table 1). Most notably patients with COVID-19 were more likely to be male, be more unwell at admission (NEWS score 6 vs 2 for patients without COVID-19) and more likely to need supplementary oxygen. On chest radiograph, patients with COVID-19 were more likely to have lung infiltrates (79% vs 48%) and less likely to have clear lung fields (7% vs 33%).

Outcomes

Overall 248 (6.2%) of medical admissions were admitted to intensive care unit (ICU) for level 2 or 3 support. Patients with COVID-19 diagnosis were more likely to be admitted to ICU (12.7% compared to 1.0%, p<0.0001). Median time to intensive care admission was 1 day (IQR 0-3) from admission. Inpatient mortality was 15.6% overall with substantially higher mortality in patients with COVID-19 diagnosis (26.9% compared to 6.4%). 0.4% [n=16] remained admitted at the time of data extraction or were missing mortality status. Inpatient death occurred a median of 5 (IQR 2-10) days after admission for patients with COVID-19, and hospital stay was longer than for those without COVID-19 (median 5 [IQR 3-11] days compared to median 3 [IQR 1-7] days, P<0.0001).

Sensitivity of SARS-CoV-2 RT-PCR

Based on COVID-19 patients with a at least one valid SARS-CoV-2 RT-PCR result (n=1674), 16.9% (n=283) diagnosed with COVID-19 had at least one false-negative RT-PCR. 217 patients had a single negative result, with 66 having two or more negative results. Median time from admission to negative swab was 0 (IQR 0-1) days. Based on a clinical COVID-19 reference standard, the sensitivity of PCR was 83.1% (95% CI 81.2-84.8%). The diagnostic yield (i.e. including those without SARS-CoV-2 PCR results) of SAR-CoV-2 PCR testing of nasopharyngeal swabs was 77.6% (95% CI 75.6-79.5%). If restricted to patients with chest radiology suggestive of COVID-19, 198/968 patients with COVID-19 were RT-PCR negative, giving a sensitivity of 79.6%.

A total of 185 patients with COVID-19 had two RT-PCR tests within 24 hours, at least one of which was positive. 35/185 had a false-negative RT-PCR, giving a sensitivity of 81.1% (95% CI

74.7-86.5%). 62/254 patients with COVID-19 and two or more RT-PCR tests within 48 hours, giving a sensitivity of 75.6% (95% CI 70.0-80.5%). 557 patients with two RT-PCR tests within 24 hours had an initial negative test, of whom 17 had a second test that was positive, giving an incremental yield of 3.1% (95% CI 1.9-4.8%). 36/669 patients with an initial negative RT-PCR had a second test that was positive within 48 hours, giving an incremental yield of 5.4% (95% CI 3.9-7.4%).

False-negative COVID-19 RT-PCR

Of patients with RT-PCR negative COVID-19, 70.0% (198/283) had chest radiography or chest CT suggestive of COVID-19 based on BSTI coding, 80.2% (227/283) had lung infiltrates on chest imaging, and only 6.7% (19/283) had normal lung fields on chest radiography. 88.0% reported cough, fever or shortness of breath at admission. Broadly, patients with false-negative RT-PCR COVID-19 and those confirmed by positive PCR had similar demographic and clinical characteristics. Distribution of NEWS score and CRP were similar to RT-PCR-confirmed COVID-19 patients, and differed from those without COVID-19 diagnosis (supplementary figure 2). Notable differences include false-negative RT-PCR COVID-19 patients being more likely to report shortness of breath, slightly longer duration of symptoms (median of 7 [IQR 3-12] days compared to 6 [IQR 3-10] days for PCR-positive patients) (table 1). False negative RT-PCR patients also had higher median lymphocyte and platelet counts.

Importantly, outcomes were worse for patients with RT-PCR confirmed COVID-19 compared to those who were had a false-negative RT-PCR, with a higher proportion admitted to ITU (13.8 [95% CI 12.1-15.7 vs 7.8 [95% CI 5.2-11.5]%, p=0.006), and more patients dying during admission (29.4 [95% CI 27.1-31.9]% vs 21.0 [95% CI 12.7-21.4]%, p<0.0001). When limited

to patients with chest radiology suggestive of COVID-19, patients with false-negative RT-PCR disease still had better outcomes than PCR-confirmed COVID-19 (ITU admission 8.4%, mortality 16.3%, n=227). In exploratory analyses adjusted for age, sex, co-morbidities, admission oxygen saturation and admission urea, OR for mortality was 0.41 (95% CI 0.27-0.61) for RT-PCR negative compared to RT-PCR positive COVID-19 (see table supplementary table 2).

Predictors of COVID-19 and diagnostic model

Several demographic and clinical variables were strongly associated with a diagnosis of COVID-19, both in univariable and multivariable analysis (table 2). Abnormal chest radiography with infiltrates (OR 7.8, 95% CI 6.3-9.6), CRP over 50 (OR 6.0, 95% CI 5.2-6.9) and NEWS 2 score 5 or more (OR 5.2, 95% CI 5.0-6.6) had the strongest associations with COVID-19 diagnosis.

The final multivariable diagnostic model included age (modelled as a binary variable being between 50 and 70 years old), sex, ethnicity, reporting anyone of cough, fever or shortness of breath, NEWS 2, CRP, and chest radiograph appearance (n=2,940 table 3). Discrimination of the full model was moderate (AUC of ROC 0.83, 95% CI 0.82-0.85), with good calibration (see figure 2). A simplified risk score was constructed based on β-coefficients (table 3), with similar calibration and discrimination to the full model (AUC 0.83, 95% CI 8.1 – 8.4). Internal validation using bootstrap samples (n=200) generated an optimism corrected AUC 0.82 (95% CI 0.80-0.84, AUC for internal validated model 0.83 [95% CI 0.81 – 0.85]). Decision curve analysis showed the diagnostic risk score model had better clinical utility across a range of thresholds than treating all or no patients as having COVID-19, using a CRP of >50, or a NEWS score ≥5

(see figure 2). The model and risk score performed similarly in sensitivity analyses using multiple imputation instead of complete case analysis, and assessing the risk score using the whole patient population (see supplementary table 3).

The number and proportion of patients with or without COVID-19 diagnosis based on the risk score is shown in figure 3. 446 (15%) of patients had a score of <4, of whom 10.9% (49/446) were diagnosed with COVID-19. Using this threshold to identify patients *without* COVID-19 had a 26.6% sensitivity, but 96.6% specificity, with an 89.0% positive predictive value (PPV, supplementary table 4). 594 (20.2%) patients were above the high-risk threshold, set at a diagnostic risk score >9. At high COVID-19 prevalence (50%), this threshold had a good PPV (>90%), and at a low prevalence (<5%), had a high NPV. However, most patients fell in between both thresholds. Potential uses for such a clinical score are highlighted in supplementary table 5.

DISCUSSION

The key findings of this study are that SARS-CoV-2 RT-PCR negative COVID-19 is common amongst patients admitted to hospital, with real-life sensitivity of RT-PCR testing from NPS being 83% compared to a clinical reference standard of clinical diagnosis of COVID-19. Patients with RT-PCR negative COVID-19 had similar clinical characteristics to RT-PCR positive patients in this and other cohorts,[17] although significantly better outcomes (lower risk of mortality and ITU admission).[13,17] The proportion and number of COVID-19 admissions was increased during a three-week period from the 22nd March to 11th April 2020, and patients with

COVID-19 were substantially more unwell than patients without COVID-19, with implications for service delivery. Mortality in patients admitted without COVID-19 was also high at 6.4%.

The current gold standard diagnostic test for COVID-19, SARS-CoV-2 PCR from nasopharyngeal swabs, has several limitations which are challenging health systems and healthcare facilities management. We demonstrate, despite high analytical sensitivity, the real-life sensitivity of PCR is inadequate (around 80% at best).[18] Repeat testing of patients with an initial negative RT-PCR only increased yield by 3-5% within 48 hours. In addition to slow turnaround times, and resource and logistical challenges, there is an urgent need for alternative rapid and accurate methods to triage and stratify patient's risk of COVID-19, to allow appropriate infection control measures and safe patient flow to cohort areas or isolation rooms, without overwhelming hospital infrastructure. CT imaging of lungs can lack specificity for COVID-19, and rapid RT-PCR platforms are expensive and have inadequate throughput for future peaks of COVID-19.[19,20] Few studies have assessed pragmatic tools to assess risk of COVID-19 based on readily available clinical or laboratory variables.[21,22]

We found several clinical, radiological and laboratory blood factors that were associated with COVID-19. Our diagnostic score had moderate performance for discriminating COVID-19 from other diagnoses (AUROC 0.83). A low risk threshold had a good specificity and PPV, therefore could be used identify patients with a low COVID risk for transfer to a low-risk cohort area. Similarly, the high-risk score had a good PPV and specificity, therefore these patients could be managed as having COVID-19, and cared for in isolation rooms or cohorts if necessary. Those patients in neither high- nor low-risk group may benefit from rapid COVID-19 RT-PCR or antigen testing, depending on capacity. However, this score would need external validation

before use. Although derived from a cohort including unselected acute medical admissions, the higher prevalence of other respiratory viral pathogens may impact performance, especially specificity.[23] Furthermore, this score does not account for the vulnerability of individual patients for severe COVID-19 (eg based on age or comorbidities), which would also impact decisions on isolation and testing.[22]

This is the first study, to our knowledge, reporting lower ITU admissions and mortality in RT-PCR negative patients with COVID-19, despite similar markers of disease severity at admission (NEWS, CRP, oxygen saturations and requirement for supplementary oxygen), and in multivariable adjusted model. Interestingly, the median duration of symptoms was slightly longer, and median lymphocyte count was slightly higher in PCR-negative patients, suggesting they presented slightly later in their disease course, and therefore may be at a phase of illness with lower viral burden in the upper respiratory tract.[24–26] This may also be associated with their better prognosis. Other potential reasons for better outcomes in PCR-negative patients with COVID-19 include misclassification bias, where other respiratory conditions may have been classified as COVID-19. However, sensitivity analysis in patients with chest radiology suggestive of COVID-19 had similar findings, and a small number of misclassifications are unlikely to lead to such substantial differences in mortality.

During the study period, the overall number of daily admissions did not increase substantially. However, the proportion of admissions that were related to COVID-19 increased substantially in late March and early April, with a fall in non-COVID-19 admissions, as previously documented.[27] This has implications for planning for future COVID peaks. Another important finding was the high mortality in patients without COVID-19, an over two-fold increase from

mortality in the previous year (2.4% compared to 6.4%).[27] Whilst we were unable to describe the causes of death amongst these patients, the increased mortality may result from late presentation to hospital due to national government-mandated 'lockdown' COVID-19 control measures and fear of nosocomial transmission risk. This has been previously documented in paediatric, cardiology, and oncology patients, but not amongst acute medical admissions.[28,29]

This study has several strengths. The cohort is in a large acute hospital trust with two sites covering a diverse population, and all consecutive medical admissions were included. This is one of the first large cohorts to report data on unselected acute medical admissions, and one of the largest cohorts of RT-PCR negative patients with COVID-19. There are also several limitations. The retrospective nature of the study has inherent limitations, including missing data. Although we included consecutive admitted patients, not all patients had SARS-CoV-2 testing, and two different RT-PCR assays were used. The decision to repeat tests on patients with negative RT-PCR results was made by the responsible clinical team. The absence of serology or other confirmatory testing introduces a risk of misclassification bias and RT-PCR inclusion in the reference standard, and the influence of variables including in the diagnostic risk score on clinical diagnosis of COVID-19 introduces incorporation bias. However there remains no perfect reference standard for COVID-19 diagnosis and these biases are unlikely to significantly impact our findings. Our diagnostic risk model needs external validation, only has moderate discrimination, and is at risk of overfitting. Systematic reviews have struggled to identify other diagnostic clinical scores with high discrimination, and effective patient management is likely to involve a combination of clinical features, radiology and rapid PCR-testing.[11]

In conclusion, we demonstrate that RT-PCR negative COVID-19 is common amongst patients admitted to hospital, and is associated with a better outcome despite similar severity at presentation. We derived and internally validated a diagnostic risk score with potential utility to help triage patients admitted from the emergency department, although prospective trials of different approaches are warranted in future peaks of COVID-19.

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Author contributions

AGW, CKM, TC, VP, GS, RT, NV, SD, AW, AM and PP made substantial contribution to the conception of the work. AGW, CKM, AW, AM and PP made substantial contribution to the design of the work. AGW, CKM, JB, SF, GS, JT, NG, HC contributed to data acquisition. AGW and CKM analysed the data. AGW, CKM, AW, AM, PP contributed to data interpretation. AGW and CFM drafted the manuscript. All authors contributed to revising the manuscript critically for important intellectual content, approved the final manuscript and are accountable for all aspects of the work.

Patient and Public Involvement Statement

Due to the retrospective nature of this study, undertaken during the COVID-19 pandemic, patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Competing interests statement

The authors have no competing interests to declare

		Not diagnosed with COVID	All COVID diagnoses	p-value	COVID negative PCR	COVID diagnosis PCR positive	p-value
		n=2215	n=1793		n=283	n=1391	
Age at admission, median years (IQR)		71 (51, 82) (n=2215)	69 (56, 81) (n=1793)	0.44	70 (54, 79) (n=283)	70 (57, 81) (n=1391)	0.27
Age 65 years or older		1266 (57.2%)	1005 (56.1%)	0.48	154 (54.4%)	800 (57.5%)	0.34
Sex	Female	1021 (46.1%)	651 (36.3%)	<0.001	112 (39.6%)	498 (35.8%)	0.23
	Male	1193 (53.9%)	1142 (63.7%)		171 (60.4%)	893 (64.2%)	
Ethnicity	South Asian	486 (21.9%)	447 (24.9%)	<0.001	57 (20.1%)	362 (26.0%)	0.15
	Asian Other	174 (7.9%)	211 (11.8%)		30 (10.6%)	162 (11.6%)	
	Black African or Caribbean	212 (9.6%)	224 (12.5%)		33 (11.7%)	181 (13.0%)	
	Mixed Ethnicity	6 (0.3%)	10 (0.6%)		2 (0.7%)	8 (0.6%)	
	Unknown	330 (14.9%)	318 (17.7%)		53 (18.7%)	233 (16.8%)	
	White European	890 (40.2%)	458 (25.5%)		81 (28.6%)	361 (26.0%)	
	Other	117 (5.3%)	125 (7.0%)		27 (9.5%)	84 (6.0%)	
Index of Multiple Deprivation Decile, median (IQR)		5 (3, 7) (n=2105)	5 (3, 6) (n=1743)	0.048	4 (3, 6) (n=277)	5 (3, 6) (n=1366)	0.043
Diabetes		563 (25.7%)	599 (33.6%)	<0.001	81 (28.9%)	482 (34.8%)	0.059
Hypertension		825 (37.7%)	739 (41.5%)	0.015	110 (39.3%)	590 (42.6%)	0.31
Ischaemic Heart Disease		413 (18.9%)	309 (17.3%)	0.21	44 (15.7%)	247 (17.8%)	0.40
Heart Failure		156 (7.1%)	70 (3.9%)	<0.001	14 (5.0%)	53 (3.8%)	0.36

Chronic Obstructive Pulmonary Disease		185 (8.5%)	112 (6.3%)	0.010	21 (7.5%)	88 (6.3%)	0.48
Asthma		200 (9.1%)	165 (9.3%)	0.89	19 (6.8%)	133 (9.6%)	0.14
Cancer		169 (7.7%)	78 (4.4%)	<0.001	11 (3.9%)	65 (4.7%)	0.58
HIV		21 (1.0%)	14 (0.8%)	0.56	3 (1.1%)	11 (0.8%)	0.64
Cerebrovascular Disease		110 (5.0%)	96 (5.4%)	0.61	15 (5.4%)	75 (5.4%)	0.97
Dementia		156 (7.1%)	188 (10.5%)	<0.001	29 (10.4%)	153 (11.0%)	0.74
Chronic Kidney Disease		263 (12.0%)	233 (13.1%)	0.31	33 (11.8%)	182 (13.1%)	0.54
Cough		537 (24.5%)	1114 (62.5%)	<0.001	177 (63.2%)	865 (62.4%)	0.80
Shortness of breath		687 (31.4%)	1171 (65.7%)	<0.001	203 (72.5%)	886 (63.9%)	0.006
Fever		547 (25.0%)	1117 (62.7%)	<0.001	184 (65.7%)	860 (62.0%)	0.25
Confusion		241 (11.0%)	195 (10.9%)	0.95	30 (10.7%)	153 (11.0%)	0.87
Symptom duration (days), median (IQR)		4 (2, 12) (n=592)	7 (3, 10) (n=1083)	0.010	7 (3, 12) (n=163)	6 (3, 10) (n=844)	0.021
Observations							
Pulse >120 bpm		203 (10.3%)	241 (14.3%)	<0.001	41 (15.4%)	177 (13.4%)	0.39
Respiratory rate >30 per minute		175 (8.9%)	568 (33.6%)	<0.001	90 (33.8%)	439 (33.3%)	0.87
Temperature >38°C		180 (9.2%)	605 (35.9%)	<0.001	72 (27.0%)	495 (37.7%)	<0.001
Systolic Blood Pressure <100 mmHg		108 (5.5%)	101 (6.1%)	0.51	16 (6.1%)	78 (6.0%)	0.97
Consciousness level	Alert	646 (95.1%)	596 (96.0%)	0.93	101 (97.1%)	449 (95.5%)	0.47
	Confusion	13 (1.9%)	11 (1.8%)		3 (2.9%)	8 (1.7%)	

	Verbal	8 (1.2%)	5 (0.8%)		0 (0.0%)	4 (0.9%)	
	Pain	5 (0.7%)	3 (0.5%)		0 (0.0%)	3 (0.6%)	
	Unresponsive	7 (1.0%)	6 (1.0%)		0 (0.0%)	6 (1.3%)	
O_2 saturations <94%		198 (10.1%)	543 (32.2%)	<0.001	79 (29.8%)	430 (32.6%)	0.37
NEWS 2 Score, median (IQR)		2 (1, 4) (n=1951)	6 (3, 8) (n=1666)	<0.001	6 (4, 7) (n=264)	6 (3, 8) (n=1299)	0.73
NEWS 2 Score ≥5		477 (24.4%)	1084 (65.1%)	<0.001	176 (66.7%)	840 (64.7%)	0.53
Supplementary oxygen		169 (8.8%)	529 (33.1%)	<0.001	96 (37.9%)	404 (32.4%)	0.091
PO_2 <8 mmHg		127 (35.4%)	251 (36.2%)	0.79	34 (27.9%)	205 (38.7%)	0.025
PCO_2 >6 mmHg		124 (34.5%)	75 (10.8%)	<0.001	12 (9.8%)	59 (11.1%)	0.68
Neutrophils >10 x10^9/L		361 (17.8%)	250 (15.6%)	0.083	52 (19.0%)	183 (14.7%)	0.078
Lymphocytes <1 x10^9/L		509 (25.1%)	736 (46.1%)	<0.001	107 (39.1%)	594 (47.8%)	0.009
Platelet count x10^9/L, median (IQR)		246.0 (193.0, 317.0) (n=2025)	231.0 (177.0, 306.0) (n=1597)	<0.001	263.0 (206.0, 343.0) (n=274)	226.0 (172.0, 297.0) (n=1242)	<0.001
Creatinine >120 mmol/L		507 (25.2%)	426 (26.9%)	0.24	64 (23.8%)	338 (27.4%)	0.23
CRP µg/mL, median (IQR)		16.1 (3.4, 66.9) (n=1928)	98.7 (46.0, 175.3) (n=1590)	<0.001	86.2 (41.7, 170.1) (n=272)	101.5 (48.3, 180.2) (n=1237)	0.15
Influenza RT-PCR	Influenza A	11 (2.3%) (n=490)	1 (0.2%) (n=528)	<0.001	0 (n=72)	1 (0.2%) (n=445)	0.31
	Influenza B	9 (1.9%)	2 (0.4%)		1 (1.4%)	1 (0.2%)	

Table 1. Baseline characteristics for patients, including demographics, co-morbidities, admission vital signs and laboratory blood tests, stratified by diagnosis and SARS- CoV-2 RT-PCR status. Data on com-morbidities represents number with each condition. Where data are missing,

numbers in each category are presented. P-values are calculated using chi-squared for proportions, t-tests for means and Wilcoxon rank sum for medians. CRP C-reactive Protein, IQR inter quartile range. NEWS National Early Warning Score. PO2 partial pressure of oxygen, PCO2 partial pressure of carbon dioxide.

			Univariable Regression			Multivariable regression	
Variable		N	Odds Ratio (95% CI)	p		Odds Ratio (95% CI)	p
Age	increase 10 years	4,008	1.05 (1 - 1.08)	0.015			
	50-70	4,008	1.62 (1.4 - 1.86)	<0.0001		1.7 (1.4 - 2.08)	<0.0001
Sex	Male	4,008	1.5 (1.3 - 1.71)	<0.0001		1.26 (1.1 - 1.52)	<0.0001
IMD Decile		3,848	0.97 (0.9 - 1)	0.013			
Diabetes		3,971	1.46 (1.3 - 1.68)	<0.0001			

Hypertension		3,971	1.17 (1 - 1.33)	0.007			
Ethnicity		4,008					
	White	1,348	1	<0.0001		1	<0.0001
	Asian	1,318	1.94 (1.7 - 2.26)			1.82 (1.5 - 2.27)	
	Black	436	2.05 (1.6 - 2.56)			1.85 (1.4 - 2.53)	
	Mixed/ Other	258	2.13 (1.6 - 2.79)			2.25 (1.5 - 3.33)	
	Unknown	648	1.87 (1.5 - 2.27)			1.77 (1.3 - 2.34)	
Symptoms		3,971					
	Cough		5.13 (4.5 - 5.88)	<0.0001			
	Shortness of breath		4.19 (3.7 - 4.79)	<0.0001			
	Fever		5.04 (4.4 - 5.78)	<0.0001			
Respiratory rate	Any of above	4,008	6.29 (5.4 - 7.36)	<0.0001		3.11 (2.5 - 3.85)	<0.0001
Oxygen saturations		3,654	1.14 (1.1 - 1.15)	<0.0001			

NEWS Score	Continuous (linear)	3,647	0.89 (0.9 - 0.9)	<0.0001			
	Continuous (linear)	3,617	1.39 (1.3 - 1.42)	<0.0001			
CRP	>5		5.76 (5 - 6.65)	<0.0001		2.39 (2 - 2.87)	<0.0001
	every 10 increase	3,518	1.01 (1 - 1.01)	<0.0001			
Lymphocytes	>50		5.99 (5.2 - 6.93)	<0.0001		3.11 (2.6 - 3.75)	<0.0001
	Continuous (linear)	3,624	0.66 (0.6 - 0.72)	<0.0001			
Chest x-ray	<1		2.54 (2.2 - 2.93)	<0.0001		1.72 (1.4 - 2.08)	<0.0001
		3,581					
	Normal	718	1	<0.0001		1	<0.0001
	lung infiltrates	2,262	7.79 (6.3 - 9.65)			3.75 (2.9 - 4.91)	
	other abnormality	601	3.56 (2.8 - 4.6)			1.94 (1.4 - 2.68)	
	CVCX0	424	1	<0.0001			

	CVCX1	1,040	25.85 (18.7 - 35.66)				
	CVCX2	435	2.98 (2.3 - 3.93)				
	CVCX3	129	1.64 (1.1 - 2.44)				

Table 2. Univariable and multivariable logistic regression analysis for risk of COVID-19 diagnosis. P-values calculated using likelihood ratio tests. There was no evidence of interaction between variables in the final multivariable model. N=2,490 for multivariable model. CVCX represents British Society of Thoracic Imaging (BSTI) classification of chest x-ray. CRP C-reactive Protein

Variable		Coefficient	Standard error	Diagnsotic score points
Age	50-70	0.53 (0 - 0.41)	0.09	1
Sex	Male	0.23 (0.3 - 0.73)	0.10	1
Ethnicity	Asian	0.6 (0.4 - 0.82)	0.11	1
	Black	0.62 (0.3 - 0.93)	0.16	1
	Mixed/Other	0.81 (0.4 - 1.2)	0.20	1
	Unknown	0.57 (0.3 - 0.85)	0.14	1
Cough, fever or shortness of breath		1.13 (0.9 - 1.35)	0.11	2
NEWS2 Score	>5	0.87 (0.7 - 1.05)	0.09	2
CRP	>50	1.13 (1 - 1.32)	0.09	2
Lymphocytes	<1	0.54 (0.4 - 0.73)	0.10	1
Chest x-ray	lung infiltrates	1.32 (1.1 - 1.59)	0.14	2
	other abnormality	0.66 (0.3 - 0.98)	0.16	1

Table 3. Multivariable logistic regression diagnostic model for COVID-19, with regression (β) co-efficients and diagnostic score points. The constant (intercept) was -4.0 (95% cI -4.4 to -3.6). N= 2,940.

		Prevalence				
Low-risk diagnostic score threshold (<4)	Study population	0.5	0.2	0.1	0.05	0.01
Sensitivity	26.6%	-	-	-	-	-
Specificity	96.6%	-	-	-	-	-
PPV	89.0%	88.7%	66.2%	46.6%	29.2%	7.3%
NPV	56.0%	56.8%	84.0%	92.2%	96.2%	99.2%

High-risk diagnostic score threshold (>9)						
Sensitivity	37.0%	-	-	-	-	-
Specificity	96.1%	-	-	-	-	-
PPV	90.1%	90.4%	70.1%	51.0%	33.0%	8.6%
NPV	61.2%	60.4%	85.9%	93.2%	96.7%	99.3%

Table 4. Diagnostic performance of a low COVID-19 risk threshold (less than 4 points on the diagnostic score) and high-risk threshold (greater than 9 points). Low-risk threshold diagnostic accuracy is for identifying patients without COVID-19, whereas high-risk threshold is for identifying patients with COVID-19

Figure 1. Patient flow diagram by final diagnosis and SARS-CoV-2 RT-PCR status with outcomes. Note 'presumed COVID' includes patients who were RT-PCR negative (n=293) and those who did not have a valid RT-PCR results (n=109)

Figure 2. (A) Receiver operator curve for the full diagnostic predictive model. Area under the curve (AUC) 0.839 (95%CI 0.824-0.853), N=2,940. (B) Calibration plot showing observed compared to predicted risk of COVID-19 diagnosis as deciles, with 95% confidence interval. The dashed green line shows perfect calibration. (C) Decision curve analysis showing standardised net benefit at different threshold probabilities for diagnosing patients with COVID-19 , comparing diagnosing all patients as COVID-19 (blue solid line), diagnosing no patients with COVID-19 (solid red line), and various diagnostic risk models, including the COVID diagnostic score (full model and simplified risk score), C-reactive protein over 50, and National Early Warning Score of 5 or more. CRP C-reactive Protein, NEWS National Early Warning Score

Figure 3. (A) Overlaid histogram of COVID diagnostic risk score and number of patients with COVID-19 (white) and alternative (not COVID-19) diagnoses. (B) Proportion (%) of patients with COVID-19 (orange) or alternative (not COVID-19, blue) diagnoses by COVID diagnostic risk score. N=2,940

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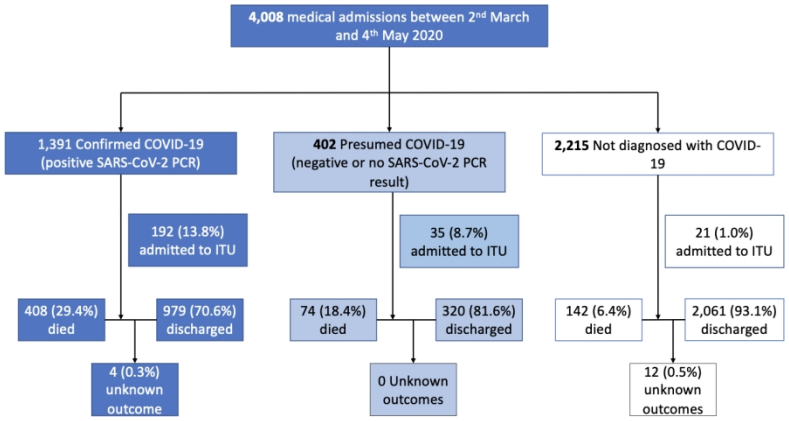

Figure 1

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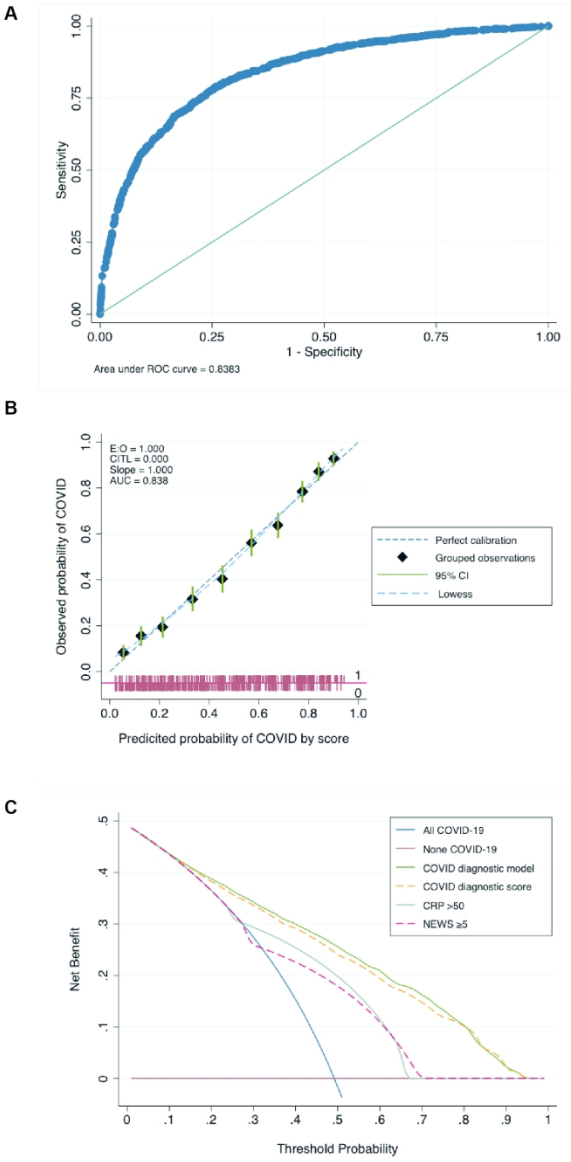

A
Sensitivity
1 - Specificity
Area under ROC curve = 0.8383
B
E:O = 1.000
CITL = 0.000
Slope = 1.000
AUC = 0.838
Observed probability of COVID
Predicited probability of COVID by score
Perfect calibration
Grouped observations
95% CI
Lowess
C
Net Benefit
Threshold Probability
All COVID-19
None COVID-19
COVID diagnostic model
COVID diagnostic score
CRP >50
NEWS ≥5

Figure 2

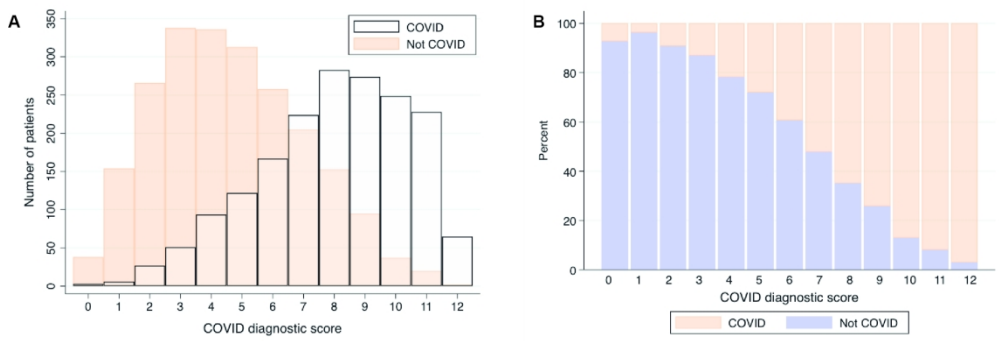

A
Number of patients
0
50
100
150
200
250
300
350
0 1 2 3 4 5 6 7 8 9 10 11 12
COVID diagnostic score
COVID
Not COVID
B
Percent
0
20
40
60
80
100
0 1 2 3 4 5 6 7 8 9 10 11 12
COVID diagnostic score
COVID
Not COVID

Figure 3

Supplementary Appendix- False-negative RT-PCR for COVID-19 and a diagnostic risk score: a retrospective cohort study among patients admitted to hospital

		Not diagnosed with COVID	All COVID diagnoses	p-value	COVID negative PCR	COVID diagnosis PCR positive	p-value
		n=2215	n=1793		n=283	n=1391	
Symptoms							
Cough		537 (24.5%)	1114 (62.5%)	<0.001	177 (63.2%)	865 (62.4%)	0.80
Chest pain		335 (15.3%)	109 (6.1%)	<0.001	23 (8.2%)	80 (5.8%)	0.12
Diarrhoea		152 (6.9%)	131 (7.4%)	0.62	25 (8.9%)	96 (6.9%)	0.24
Fall		277 (12.7%)	166 (9.3%)	<0.001	24 (8.6%)	129 (9.3%)	0.70
Symptom duration (days), median (IQR)		4 (2, 12) (n=592)	7 (3, 10) (n=1083)	0.010	7 (3, 12) (n=163)	6 (3, 10) (n=844)	0.021
Observations							
Pulse, median (IQR)		89 (75, 106) (n=1964)	96 (83, 110) (n=1689)	<0.001	98 (85, 110) (n=266)	96 (83, 110) (n=1319)	0.050
Pulse >120 bpm		203 (10.3%)	241 (14.3%)	<0.001	41 (15.4%)	177 (13.4%)	0.39
Respiratory rate per minute, median (IQR)		20 (18, 23) (n=1966)	26 (21, 32) (n=1688)	<0.001	26 (22, 32) (n=266)	26 (20, 32) (n=1318)	0.59
Respiratory rate >30 per minute		175 (8.9%)	568 (33.6%)	<0.001	90 (33.8%)	439 (33.3%)	0.87
Temperature °C, median (IQR)		36.7 (36.4, 37.1) (n=1961)	37.5 (36.8, 38.4) (n=1684)	<0.001	37.3 (36.7, 38) (n=267)	37.5 (36.8, 38.4) (n=1313)	0.007
Temperature >38°C		180 (9.2%)	605 (35.9%)	<0.001	72 (27.0%)	495 (37.7%)	<0.001
Systolic Blood Pressure mmHg, median (IQR)		136 (119, 154) (n=1948)	132 (117, 147) (n=1666)	<0.001	131 (118, 146.5) (n=264)	132 (117, 148) (n=1299)	0.88
Systolic Blood Pressure mmHg <100		108 (5.5%)	101 (6.1%)	0.51	16 (6.1%)	78 (6.0%)	0.97
O_2 saturations %, median (IQR)		97 (96, 99) (n=1961)	96 (92, 97) (n=1686)	<0.001	95 (93, 98) (n=265)	96 (92, 97) (n=1317)	0.55
O_2 saturations <94%		198 (10.1%)	543 (32.2%)	<0.001	79 (29.8%)	430 (32.6%)	0.37
NEWS 2 Score, median (IQR)		2 (1, 4) (n=1951)	6 (3, 8) (n=1666)	<0.001	6 (4, 7) (n=264)	6 (3, 8) (n=1299)	0.73
NEWS 2 Score ≥5		477 (24.4%)	1084 (65.1%)	<0.001	176 (66.7%)	840 (64.7%)	0.53
Supplementary oxygen	Yes	169 (8.8%)	529 (33.1%)	<0.001	96 (37.9%)	404 (32.4%)	0.091

Blood gas and pathology							
PO_2 (KPa), median (IQR)		8.8 (7.3, 11.1) (n=359)	8.7 (7.4, 10.7) (n=693)	0.51	9.1 (7.7, 10.6) (n=122)	8.5 (7.3, 10.7) (n=530)	0.18
PO_2 <8 v		127 (35.4%)	251 (36.2%)	0.79	34 (27.9%)	205 (38.7%)	0.025
pCO_2 (KPa), median (IQR)		5.2 (4.4, 6.7) (n=359)	4.6 (4.1, 5.2) (n=693)	<0.001	4.6 (4.1, 5.2) (n=122)	4.6 (4.1, 5.2) (n=530)	0.83
pCO_2 >6		124 (34.5%)	75 (10.8%)	<0.001	12 (9.8%)	59 (11.1%)	0.68
Haemoglobin (g/L), mean (SD)		121.7 (23.2) (n=2026)	124.4 (21.1) (n=1598)	<0.001	122.2 (21.0) (n=274)	124.6 (20.9) (n=1243)	0.085
Neutrophil count (x10^9/L), median (IQR)		5.9 (4.1, 8.6) (n=2026)	5.8 (4.0, 8.3) (n=1598)	0.20	6.7 (4.5, 9.1) (n=274)	5.6 (3.9, 8.0) (n=1243)	<0.001
Neutrophils >10 x10^9/L		361 (17.8%)	250 (15.6%)	0.083	52 (19.0%)	183 (14.7%)	0.078
Lymphocyte count (x10^9/L), median (IQR)		1.4 (0.9, 2.0) (n=2026)	1.0 (0.7, 1.4) (n=1598)	<0.001	1.1 (0.8, 1.4) (n=274)	1.0 (0.7, 1.4) (n=1243)	0.013
Lymphocytes <1 x10^9/L		509 (25.1%)	736 (46.1%)	<0.001	107 (39.1%)	594 (47.8%)	0.009
Platelet count (x10^9/L), median (IQR)		246.0 (193.0, 317.0) (n=2025)	231.0 (177.0, 306.0) (n=1597)	<0.001	263.0 (206.0, 343.0) (n=274)	226.0 (172.0, 297.0) (n=1242)	<0.001
Platelets <100 x10^9/L		80 (4.0%)	62 (3.9%)	0.92	11 (4.0%)	50 (4.0%)	0.99
ALT, median (IQR)		22.0 (15.0, 36.0) (n=1755)	31.0 (18.0, 51.0) (n=1412)	<0.001	31.0 (18.0, 55.0) (n=245)	30.0 (19.0, 51.0) (n=1096)	0.71
Creatinine (mmol/L), median (IQR)		84.0 (65.0, 121.0) (n=2011)	86.0 (67.0, 124.0) (n=1582)	0.057	80.0 (65.0, 117.0) (n=269)	87.0 (68.0, 127.0) (n=1235)	0.011
Creatinine >120 mmol/L		507 (25.2%)	426 (26.9%)	0.24	64 (23.8%)	338 (27.4%)	0.23
Urea (mmol/L), median (IQR)		6.0 (4.0, 9.8) (n=2025)	6.1 (4.0, 10.6) (n=1584)	0.58	5.5 (3.8, 8.9) (n=270)	6.4 (4.1, 11.0) (n=1236)	0.007
CRP µg/mL, median (IQR)		16.1 (3.4, 66.9) (n=1928)	98.7 (46.0, 175.3) (n=1590)	<0.001	86.2 (41.7, 170.1) (n=272)	101.5 (48.3, 180.2) (n=1237)	0.15
CRP >50 µg/mL		599 (31.1%)	1160 (73.0%)	<0.001	191 (70.2%)	917 (74.1%)	0.19
Glucose (mmol/L), median (IQR)		6.6 (5.6, 8.5) (n=1182)	7.1 (5.9, 9.3) (n=910)	<0.001	6.7 (5.9, 9.1) (n=147)	7.1 (5.9, 9.3) (n=710)	0.49
Lactate >2 mmol/L		41 (3.5%)	30 (3.3%)	0.83	5 (3.4%)	21 (3.0%)	0.78

Supplementary Table 1. Baseline characteristics for patients, including co-morbidities, admission vital signs and laboratory blood tests, stratified by diagnosis and SARS- CoV-2 RT-PCR status. Data on com-morbidities represents number with each condition. Where data are missing, numbers in each category are presented. P-values are calculated using chi-squared for proportions, t-tests for means and Wilcoxon rank sum for medians. CRP C-reactive Protein, IQR inter quartile range. NEWS National Early Warning Score. PO2 partial pressure of oxygen, PCO2 partial pressure of carbon dioxide.

Variable		Odds ratio (95% CI)	P-value
COVID-19 RT-PCR negative		0.41 (0.3 - 0.6)	<0.0001
Age, years		1.06 (1.0 - 1.1)	<0.0001
Sex	Female	0.90 (0.7 - 1.2)	0.446
Co-morbidities	1	1.13 (0.8 - 1.7)	0.552
	2 or more	1.45 (1.0 - 2.1)	0.042
CRP		1.00 (1.0 – 1.0)	<0.0001
Oxygen Saturations <94%		1.41 (1.1 - 1.9)	0.016
Urea		1.04 (1 - 1.1)	<0.0001

Supplementary Table 2. Multivariable logistic regression model assessing association between COVID-19 PCR-status and mortality, adjusting for other variables known to be risk-factors for mortality in COVID-19. Continuous variables modelled as linear. No interactions in the final model. P-vales calculated by likelihood ratio tests. N= 1,414.

Variable		ß-Coefficient	Odds ratio (95% CI)	Diagnostic score points
Age	50-70	0.4 (0.2 - 0.6)	1.5 (1.2-1.8)	1
Sex	Male	0.2 (0.0 - 0.3)	1.2 (1.0-1.4)	1
Ethnicity	Asian	0.6 (0.4 - 0.8)	1.8 (1.4-2.1)	1
	Black	0.6 (0.4 - 0.9)	1.9 (1.4-2.5)	1
	Mixed/Other	0.8 (0.4 - 1.1)	2.2 (1.5-3.1)	1
	Unknown	0.5 (0.3 - 0.8)	1.7 (1.3-2.2)	1
Cough, fever or shortness of breath		1.3 (1.2 - 1.5)	3.8 (3.2-4.5)	2
NEWS2 Score	>5	0.9 (0.7 - 1.1)	2.4 (2.0-2.9)	2
CRP	>50	1.1 (1.0 - 1.3)	3.0 (2.6-3.7)	2
Lymphocytes	<1	0.6 (0.4 - 0.8)	1.8 (1.5 – 2.2)	1
Chest x-ray	lung infiltrates	1.3 (1.0 - 1.5)	3.6 (2.8 -4.5)	2
	other abnormality	0.7 (0.4 - 0.9)	1.9 (1.4-2.6)	1

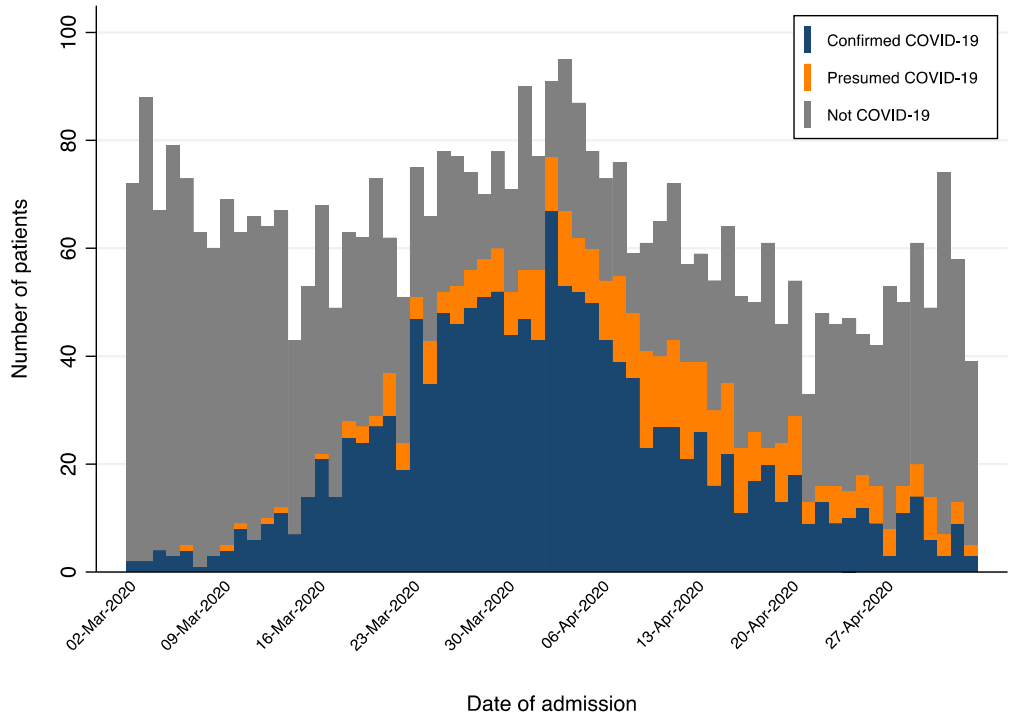
Supplementary Table 3. Logistic regression multivariable model for COVID-19 diagnosis using multivariate multiple imputation using chained equations for missing data in candidate predictor variables, with odds ratio and ß co-efficents. N=3,968. Area under the receiver operator curve (ROC) = 0.86 (95% CI 0.84 – 0.87).

		Prevalence				
Low-risk diagnostic score threshold (<4)	Study population	0.5	0.2	0.1	0.05	0.01
Sensitivity	26.6%	-	-	-	-	-
Specificity	96.6%	-	-	-	-	-
PPV	89.0%	88.7%	66.2%	46.6%	29.2%	7.3%
NPV	56.0%	56.8%	84.0%	92.2%	96.2%	99.2%
High-risk diagnostic score threshold (>9)						
Sensitivity	37.0%	-	-	-	-	-
Specificity	96.1%	-	-	-	-	-
PPV	90.1%	90.4%	70.1%	51.0%	33.0%	8.6%
NPV	61.2%	60.4%	85.9%	93.2%	96.7%	99.3%

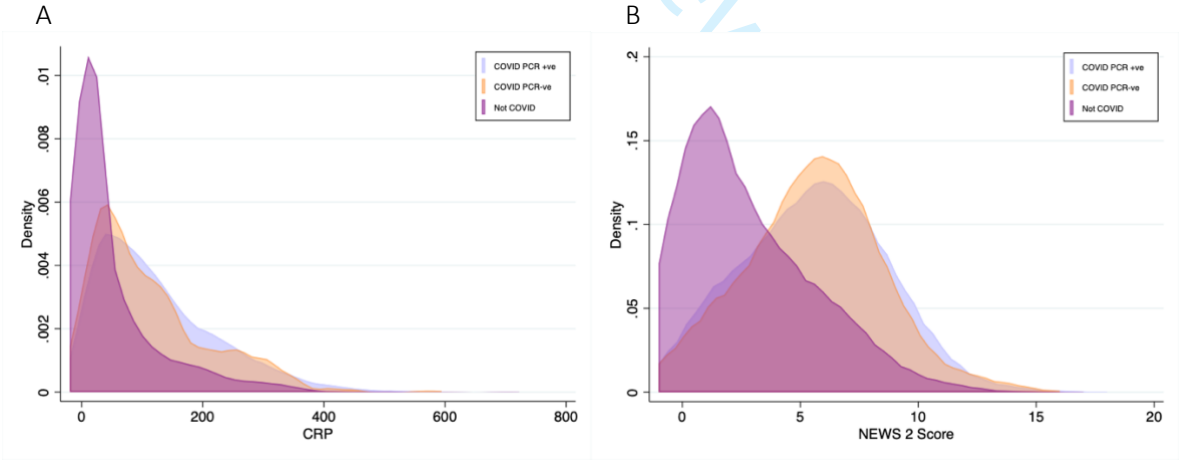
Supplementary Table 4. Diagnostic performance of a low COVID-19 risk threshold (less than 4 points on the diagnostic score) and high-risk threshold (greater than 9 points). Low-risk threshold diagnostic accuracy is for identifying patients without COVID-19, whereas high-risk threshold is for identifying patients with COVID-19

COVID status based on diagnostic risk score (proportion of patients expected during 'peak')	Management
Low risk, COVID-19 diagnostic risk score <4	• Alternative diagnosis most likely • Rapid RT-PCR or antigen test, if negative send to 'COVID-negative' area
Medium risk, COVID-19 diagnostic score 4-9	• Uncertain if COVID-19 is cause for presentation • Will need further testing to determine COVID-19 diagnosis • Either test with Rapid RT-PCR or antigen test, or consider CT imaging, or standard COVID-19 RT-PCR testing and move to isolation in
High risk, COVID-19 diagnostic score >9	• COVID-19 most likely • Isolate patient in COVID-19 area or isolation room and standard COVID-19 RT-PCR testing

Supplementary Table 5. Potential application of COVID-19 diagnostic risk score



Supplementary Figure 1. Number of patients admitted and final diagnosis by date of admission. Confirmed COVID-19 is patients with a positive SARS-CoV-2 PCR from nasopharyngeal swab, presumed COVID-19 is patients without a positive SARS-CoV-2 PCR but a discharge diagnosis of COVID-19. Not COVID-19 are patients without a positive SARS-CoV-2 PCR and an alternative diagnosis. N=4008.



Supplementary Figure 2. Distribution of (A) C-reactive protein (N=3518) and (B) National Early Warning Score (NEWS) (N=3889) by diagnosis at the time of hospital admission.

False-negative RT-PCR for COVID-19 and diagnostic risk score: a retrospective cohort study among patients admitted to hospital

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4-5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4-5*
Bias	9	Describe any efforts to address potential sources of bias	5-6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5-6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	5-6

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1/page 7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1, page 7
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	Report numbers of outcome events or summary measures over time	7, figure S2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-8
		(*b*) Report category boundaries when continuous variables were categorized	7-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7-8
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

False-negative RT-PCR for COVID-19 and diagnostic risk score: a retrospective cohort study among patients admitted to hospital

TRIPOD Checklist: Prediction Model Development and Validation

Section/Topic	Item		Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	2
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	3
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	3
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	4
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	4
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	4
	5b	D;V	Describe eligibility criteria for participants.	4
	5c	D;V	Give details of treatments received, if relevant.	NA
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	6
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	NA
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	6
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	NA
Sample size	8	D;V	Explain how the study size was arrived at.	6
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	6-7
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	6
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	6
	10c	V	For validation, describe how the predictions were calculated.	6-7
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	6
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	NA
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	6-7
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	NA
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	Figure 1, page 7
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	Table 1
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	NA
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	7
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	Table 2
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	Table 3
	15b	D	Explain how to the use the prediction model.	9
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	9, supplement
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	NA
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	10-11
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	10-11
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	10-11
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	10-11, table S5
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Supplement

False-negative RT-PCR for COVID-19 and diagnostic risk score: a retrospective cohort study among patients admitted to hospital

TRIPOD Checklist: Prediction Model Development and Validation

				ary appen dix
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	12

*Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D;V. We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.

BMJ Open

False-negative RT-PCR for COVID-19 and a diagnostic risk score: a retrospective cohort study among patients admitted to hospital

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Keywords:	COVID-19, Molecular diagnostics < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES

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BMJ Open: Original Research Article

Title:

False-negative RT-PCR for COVID-19 and a diagnostic risk score: a retrospective cohort study among patients admitted to hospital

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Keywords: COVID-19, diagnosis, risk score, hospital

ABSTRACT

Objective: To describe the characteristics and outcomes of patients with a clinical diagnosis of COVID-19 and false negative SARS-CoV-2 RT-PCR, and develop and internally validate a diagnostic risk score to predict risk of COVID-19 (including RT-PCR negative COVID-19) amongst medical admissions

Design: Retrospective cohort study

Setting: Two hospitals within an acute NHS trust in London, UK

Participants: All patients admitted to medical wards between 2nd March and 3rd May 2020.

Outcomes: Main outcomes were diagnosis of COVID-19, SARS-CoV-2 RT-PCR results, sensitivity of SARS-CoV-2 RT-PCR and mortality during hospital admission. For the diagnostic risk score, we report discrimination, calibration and diagnostic accuracy of the model and simplified risk score, and internal validation.

Results: 4008 patients were admitted between 2nd March and 3rd May 2020. 1792 patients (44.8%) were diagnosed with COVID-19, of whom 1391 were SARS-CoV-2 RT-PCR positive, and 283 had only negative RT-PCRs. Compared to a clinical reference standard, sensitivity of RT-PCR in hospital patients was 83.1% (95% CI 81.2-84.8%). Broadly, patients with false-negative RT-PCR COVID-19 and those confirmed by positive PCR had similar demographic and clinical characteristics, but lower risk of ICU admission and lower in-hospital mortality (adjusted odds ratio 0.41, 95% CI 0.27-0.61). A simple diagnostic risk score comprising of age,

sex, ethnicity, cough, fever or shortness of breath, National Early Warning Score (NEWS2), C-Reactive Protein, and chest radiograph appearance had moderate discrimination (area under the receiver-operator-curve 0.83, 95% CI 0.82-0.85), good calibration and was internally validated.

Conclusion: RT-PCR negative COVID-19 is common and is associated with lower mortality despite similar presentation. Diagnostic risk scores could potentially help triage patients requiring admission, but need external validation.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Large cohort of consecutive acute medical admissions in two hospitals covering a diverse population in London, UK, during first COVID-19 'peak'
- Assessment of 'real world' performance of SARS CoV-2 RT-PCR from nasopharyngeal swabs for diagnosis of COVID-19
- Inherent limitations of retrospective cohort study design, including some missing data
- Not all patients had SARS-CoV-2 RT-PCR testing

INTRODUCTION

The coronavirus disease 2019 (COVID-19) global pandemic, caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to unprecedented numbers of unwell and infectious patients requiring admission to hospital. The symptoms of COVID-19 can be non-specific, so diagnostic confirmation in hospital is often sought by detection of SARS-CoV-2 ribonucleic acid (RNA) sequences by reverse transcription-polymerase chain reaction (RT-PCR) from a clinical specimen.

Since the beginning of the pandemic, the standard sample for PCR testing has been a nasopharyngeal swab (NPS) or aspirate, but there are concerns that a significant proportion of cases test negative on initial RT-PCR of an NPS sample, with many patients having repeated sampling to confirm the diagnosis.[1] A systematic review of real-world diagnostic sensitivity of SARS-CoV-2 RT-PCR reports that up to 33% of patients with COVID-19 may have an initial false negative NPS result despite a compatible clinical illness, consistent thoracic imaging and/or subsequent positive antibodies to COVID-19.[2–5] False negative RT-PCR may result from inadequate nasopharyngeal sampling technique, delayed time to analysis, ineffective sample storage, variable gene targets in RT-PCR assays leading to imperfect analytic sensitivity, or if a patient is tested at a point when viral throat carriage is absent or below the detectable threshold (either too early or too late).[6,7] This high false negative rate complicates both hospital infection control and clinical decision making. Being able to identify patients with a high probability of COVID-19 despite a negative RT-PCR is crucial for effective clinical care.

The clinical characteristics and outcomes of hospitalised patients with COVID-19 have been well described globally, but these studies are limited to patients with RT-PCR confirmed

COVID-19.[8–10] The pattern of disease and outcomes of patients with false negative COVID-19 tests has not been well reported to date, nor has the diagnostic accuracy of RT-PCR assays in secondary care settings in the United Kingdom (UK). Several studies have derived and validated risk scores to assess severity and prognosis amongst patients with COVID-19. However few risk scores focus on identifying patients with COVID-19 amongst those needing hospital admission and those that do are from outside the UK, do not consider all hospital admissions, rely on high-resolution computerised tomography (CT) scanning of the lungs, and exclude patients without RT-PCR-confirmed disease.[11]

We therefore aim to describe the characteristics and outcomes of patients with a clinical diagnosis of COVID-19 but with negative RT-PCR from NPS, and the real-world sensitivity of RT-PCR for COVID-19. Secondly, we describe predictors of COVID-19 amongst general medical admissions, including assessing whether a simple diagnostic risk score could be derived, internally validated, and used to predict which patients admitted to medical wards will have COVID-19.

METHODS

Study design

This is a retrospective observational cohort study of consecutive admissions in London North West University Healthcare NHS Trust, comprising two hospitals, Northwick Park and Ealing. Patients were included in this study if they were admitted via the acute medical team between 2nd March and 3rd May 2020 inclusive.

Data collection

Cases were identified retrospectively through electronic medical admission lists. De-identified data on patient demographics, co-morbidities, clinical characteristics, vital signs, routine biochemical, haematological and microbiological tests, diagnosis and clinical outcomes were extracted from routinely collected clinical data using electronic patient record systems, and other NHS Trust health information systems. Physiological observations were those first recorded on admission to the emergency department. All biochemical and haematological data were from the first samples taken within 48 hours of admission. Thoracic imaging (chest radiographs and CT) were reported by consultant radiologists and coded based upon COVID-19 guidelines from the British Society of Thoracic Imaging (BSTI).[12]

RT-PCR of a clinical specimen from NPS was the only SARS-CoV-2 testing available during the study period. The decision to test was based on a clinical suspicion of COVID-19. Testing was performed at the point of admission or as soon as possible afterwards. Due to high demand and limited capacity, some patients with high clinical suspicion did not undergo SARS-CoV-2 testing. Routine testing for all admissions was introduced after the study period. Most SARS-CoV-2 testing was done using Panther Fusion™ (Hologic; ORF1ab Region 1 / 2 target) or Abbott RealTime™ (RNA-dependent RNA polymerase, Nucleocapsid target) assays on NPS.

Approval for this study was provided by London North West University Healthcare NHS Trust research and governance department, and the NHS Health Regulatory Authority (IRAS ID 285815). Written informed consent from participants was not obtained in compliance with Secretary of State for Health and Social Care 'Notice' under Regulation 3(4) of the Health Service Control of Patient Information Regulations 20021 (COPI) requiring health providers to

process confidential patient and Control of Patient Information Regulations due to the COVID-19 pandemic.

Definitions

Patients were assigned as having RT-PCR confirmed COVID-19 if they had a positive SARS-CoV-2 RT-PCR within 7 days before or after the date of admission, and had a discharge diagnosis of COVID-19 recorded by the clinical team. False-negative RT-PCR COVID-19 was defined as patients with a discharge diagnosis of COVID-19 made by the clinical team and one or more negative SARS-CoV-2 RT-PCR within 48 hours of admission in the absence of any positive SARS-CoV-2 RT-PCR results. Patients with evidence of alternative diagnoses (i.e. not COVID-19) made by the clinical team and no positive SARS-CoV-2 RT-PCR results were defined as not having COVID-19. Medical records for patients with positive SARS-CoV-2 tests greater than 7 days after admission but before discharge, and a diagnosis of COVID-19 were reviewed as to whether the admission was likely to represent a missing or delayed SARS-CoV-2 RT-PCR result (i.e. patients with community-acquired COVID-19) or nosocomial COVID-19 transmission. Mortality was assessed at discharge from hospital.

Statistical methods

Basic descriptive statistics were performed, with continuous data presented as median (interquartile range) and categorical data as frequency (%). Comparisons were made using chi-squared tests for proportions, t-tests for means and Wilcoxon rank sum for medians. Logistic regression was used to assess associations between variables and diagnosis of COVID-19. In exploratory analyses to assess association between RT-PCR negative COVID-19 and

mortality, a multivariable logistic regression model was used adjusting for other variable associated with poor outcomes in COVID-19.[13]

Sensitivity and false-negative RT-PCR

The real-world sensitivity of SARS-CoV-2 RT-PCR from NPS against a reference standard of a clinical diagnosis of COVID-19 was estimated as the proportion of patients positive from any RT-PCR, excluding those without any valid RT-PCR results. Sensitivity was also calculated by restricting analyses to patients with two or more RT-PCR results from NPS taken in a 24- and 48-hour period. The reference standard was patients with at least one positive RT-PCR in the time period. Incremental yield of a second RT-PCR following an initial negative result in patients was also calculated. Specificity of SARS-CoV-2 RT-PCR was assumed to be 100%.

Diagnostic Risk Score

In development of a score to predict COVID-19 among medical admissions, candidate predictor variables were selected based on *a priori* knowledge, published literature, clinical reasoning and the need for variables to be objective, reproducible, available in the emergency department soon after presentation. We considered demographic characteristics (age, sex, ethnicity), clinical symptoms associated with COVID-19 (cough, fever or shortness of breath), vital signs (including National Early Warning [NEWS] Score 2), and laboratory bloods (including C-reactive protein (CRP) and arterial/venous blood gas) at the time of presentation to hospital.

Continuous variables were assessed for non-linearity using fractional polynomials, and categorised based on established cut-off values and/or fractional polynomials. Complete case analysis was chosen for derivation and internal validation of the score, given most key variables

had fewer than 10% missing data. To derive a prediction model, we undertook univariable logistic regression analysis assessing associations between candidate variables and COVID-19 diagnosis (including all COVID-19 irrespective of RT-PCT status). We then used a backward elimination approach to create a multivariable predictive model, with stepwise elimination of variables, using likelihood ratio tests and Akaike information criterion to compare models. Interaction in the model were also assessed using likelihood ratio testing.

Points were assigned to each variable by identifying clusters of regression coefficients from the final model, then taking the median of those clustered coefficients and scaling so the lowest point score is at least one, and then rounding to the nearest integer.[14] A COVID-19 diagnostic risk score was then derived by combining the points based on patient characteristics. Performance of both the full predictive model and risk score was assessed using the area under the receiver operating characteristic (ROC) curve (AUROC curve, also known as concordance-statistic) for discrimination, and plots of predicted probability of COVID-19 against observed risk of COVID-19 for calibration (calibration plots). Decision curve analysis was also conducted to help weigh benefits of using the model, compared to assuming all or no patients were diagnosed with COVID-19, and comparison with other single variables with strong associations with COVID-19.

Internal model validation was done using the bootstrap procedure, with final model applied to each bootstrap sample (n=200), and an optimism corrected AUROC curve calculated.[15] A prediction model was also generated using bootstrap samples and tested on the original dataset. Cut-off thresholds were defined to identify patients at high- and low-risk of COVID-19 after plotting risk score against observed COVID risk such that the high-risk group accounted

for as many COVID-19 cases as the low-risk as few as possible. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each threshold, and NPV and PPV calculated for varying prevalence of COVID-19 amongst medical admissions. Sensitivity analysis used multivariate multiple imputation with chained equations for missing data, assuming they were missing at random. Imputation was done for missing candidate predictor variables using 20 imputations, and model generation and performance repeated. All analyses were done using Stata version 16 (StataCorp 2019). Predictive modelling elements are presented in accordance with TRIPOD guidance.[16]

RESULTS

Patient characteristics

Between 2nd March and 3rd May 2020, 4008 patients were admitted (2536 at Northwick Park Hospital, and 1472 at Ealing Hospital), with 1792 (44.7%) diagnosed with COVID-19 (figure 1). There were a median of 65 (IQR 57-76) admissions daily, including median daily admission of 47 (IQR 28-56) patients diagnosed with COVID-19 (supplementary figure 1). 1391 (77.6%) COVID-19 diagnoses had at least one positive SARS-CoV-2 RT-PCR. 283 (15.8%) had at least one negative and no positive RT-PCR, and 119 (6.6%) did not have a RT-PCR result.

There were several differences between patients with and without a COVID-19 diagnosis at discharge (including those with false negative RT-PCR results, table 1 and supplementary table 1). Most notably patients with COVID-19 were more likely to be male, be more unwell at admission (NEWS score 6 vs 2 for patients without COVID-19) and more likely to need supplementary oxygen. On chest radiograph, patients with COVID-19 were more likely to have lung infiltrates (79% vs 48%) and less likely to have clear lung fields (7% vs 33%).

Outcomes

Overall 248 (6.2%) of medical admissions were admitted to intensive care unit (ICU) for level 2 or 3 support. Patients with COVID-19 diagnosis were more likely to be admitted to ICU (12.7% compared to 1.0%, p<0.0001). Median time to intensive care admission was 1 day (IQR 0-3) from admission. Inpatient mortality was 15.6% overall with substantially higher mortality in patients with COVID-19 diagnosis (26.9% compared to 6.4%). 0.4% [n=16] remained admitted at the time of data extraction or were missing mortality status. Inpatient death occurred a median of 5 (IQR 2-10) days after admission for patients with COVID-19, and hospital stay was longer than for those without COVID-19 (median 5 [IQR 3-11] days compared to median 3 [IQR 1-7] days, P<0.0001).

Sensitivity of SARS-CoV-2 RT-PCR

Based on COVID-19 patients with a at least one valid SARS-CoV-2 RT-PCR result (n=1674), 16.9% (n=283) diagnosed with COVID-19 had at least one false-negative RT-PCR. 217 patients had a single negative result, with 66 having two or more negative results. Median time from admission to negative swab was 0 (IQR 0-1) days. Based on a clinical COVID-19 reference standard, the sensitivity of PCR was 83.1% (95% CI 81.2-84.8%). The diagnostic yield (i.e. including those without SARS-CoV-2 PCR results) of SAR-CoV-2 PCR testing of nasopharyngeal swabs was 77.6% (95% CI 75.6-79.5%). If restricted to patients with chest radiology suggestive of COVID-19, 198/968 patients with COVID-19 were RT-PCR negative, giving a sensitivity of 79.6%.

A total of 185 patients with COVID-19 had two RT-PCR tests within 24 hours, at least one of which was positive. 35/185 had a false-negative RT-PCR, giving a sensitivity of 81.1% (95% CI 74.7-86.5%). 62/254 patients with COVID-19 and two or more RT-PCR tests within 48 hours, giving a sensitivity of 75.6% (95% CI 70.0-80.5%). 557 patients with two RT-PCR tests within 24 hours had an initial negative test, of whom 17 had a second test that was positive, giving an incremental yield of 3.1% (95% CI 1.9-4.8%). 36/669 patients with an initial negative RT-PCR had a second test that was positive within 48 hours, giving an incremental yield of 5.4% (95% CI 3.9-7.4%).

False-negative COVID-19 RT-PCR

Of patients with RT-PCR negative COVID-19, 70.0% (198/283) had chest radiography or chest CT suggestive of COVID-19 based on BSTI coding, 80.2% (227/283) had lung infiltrates on chest imaging, and only 6.7% (19/283) had normal lung fields on chest radiography. 88.0% reported cough, fever or shortness of breath at admission. Broadly, patients with false-negative RT-PCR COVID-19 and those confirmed by positive PCR had similar demographic and clinical characteristics. Distribution of NEWS score and CRP were similar to RT-PCR-confirmed COVID-19 patients, and differed from those without COVID-19 diagnosis (supplementary figure 2). Notable differences include false-negative RT-PCR COVID-19 patients being more likely to report shortness of breath, slightly longer duration of symptoms (median of 7 [IQR 3-12] days compared to 6 [IQR 3-10] days for PCR-positive patients) (table 1). False negative RT-PCR patients also had higher median lymphocyte and platelet counts.

Importantly, outcomes were worse for patients with RT-PCR confirmed COVID-19 compared to those who were had a false-negative RT-PCR, with a higher proportion admitted to ICU (13.8

[95% CI 12.1-15.7 vs 7.8 [95% CI 5.2-11.5]%, p=0.006), and more patients dying during admission (29.3 [95% CI 27.0-31.8]% vs 16.6 [95% CI 12.7-21.4]%, p<0.0001). When limited to patients with chest radiology suggestive of COVID-19, patients with false-negative RT-PCR disease still had better outcomes than PCR-confirmed COVID-19 (ICU admission 8.4%, mortality 16.3%, n=227). In exploratory analyses adjusted for age, sex, co-morbidities, admission oxygen saturation and admission urea, OR for mortality was 0.41 (95% CI 0.27-0.61) for RT-PCR negative compared to RT-PCR positive COVID-19 (see table supplementary table 2).

Predictors of COVID-19 and diagnostic model

Several demographic and clinical variables were strongly associated with a diagnosis of COVID-19, both in univariable and multivariable analysis (table 2). Abnormal chest radiography with infiltrates (OR 7.8, 95% CI 6.3-9.6), CRP over 50 (OR 6.0, 95% CI 5.2-6.9) and NEWS 2 score 5 or more (OR 5.2, 95% CI 5.0-6.6) had the strongest associations with COVID-19 diagnosis.

The final multivariable diagnostic model included age (modelled as a binary variable being between 50 and 70 years old), sex, ethnicity, reporting anyone of cough, fever or shortness of breath, NEWS 2, CRP, and chest radiograph appearance (n=2,940 table 3). Discrimination of the full model was moderate (AUROC curve 0.83, 95% CI 0.82-0.85), with good calibration (see figure 2). A simplified risk score was constructed based on β-coefficients (table 3), with similar calibration and discrimination to the full model (AUROC curve 0.83, 95% CI 0.81 – 0.84). Internal validation using bootstrap samples (n=200) generated an optimism corrected AUC 0.82 (95% CI 0.80-0.84, AUC for internal validated model 0.83 [95% CI 0.81 – 0.85]). Decision curve

analysis showed the diagnostic risk score model had better clinical utility across a range of thresholds than treating all or no patients as having COVID-19, using a CRP of >50, or a NEWS score ≥5 (see figure 2). The model and risk score performed similarly in sensitivity analyses using multiple imputation instead of complete case analysis, and assessing the risk score using the whole patient population (see supplementary table 3).

The number and proportion of patients with or without COVID-19 diagnosis based on the risk score is shown in figure 3. 446 (15%) of patients had a score of <4, of whom 10.9% (49/446) were diagnosed with COVID-19. Using this threshold to identify patients *without* COVID-19 had a 26.6% sensitivity, but 96.6% specificity, with an 89.0% positive predictive value (PPV, table 4). 594 (20.2%) patients were above the high-risk threshold, set at a diagnostic risk score >9. At high COVID-19 prevalence (50%), this threshold had a good PPV (>90%), and at a low prevalence (<5%), had a high NPV. However, most patients fell in between both thresholds. Potential uses for such a clinical score are highlighted in supplementary table 4.

DISCUSSION

The key findings of this study are that SARS-CoV-2 RT-PCR negative COVID-19 is common amongst patients admitted to hospital, with real-life sensitivity of RT-PCR testing from NPS being 83% compared to a clinical reference standard of clinical diagnosis of COVID-19. Patients with RT-PCR negative COVID-19 had similar clinical characteristics to RT-PCR positive patients in this and other cohorts,[17] although significantly better outcomes (lower risk of mortality and ICU admission).[13,17] The proportion and number of COVID-19 admissions was increased during a three-week period from the 22nd March to 11th April 2020, and patients with

COVID-19 were substantially more unwell than patients without COVID-19, with implications for service delivery. Mortality in patients admitted without COVID-19 was also high at 6.4%.

The current gold standard diagnostic test for COVID-19, SARS-CoV-2 PCR from nasopharyngeal swabs, has several limitations which are challenging health systems and healthcare facilities management. We demonstrate, despite high analytical sensitivity, the real-life sensitivity of PCR is inadequate (around 80% at best).[18] Repeat testing of patients with an initial negative RT-PCR only increased yield by 3-5% within 48 hours. In addition to slow turnaround times, and resource and logistical challenges, there is an urgent need for alternative rapid and accurate methods to triage and stratify patient's risk of COVID-19, to allow appropriate infection control measures and safe patient flow to cohort areas or isolation rooms, without overwhelming hospital infrastructure. CT imaging of lungs can lack specificity for COVID-19, and rapid RT-PCR platforms are expensive and have inadequate throughput for future peaks of COVID-19.[19,20] Few studies have assessed pragmatic tools to assess risk of COVID-19 based on readily available clinical or laboratory variables.[21,22]

We found several clinical, radiological and laboratory blood factors that were associated with COVID-19. Our diagnostic score had moderate performance for discriminating COVID-19 from other diagnoses (AUROC curve 0.83). A low risk threshold had a good specificity and PPV, therefore could be used identify patients with a low COVID risk for transfer to a low-risk cohort area. Similarly, the high-risk score had a good PPV and specificity, therefore these patients could be managed as having COVID-19, and cared for in isolation rooms or cohorts if necessary. Those patients in neither high- nor low-risk group may benefit from rapid COVID-19 RT-PCR or antigen testing, depending on capacity. However, this score would need external

validation before use. Although derived from a cohort including unselected acute medical admissions, the higher prevalence of other respiratory viral pathogens may impact performance, especially specificity.[23] Furthermore, this score does not account for the vulnerability of individual patients for severe COVID-19 (eg based on age or comorbidities), which would also impact decisions on isolation and testing.[22]

This is the first study, to our knowledge, reporting lower ICU admissions and mortality in RT-PCR negative patients with COVID-19, despite similar markers of disease severity at admission (NEWS, CRP, oxygen saturations and requirement for supplementary oxygen), and in multivariable adjusted model. Interestingly, the median duration of symptoms was slightly longer, and median lymphocyte count was slightly higher in PCR-negative patients, suggesting they presented slightly later in their disease course, and therefore may be at a phase of illness with lower viral burden in the upper respiratory tract.[24–26] This may also be associated with their better prognosis. Other potential reasons for better outcomes in PCR-negative patients with COVID-19 include misclassification bias, where other respiratory conditions may have been classified as COVID-19. However, sensitivity analysis in patients with chest radiology suggestive of COVID-19 had similar findings, and a small number of misclassifications are unlikely to lead to such substantial differences in mortality. RT-PCR result may therefore be important in prognostic scores for COVID-19, especially as its association with mortality was independent of other key predictors such as age and sex. Patients with RT-PCR negative COVID-19 should also be included in treatment trials, and the efficacy of treatment could be analysed separately given their different outcomes.

During the study period, the overall number of daily admissions did not increase substantially. However, the proportion of admissions that were related to COVID-19 increased substantially in late March and early April, with a fall in non-COVID-19 admissions, as previously documented.[27] This has implications for planning for future COVID peaks. Another important finding was the high mortality in patients without COVID-19, an over two-fold increase from mortality in the previous year (2.4% compared to 6.4%).[27] Whilst we were unable to describe the causes of death amongst these patients, the increased mortality may result from late presentation to hospital due to national government-mandated 'lockdown' COVID-19 control measures and fear of nosocomial transmission risk. This has been previously documented in paediatric, cardiology, and oncology patients, but not amongst acute medical admissions.[28,29]

This study has several strengths. The cohort is in a large acute hospital trust with two sites covering a diverse population, and all consecutive medical admissions were included. This is one of the first large cohorts to report data on unselected acute medical admissions, and one of the largest cohorts of RT-PCR negative patients with COVID-19. There are also several limitations. The retrospective nature of the study has inherent limitations, including missing data. Although we included consecutive admitted patients, not all patients had SARS-CoV-2 testing, and two different RT-PCR assays were used which may have slightly different primer targets and analytical sensitivities, and may impact generalisability. The decision to repeat tests on patients with negative RT-PCR results was made by the responsible clinical team. The absence of serology or other confirmatory testing introduces a risk of misclassification bias and RT-PCR inclusion in the reference standard, and the influence of variables including in the diagnostic risk score on clinical diagnosis of COVID-19 introduces incorporation bias. However there remains no perfect reference standard for COVID-19 diagnosis and these biases are

unlikely to significantly impact our findings. Our diagnostic risk model needs external validation, only has moderate discrimination, and is at risk of overfitting. Systematic reviews have struggled to identify other diagnostic clinical scores with high discrimination, and effective patient management is likely to involve a combination of clinical features, radiology and rapid PCR-testing.[11]

In conclusion, we demonstrate that RT-PCR negative COVID-19 is common amongst patients admitted to hospital, and is associated with a better outcome despite similar severity at presentation. We derived and internally validated a diagnostic risk score with potential utility to help triage patients admitted from the emergency department, although prospective trials of different approaches are warranted in future peaks of COVID-19.

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Author contributions

AGW, CKM, TC, VP, GS, RT, NV, SD, AW, AM, MH and PP made substantial contribution to the conception of the work. AGW, CKM, AW, AM and PP made substantial contribution to the

design of the work. AGW, CKM, JB, SF, GS, JT, NG, HC, MH contributed to data acquisition. AGW and CKM analysed the data. AGW, CKM, AW, AM, PP contributed to data interpretation. AGW and CKM drafted the manuscript. All authors contributed to revising the manuscript critically for important intellectual content, approved the final manuscript and are accountable for all aspects of the work.

Data availability statement

Data are available upon reasonable request, subject to approval by the London North West University Healthcare NHS Trust Research and Governance Department and approval from relevant ethics and regulatory bodies.

Patient and Public Involvement Statement

Due to the retrospective nature of this study, undertaken during the COVID-19 pandemic, patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Competing interests statement

The authors have no competing interests to declare

		Not diagnosed with COVID	All COVID diagnoses	p-value	COVID diagnosis PCR negative	COVID diagnosis PCR positive	p-value
		n=2215	n=1793		n=283	n=1391	
Age at admission, median years (IQR)		71 (51, 82) (n=2215)	69 (56, 81) (n=1793)	0.44	70 (54, 79) (n=283)	70 (57, 81) (n=1391)	0.27
Age 65 years or older		1266 (57.2%)	1005 (56.1%)	0.48	154 (54.4%)	800 (57.5%)	0.34
Sex	Female	1021 (46.1%)	651 (36.3%)	<0.0001	112 (39.6%)	498 (35.8%)	0.23
	Male	1193 (53.9%)	1142 (63.7%)		171 (60.4%)	893 (64.2%)	
Ethnicity	South Asian	486 (21.9%)	447 (24.9%)	<0.0001	57 (20.1%)	362 (26.0%)	0.15
	Asian Other	174 (7.9%)	211 (11.8%)		30 (10.6%)	162 (11.6%)	
	Black African or Caribbean	212 (9.6%)	224 (12.5%)		33 (11.7%)	181 (13.0%)	
	Mixed Ethnicity	6 (0.3%)	10 (0.6%)		2 (0.7%)	8 (0.6%)	
	Unknown	330 (14.9%)	318 (17.7%)		53 (18.7%)	233 (16.8%)	
	White European	890 (40.2%)	458 (25.5%)		81 (28.6%)	361 (26.0%)	
	Other	117 (5.3%)	125 (7.0%)		27 (9.5%)	84 (6.0%)	
Index of Multiple Deprivation Decile, median (IQR)		5 (3, 7) (n=2105)	5 (3, 6) (n=1743)	0.048	4 (3, 6) (n=277)	5 (3, 6) (n=1366)	0.043
Diabetes		563 (25.7%)	599 (33.6%)	<0.0001	81 (28.9%)	482 (34.8%)	0.059
Hypertension		825 (37.7%)	739 (41.5%)	0.015	110 (39.3%)	590 (42.6%)	0.31

Ischaemic Heart Disease		413 (18.9%)	309 (17.3%)	0.21	44 (15.7%)	247 (17.8%)	0.40
Heart Failure		156 (7.1%)	70 (3.9%)	<0.0001	14 (5.0%)	53 (3.8%)	0.36
Chronic Obstructive Pulmonary Disease		185 (8.5%)	112 (6.3%)	0.010	21 (7.5%)	88 (6.3%)	0.48
Asthma		200 (9.1%)	165 (9.3%)	0.89	19 (6.8%)	133 (9.6%)	0.14
Cancer		169 (7.7%)	78 (4.4%)	<0.0001	11 (3.9%)	65 (4.7%)	0.58
HIV		21 (1.0%)	14 (0.8%)	0.56	3 (1.1%)	11 (0.8%)	0.64
Cerebrovascular Disease		110 (5.0%)	96 (5.4%)	0.61	15 (5.4%)	75 (5.4%)	0.97
Dementia		156 (7.1%)	188 (10.5%)	<0.0001	29 (10.4%)	153 (11.0%)	0.74
Chronic Kidney Disease		263 (12.0%)	233 (13.1%)	0.31	33 (11.8%)	182 (13.1%)	0.54
Cough		537 (24.5%)	1114 (62.5%)	<0.0001	177 (63.2%)	865 (62.4%)	0.80
Shortness of breath		687 (31.4%)	1171 (65.7%)	<0.0001	203 (72.5%)	886 (63.9%)	0.006
Fever		547 (25.0%)	1117 (62.7%)	<0.0001	184 (65.7%)	860 (62.0%)	0.25
Confusion		241 (11.0%)	195 (10.9%)	0.95	30 (10.7%)	153 (11.0%)	0.87
Symptom duration (days), median (IQR)		4 (2, 12) (n=592)	7 (3, 10) (n=1083)	0.010	7 (3, 12) (n=163)	6 (3, 10) (n=844)	0.021
Observations							

Pulse >120 bpm		203 (10.3%)	241 (14.3%)	<0.0001	41 (15.4%)	177 (13.4%)	0.39
Respiratory rate >30 per minute		175 (8.9%)	568 (33.6%)	<0.0001	90 (33.8%)	439 (33.3%)	0.87
Temperature >38°C		180 (9.2%)	605 (35.9%)	<0.0001	72 (27.0%)	495 (37.7%)	<0.001
Systolic Blood Pressure <100 mmHg		108 (5.5%)	101 (6.1%)	0.51	16 (6.1%)	78 (6.0%)	0.97
Consciousness level	Alert	646 (95.1%)	596 (96.0%)	0.93	101 (97.1%)	449 (95.5%)	0.47
	Confusion	13 (1.9%)	11 (1.8%)		3 (2.9%)	8 (1.7%)	
	Verbal	8 (1.2%)	5 (0.8%)		0 (0.0%)	4 (0.9%)	
	Pain	5 (0.7%)	3 (0.5%)		0 (0.0%)	3 (0.6%)	
	Unresponsive	7 (1.0%)	6 (1.0%)		0 (0.0%)	6 (1.3%)	
O_2 saturations <94%		198 (10.1%)	543 (32.2%)	<0.0001	79 (29.8%)	430 (32.6%)	0.37
NEWS 2 Score, median (IQR)		2 (1, 4) (n=1951)	6 (3, 8) (n=1666)	<0.0001	6 (4, 7) (n=264)	6 (3, 8) (n=1299)	0.73
NEWS 2 Score ≥5		477 (24.4%)	1084 (65.1%)	<0.0001	176 (66.7%)	840 (64.7%)	0.53
Supplementary oxygen		169 (8.8%)	529 (33.1%)	<0.0001	96 (37.9%)	404 (32.4%)	0.091
PO_2 <8 mmHg		127 (35.4%)	251 (36.2%)	0.79	34 (27.9%)	205 (38.7%)	0.025
PCO_2 >6 mmHg		124 (34.5%)	75 (10.8%)	<0.0001	12 (9.8%)	59 (11.1%)	0.68

Neutrophils >10 x10^9/L		361 (17.8%)	250 (15.6%)	0.083	52 (19.0%)	183 (14.7%)	0.078
Lymphocytes <1 x10^9/L		509 (25.1%)	736 (46.1%)	<0.0001	107 (39.1%)	594 (47.8%)	0.009
Platelet count x10^9/L, median (IQR)		246.0 (193.0, 317.0) (n=2025)	231.0 (177.0, 306.0) (n=1597)	<0.0001	263.0 (206.0, 343.0) (n=274)	226.0 (172.0, 297.0) (n=1242)	<0.0001
Creatinine >120 mmol/L		507 (25.2%)	426 (26.9%)	0.24	64 (23.8%)	338 (27.4%)	0.23
CRP µg/mL, median (IQR)		16.1 (3.4, 66.9) (n=1928)	98.7 (46.0, 175.3) (n=1590)	<0.0001	86.2 (41.7, 170.1) (n=272)	101.5 (48.3, 180.2) (n=1237)	0.15
Influenza RT-PCR	Influenza A	11 (2.3%) (n=490)	1 (0.2%) (n=528)	<0.0001	0 (n=72)	1 (0.2%) (n=445)	0.31
	Influenza B	9 (1.9%)	2 (0.4%)		1 (1.4%)	1 (0.2%)	
Admitted to ICU		21 (1.0%)	227 (12.7%)	<0.0001	22 (7.8%)	192 (13.8%)	0.006
Died during hospital admission		142 (6.4%) (n=2,202)	482 (26.9%) (n=1,789)	<0.0001	47 (16.6%)	408 (29.3%) (n=1,387)	<0.0001

Table 1. Baseline characteristics and outcomes for patients, including demographics, co-morbidities, admission vital signs and laboratory blood tests, stratified by diagnosis and SARS- CoV-2 RT-PCR status. Data on co-morbidities represents number with each condition. Where data are missing, total numbers in each category are presented in brackets. P-values are calculated using chi-squared for proportions, t-tests for means and Wilcoxon rank sum for medians. CRP C-reactive Protein, IQR inter quartile range. NEWS National Early Warning Score. PO2 partial pressure of oxygen, PCO2 partial pressure of carbon dioxide.

			Univariable Regression			Multivariable regression	
Variable		N	Odds Ratio (95% CI)	p		Odds Ratio (95% CI)	p
Age	increase 10 years	4,008	1.05 (1 - 1.08)	0.015			
	50-70	4,008	1.62 (1.4 - 1.86)	<0.0001		1.7 (1.4 - 2.08)	<0.0001
Sex	Male	4,008	1.5 (1.3 - 1.71)	<0.0001		1.26 (1.1 - 1.52)	<0.0001
IMD Decile		3,848	0.97 (0.9 - 1)	0.013			
Diabetes		3,971	1.46 (1.3 - 1.68)	<0.0001			
Hypertension		3,971	1.17 (1 - 1.33)	0.007			
Ethnicity		4,008					
	White	1,348	1	<0.0001		1	<0.0001

	Asian	1,318	1.94 (1.7 - 2.26)			1.82 (1.5 - 2.27)	
	Black	436	2.05 (1.6 - 2.56)			1.85 (1.4 - 2.53)	
	Mixed/ Other	258	2.13 (1.6 - 2.79)			2.25 (1.5 - 3.33)	
	Unknown	648	1.87 (1.5 - 2.27)			1.77 (1.3 - 2.34)	
Symptoms		3,971					
	Cough		5.13 (4.5 - 5.88)	<0.0001			
	Shortness of breath		4.19 (3.7 - 4.79)	<0.0001			
	Fever		5.04 (4.4 - 5.78)	<0.0001			
Respiratory rate	Any of above	4,008	6.29 (5.4 - 7.36)	<0.0001		3.11 (2.5 - 3.85)	<0.0001
Oxygen saturations		3,654	1.14 (1.1 - 1.15)	<0.0001			
NEWS Score	Continuous (linear)	3,647	0.89 (0.9 - 0.9)	<0.0001			
	Continuous (linear)	3,617	1.39 (1.3 - 1.42)	<0.0001			
CRP	>5		5.76 (5 - 6.65)	<0.0001		2.39 (2 - 2.87)	<0.0001

	every 10 increase	3,518	1.01 (1 - 1.01)	<0.0001			
Lymphocytes	>50		5.99 (5.2 - 6.93)	<0.0001		3.11 (2.6 - 3.75)	<0.0001
	Continuous (linear)	3,624	0.66 (0.6 - 0.72)	<0.0001			
Chest x-ray	<1		2.54 (2.2 - 2.93)	<0.0001		1.72 (1.4 - 2.08)	<0.0001
		3,581					
	Normal	718	1	<0.0001		1	<0.0001
	lung infiltrates	2,262	7.79 (6.3 - 9.65)			3.75 (2.9 - 4.91)	
	other abnormality	601	3.56 (2.8 - 4.6)			1.94 (1.4 - 2.68)	
	CVCX0	424	1	<0.0001			
	CVCX1	1,040	25.85 (18.7 - 35.66)				
	CVCX2	435	2.98 (2.3 - 3.93)				
	CVCX3	129	1.64 (1.1 - 2.44)				

Table 2. Univariable and multivariable logistic regression analysis for risk of COVID-19 diagnosis. P-values calculated using likelihood ratio tests. There was no evidence of interaction between variables in the final multivariable model. N=2,490 for multivariable model. CVCX represents British Society of Thoracic Imaging (BSTI) classification of chest x-ray. CRP C-reactive Protein

Variable		Coefficient	Standard error	Diagnsotic score points
Age	50-70	0.53 (0 - 0.41)	0.09	1
Sex	Male	0.23 (0.3 - 0.73)	0.10	1
Ethnicity	Asian	0.6 (0.4 - 0.82)	0.11	1
	Black	0.62 (0.3 - 0.93)	0.16	1
	Mixed/Other	0.81 (0.4 - 1.2)	0.20	1
	Unknown	0.57 (0.3 - 0.85)	0.14	1
Cough, fever or shortness of breath		1.13 (0.9 - 1.35)	0.11	2
NEWS2 Score	>5	0.87 (0.7 - 1.05)	0.09	2
CRP	>50	1.13 (1 - 1.32)	0.09	2
Lymphocytes	<1	0.54 (0.4 - 0.73)	0.10	1
Chest x-ray	lung infiltrates	1.32 (1.1 - 1.59)	0.14	2
	other abnormality	0.66 (0.3 - 0.98)	0.16	1

Table 3. Multivariable logistic regression diagnostic model for COVID-19, with regression (β) co-efficients and diagnostic score points. The constant (intercept) was -4.0 (95% cl -4.4 to -3.6). N= 2,940.

		Prevalence				
Low-risk diagnostic score threshold (<4)	Study population	0.5	0.2	0.1	0.05	0.01
Sensitivity	26.6%	-	-	-	-	-
Specificity	96.6%	-	-	-	-	-
PPV	89.0%	88.7%	66.2%	46.6%	29.2%	7.3%
NPV	56.0%	56.8%	84.0%	92.2%	96.2%	99.2%

High-risk diagnostic score threshold (>9)						
Sensitivity	37.0%	-	-	-	-	-
Specificity	96.1%	-	-	-	-	-
PPV	90.1%	90.4%	70.1%	51.0%	33.0%	8.6%
NPV	61.2%	60.4%	85.9%	93.2%	96.7%	99.3%

Table 4. Diagnostic performance of a low COVID-19 risk threshold (less than 4 points on the diagnostic score) and high-risk threshold (greater than 9 points). Low-risk threshold diagnostic accuracy is for identifying patients without COVID-19, whereas high-risk threshold is for identifying patients with COVID-19

Figure 1. Patient flow diagram by final diagnosis and SARS-CoV-2 RT-PCR status with outcomes. Note 'presumed COVID' includes patients who were RT-PCR negative (n=293) and those who did not have a valid RT-PCR results (n=109)

Figure 2. (A) Receiver operating characteristic curve for the full diagnostic predictive model. Area under the curve (AUC) 0.839 (95%CI 0.824-0.853), N=2,940. (B) Calibration plot showing observed compared to predicted risk of COVID-19 diagnosis as deciles, with 95% confidence interval. The dashed green line shows perfect calibration. (C) Decision curve analysis showing standardised net benefit at different threshold probabilities for diagnosing patients with COVID-19 , comparing diagnosing all patients as COVID-19 (blue solid line), diagnosing no patients with COVID-19 (solid red line), and various diagnostic risk models, including the COVID diagnostic score (full model and simplified risk score), C-reactive protein over 50, and National Early Warning Score of 5 or more. CRP C-reactive Protein, NEWS National Early Warning Score

Figure 3. (A) Overlaid histogram of COVID diagnostic risk score and number of patients with COVID-19 (white) and alternative (not COVID-19) diagnoses. (B) Proportion (%) of patients with COVID-19 (orange) or alternative (not COVID-19, blue) diagnoses by COVID diagnostic risk score. N=2,940

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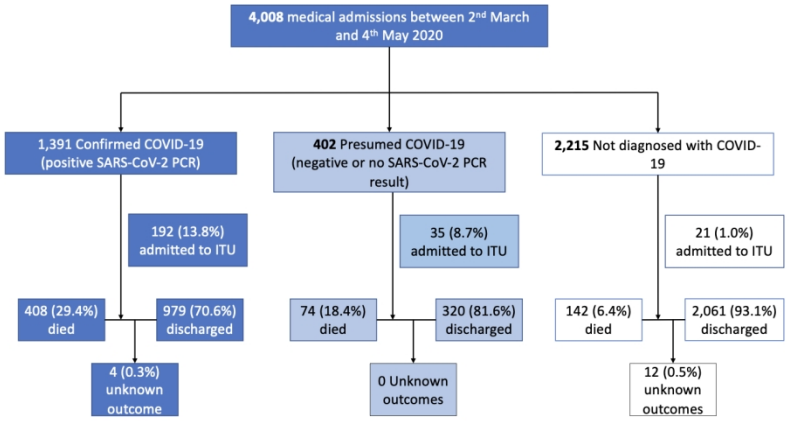

Figure 1

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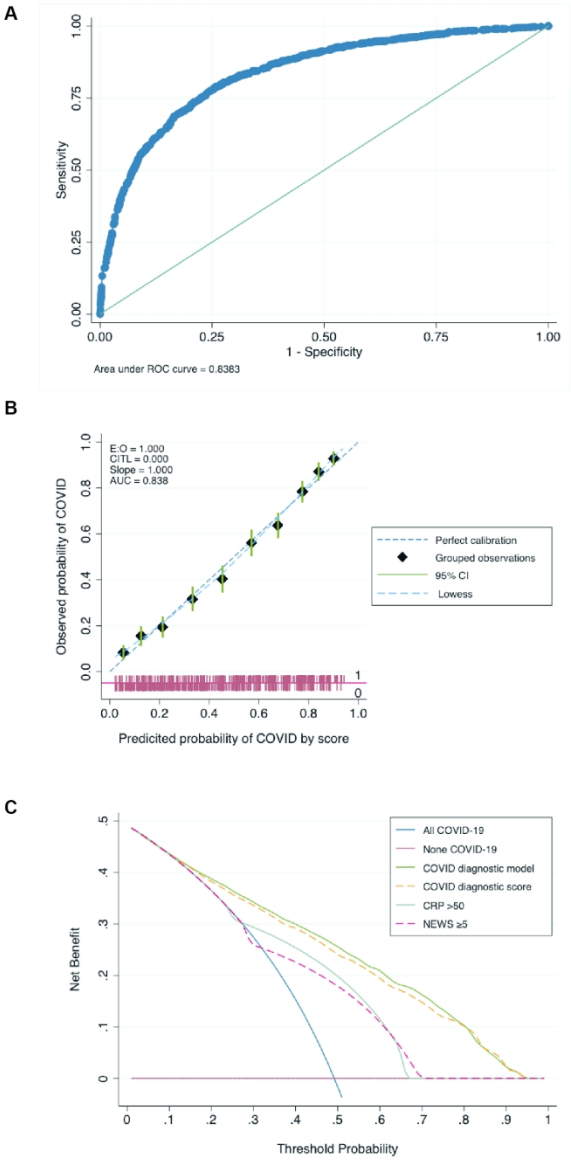

A
Sensitivity
1 - Specificity
Area under ROC curve = 0.8383
B
E:O = 1.000
CITL = 0.000
Slope = 1.000
AUC = 0.838
Observed probability of COVID
Predicited probability of COVID by score
Perfect calibration
Grouped observations
95% CI
Lowess
C
Net Benefit
Threshold Probability
All COVID-19
None COVID-19
COVID diagnostic model
COVID diagnostic score
CRP >50
NEWS ≥5

Figure 2

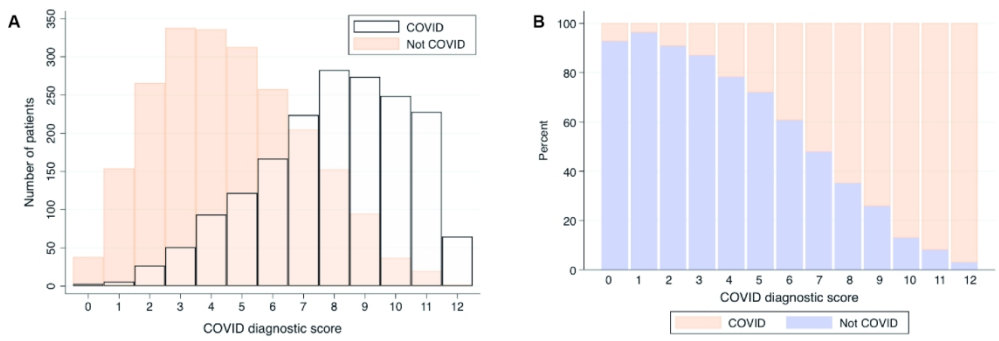

A
Number of patients
0
50
100
150
200
250
300
350
COVID
Not COVID
0 1 2 3 4 5 6 7 8 9 10 11 12
COVID diagnostic score
B
Percent
0
20
40
60
80
100
0 1 2 3 4 5 6 7 8 9 10 11 12
COVID diagnostic score
COVID
Not COVID

Figure 3

Supplementary Appendix- False-negative RT-PCR for COVID-19 and a diagnostic risk score: a retrospective cohort study among patients admitted to hospital

		Not diagnosed with COVID	All COVID diagnoses	p-value	COVID negative PCR	COVID diagnosis PCR positive	p-value
		n=2215	n=1793		n=283	n=1391	
Symptoms							
Cough		537 (24.5%)	1114 (62.5%)	<0.001	177 (63.2%)	865 (62.4%)	0.80
Chest pain		335 (15.3%)	109 (6.1%)	<0.001	23 (8.2%)	80 (5.8%)	0.12
Diarrhoea		152 (6.9%)	131 (7.4%)	0.62	25 (8.9%)	96 (6.9%)	0.24
Fall		277 (12.7%)	166 (9.3%)	<0.001	24 (8.6%)	129 (9.3%)	0.70
Symptom duration (days), median (IQR)		4 (2, 12) (n=592)	7 (3, 10) (n=1083)	0.010	7 (3, 12) (n=163)	6 (3, 10) (n=844)	0.021
Observations							
Pulse, median (IQR)		89 (75, 106) (n=1964)	96 (83, 110) (n=1689)	<0.001	98 (85, 110) (n=266)	96 (83, 110) (n=1319)	0.050
Pulse >120 bpm		203 (10.3%)	241 (14.3%)	<0.001	41 (15.4%)	177 (13.4%)	0.39
Respiratory rate per minute, median (IQR)		20 (18, 23) (n=1966)	26 (21, 32) (n=1688)	<0.001	26 (22, 32) (n=266)	26 (20, 32) (n=1318)	0.59
Respiratory rate >30 per minute		175 (8.9%)	568 (33.6%)	<0.001	90 (33.8%)	439 (33.3%)	0.87
Temperature °C, median (IQR)		36.7 (36.4, 37.1) (n=1961)	37.5 (36.8, 38.4) (n=1684)	<0.001	37.3 (36.7, 38) (n=267)	37.5 (36.8, 38.4) (n=1313)	0.007
Temperature >38°C		180 (9.2%)	605 (35.9%)	<0.001	72 (27.0%)	495 (37.7%)	<0.001
Systolic Blood Pressure mmHg, median (IQR)		136 (119, 154) (n=1948)	132 (117, 147) (n=1666)	<0.001	131 (118, 146.5) (n=264)	132 (117, 148) (n=1299)	0.88
Systolic Blood Pressure mmHg <100		108 (5.5%)	101 (6.1%)	0.51	16 (6.1%)	78 (6.0%)	0.97
O_2 saturations %, median (IQR)		97 (96, 99) (n=1961)	96 (92, 97) (n=1686)	<0.001	95 (93, 98) (n=265)	96 (92, 97) (n=1317)	0.55
O_2 saturations <94%		198 (10.1%)	543 (32.2%)	<0.001	79 (29.8%)	430 (32.6%)	0.37
NEWS 2 Score, median (IQR)		2 (1, 4) (n=1951)	6 (3, 8) (n=1666)	<0.001	6 (4, 7) (n=264)	6 (3, 8) (n=1299)	0.73
NEWS 2 Score ≥5		477 (24.4%)	1084 (65.1%)	<0.001	176 (66.7%)	840 (64.7%)	0.53
Supplementary oxygen	Yes	169 (8.8%)	529 (33.1%)	<0.001	96 (37.9%)	404 (32.4%)	0.091

Blood gas and pathology							
PO_2 (KPa), median (IQR)		8.8 (7.3, 11.1) (n=359)	8.7 (7.4, 10.7) (n=693)	0.51	9.1 (7.7, 10.6) (n=122)	8.5 (7.3, 10.7) (n=530)	0.18
PO_2 <8 v		127 (35.4%)	251 (36.2%)	0.79	34 (27.9%)	205 (38.7%)	0.025
pCO_2 (KPa), median (IQR)		5.2 (4.4, 6.7) (n=359)	4.6 (4.1, 5.2) (n=693)	<0.001	4.6 (4.1, 5.2) (n=122)	4.6 (4.1, 5.2) (n=530)	0.83
pCO_2 >6		124 (34.5%)	75 (10.8%)	<0.001	12 (9.8%)	59 (11.1%)	0.68
Haemoglobin (g/L), mean (SD)		121.7 (23.2) (n=2026)	124.4 (21.1) (n=1598)	<0.001	122.2 (21.0) (n=274)	124.6 (20.9) (n=1243)	0.085
Neutrophil count (x10^9/L), median (IQR)		5.9 (4.1, 8.6) (n=2026)	5.8 (4.0, 8.3) (n=1598)	0.20	6.7 (4.5, 9.1) (n=274)	5.6 (3.9, 8.0) (n=1243)	<0.001
Neutrophils >10 x10^9/L		361 (17.8%)	250 (15.6%)	0.083	52 (19.0%)	183 (14.7%)	0.078
Lymphocyte count (x10^9/L), median (IQR)		1.4 (0.9, 2.0) (n=2026)	1.0 (0.7, 1.4) (n=1598)	<0.001	1.1 (0.8, 1.4) (n=274)	1.0 (0.7, 1.4) (n=1243)	0.013
Lymphocytes <1 x10^9/L		509 (25.1%)	736 (46.1%)	<0.001	107 (39.1%)	594 (47.8%)	0.009
Platelet count (x10^9/L), median (IQR)		246.0 (193.0, 317.0) (n=2025)	231.0 (177.0, 306.0) (n=1597)	<0.001	263.0 (206.0, 343.0) (n=274)	226.0 (172.0, 297.0) (n=1242)	<0.001
Platelets <100 x10^9/L		80 (4.0%)	62 (3.9%)	0.92	11 (4.0%)	50 (4.0%)	0.99
ALT, median (IQR)		22.0 (15.0, 36.0) (n=1755)	31.0 (18.0, 51.0) (n=1412)	<0.001	31.0 (18.0, 55.0) (n=245)	30.0 (19.0, 51.0) (n=1096)	0.71
Creatinine (mmol/L), median (IQR)		84.0 (65.0, 121.0) (n=2011)	86.0 (67.0, 124.0) (n=1582)	0.057	80.0 (65.0, 117.0) (n=269)	87.0 (68.0, 127.0) (n=1235)	0.011
Creatinine >120 mmol/L		507 (25.2%)	426 (26.9%)	0.24	64 (23.8%)	338 (27.4%)	0.23
Urea (mmol/L), median (IQR)		6.0 (4.0, 9.8) (n=2025)	6.1 (4.0, 10.6) (n=1584)	0.58	5.5 (3.8, 8.9) (n=270)	6.4 (4.1, 11.0) (n=1236)	0.007
CRP µg/mL, median (IQR)		16.1 (3.4, 66.9) (n=1928)	98.7 (46.0, 175.3) (n=1590)	<0.001	86.2 (41.7, 170.1) (n=272)	101.5 (48.3, 180.2) (n=1237)	0.15
CRP >50 µg/mL		599 (31.1%)	1160 (73.0%)	<0.001	191 (70.2%)	917 (74.1%)	0.19
Glucose (mmol/L), median (IQR)		6.6 (5.6, 8.5) (n=1182)	7.1 (5.9, 9.3) (n=910)	<0.001	6.7 (5.9, 9.1) (n=147)	7.1 (5.9, 9.3) (n=710)	0.49
Lactate >2 mmol/L		41 (3.5%)	30 (3.3%)	0.83	5 (3.4%)	21 (3.0%)	0.78

Supplementary Table 1. Baseline characteristics for patients, including co-morbidities, admission vital signs and laboratory blood tests, stratified by diagnosis and SARS- CoV-2 RT-PCR status. Data on com-morbidities represents number with each condition. Where data are missing, numbers in each category are presented. P-values are calculated using chi-squared for proportions, t-tests for means and Wilcoxon rank sum for medians. CRP C-reactive Protein, IQR inter quartile range. NEWS National Early Warning Score. PO2 partial pressure of oxygen, PCO2 partial pressure of carbon dioxide.

Variable		Odds ratio (95% CI)	P-value
COVID-19 RT-PCR negative		0.41 (0.3 - 0.6)	<0.0001
Age, years		1.06 (1.0 - 1.1)	<0.0001
Sex	Female	0.90 (0.7 - 1.2)	0.446
Co-morbidities	1	1.13 (0.8 - 1.7)	0.552
	2 or more	1.45 (1.0 - 2.1)	0.042
CRP		1.00 (1.0 – 1.0)	<0.0001
Oxygen Saturations <94%		1.41 (1.1 - 1.9)	0.016
Urea		1.04 (1 - 1.1)	<0.0001

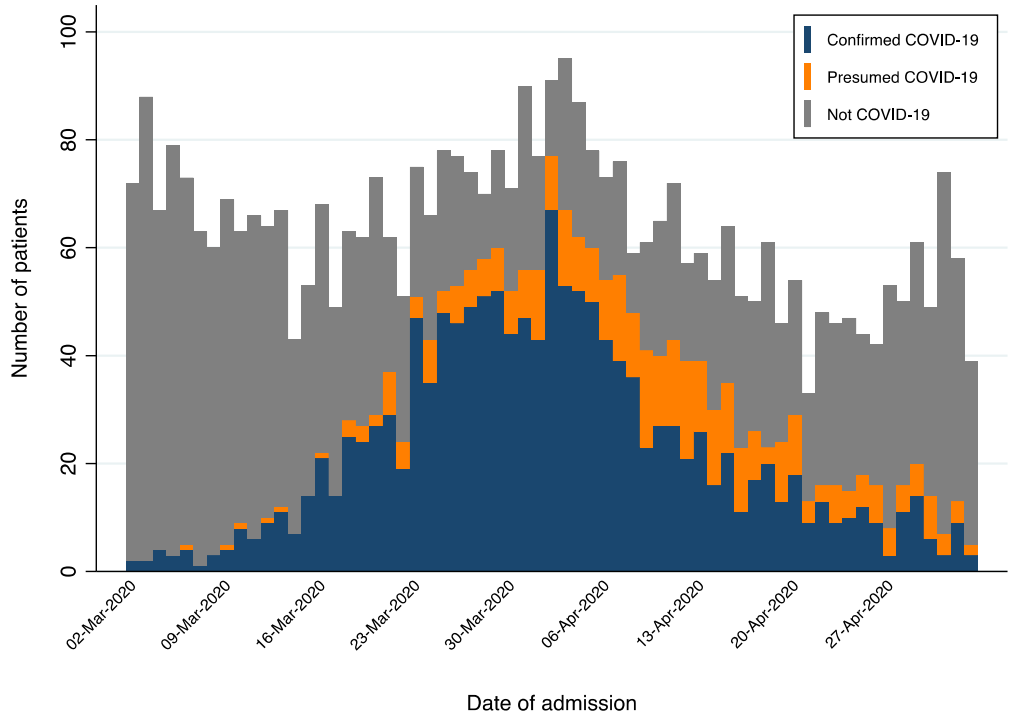
Supplementary Table 2. Multivariable logistic regression model assessing association between COVID-19 PCR-status and mortality, adjusting for other variables known to be risk-factors for mortality in COVID-19. Continuous variables modelled as linear. No interactions in the final model. P-vales calculated by likelihood ratio tests. N= 1,414.

Variable		ß-Coefficient	Odds ratio (95% CI)	Diagnostic score points
Age	50-70	0.4 (0.2 - 0.6)	1.5 (1.2-1.8)	1
Sex	Male	0.2 (0.0 - 0.3)	1.2 (1.0-1.4)	1
Ethnicity	Asian	0.6 (0.4 - 0.8)	1.8 (1.4-2.1)	1
	Black	0.6 (0.4 - 0.9)	1.9 (1.4-2.5)	1
	Mixed/Other	0.8 (0.4 - 1.1)	2.2 (1.5-3.1)	1
	Unknown	0.5 (0.3 - 0.8)	1.7 (1.3-2.2)	1
Cough, fever or shortness of breath		1.3 (1.2 - 1.5)	3.8 (3.2-4.5)	2
NEWS2 Score	>5	0.9 (0.7 - 1.1)	2.4 (2.0-2.9)	2
CRP	>50	1.1 (1.0 - 1.3)	3.0 (2.6-3.7)	2
Lymphocytes	<1	0.6 (0.4 - 0.8)	1.8 (1.5 – 2.2)	1
Chest x-ray	lung infiltrates	1.3 (1.0 - 1.5)	3.6 (2.8 -4.5)	2
	other abnormality	0.7 (0.4 - 0.9)	1.9 (1.4-2.6)	1

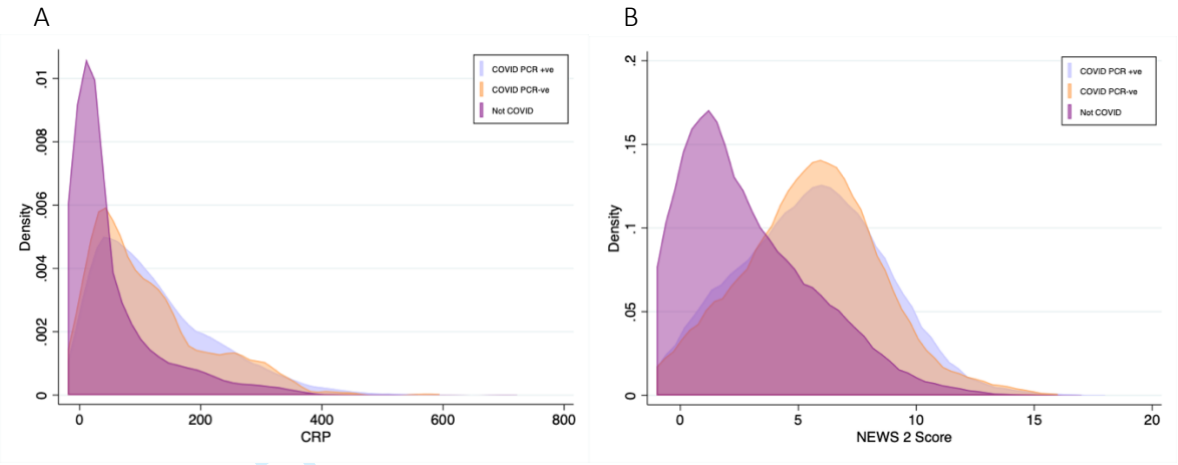
Supplementary Table 3. Logistic regression multivariable model for COVID-19 diagnosis using multivariate multiple imputation using chained equations for missing data in candidate predictor variables, with odds ratio and ß co-efficents. N=3,968. Area under the receiver operator curve (ROC) = 0.86 (95% CI 0.84 – 0.87).

COVID status based on diagnostic risk score (proportion of patients expected during 'peak')	Management
Low risk, COVID-19 diagnostic risk score <4	• Alternative diagnosis most likely • Rapid RT-PCR or antigen test, if negative send to 'COVID-negative' area
Medium risk, COVID-19 diagnostic score 4-9	• Uncertain if COVID-19 is cause for presentation • Will need further testing to determine COVID-19 diagnosis • Either test with Rapid RT-PCR or antigen test, or consider CT imaging, or standard COVID-19 RT-PCR testing and move to isolation in
High risk, COVID-19 diagnostic score >9	• COVID-19 most likely • Isolate patient in COVID-19 area or isolation room and standard COVID-19 RT-PCR testing

Supplementary Table 4. Potential application of COVID-19 diagnostic risk score



Supplementary Figure 1. Number of patients admitted and final diagnosis by date of admission. Confirmed COVID-19 is patients with a positive SARS-CoV-2 PCR from nasopharyngeal swab, presumed COVID-19 is patients without a positive SARS-CoV-2 PCR but a discharge diagnosis of COVID-19. Not COVID-19 are patients without a positive SARS-CoV-2 PCR and an alternative diagnosis. N=4008.



Supplementary Figure 2. Distribution of (A) C-reactive protein (N=3518) and (B) National Early Warning Score (NEWS) (N=3889) by diagnosis at the time of hospital admission.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4-5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4-5*
Bias	9	Describe any efforts to address potential sources of bias	5-6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5-6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	5-6

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1/page 7
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1, page 7
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	Report numbers of outcome events or summary measures over time	7, figure S2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-8
		(*b*) Report category boundaries when continuous variables were categorized	7-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7-8
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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TRIPOD Checklist: Prediction Model Development and Validation

Section/Topic	Item		Checklist Item	Page
Title and abstract				
Title	1	D;V	Identify the study as developing and/or validating a multivariable prediction model, the target population, and the outcome to be predicted.	1
Abstract	2	D;V	Provide a summary of objectives, study design, setting, participants, sample size, predictors, outcome, statistical analysis, results, and conclusions.	2
Introduction				
Background and objectives	3a	D;V	Explain the medical context (including whether diagnostic or prognostic) and rationale for developing or validating the multivariable prediction model, including references to existing models.	3
	3b	D;V	Specify the objectives, including whether the study describes the development or validation of the model or both.	3
Methods				
Source of data	4a	D;V	Describe the study design or source of data (e.g., randomized trial, cohort, or registry data), separately for the development and validation data sets, if applicable.	4
	4b	D;V	Specify the key study dates, including start of accrual; end of accrual; and, if applicable, end of follow-up.	4
Participants	5a	D;V	Specify key elements of the study setting (e.g., primary care, secondary care, general population) including number and location of centres.	4
	5b	D;V	Describe eligibility criteria for participants.	4
	5c	D;V	Give details of treatments received, if relevant.	NA
Outcome	6a	D;V	Clearly define the outcome that is predicted by the prediction model, including how and when assessed.	6
	6b	D;V	Report any actions to blind assessment of the outcome to be predicted.	NA
Predictors	7a	D;V	Clearly define all predictors used in developing or validating the multivariable prediction model, including how and when they were measured.	6
	7b	D;V	Report any actions to blind assessment of predictors for the outcome and other predictors.	NA
Sample size	8	D;V	Explain how the study size was arrived at.	6
Missing data	9	D;V	Describe how missing data were handled (e.g., complete-case analysis, single imputation, multiple imputation) with details of any imputation method.	6-7
Statistical analysis methods	10a	D	Describe how predictors were handled in the analyses.	6
	10b	D	Specify type of model, all model-building procedures (including any predictor selection), and method for internal validation.	6
	10c	V	For validation, describe how the predictions were calculated.	6-7
	10d	D;V	Specify all measures used to assess model performance and, if relevant, to compare multiple models.	6
	10e	V	Describe any model updating (e.g., recalibration) arising from the validation, if done.	NA
Risk groups	11	D;V	Provide details on how risk groups were created, if done.	6-7
Development vs. validation	12	V	For validation, identify any differences from the development data in setting, eligibility criteria, outcome, and predictors.	NA
Results				
Participants	13a	D;V	Describe the flow of participants through the study, including the number of participants with and without the outcome and, if applicable, a summary of the follow-up time. A diagram may be helpful.	Figure 1, page 7
	13b	D;V	Describe the characteristics of the participants (basic demographics, clinical features, available predictors), including the number of participants with missing data for predictors and outcome.	Table 1
	13c	V	For validation, show a comparison with the development data of the distribution of important variables (demographics, predictors and outcome).	NA
Model development	14a	D	Specify the number of participants and outcome events in each analysis.	7
	14b	D	If done, report the unadjusted association between each candidate predictor and outcome.	Table 2
Model specification	15a	D	Present the full prediction model to allow predictions for individuals (i.e., all regression coefficients, and model intercept or baseline survival at a given time point).	Table 3
	15b	D	Explain how to the use the prediction model.	9
Model performance	16	D;V	Report performance measures (with CIs) for the prediction model.	9, supplement
Model-updating	17	V	If done, report the results from any model updating (i.e., model specification, model performance).	NA
Discussion				
Limitations	18	D;V	Discuss any limitations of the study (such as nonrepresentative sample, few events per predictor, missing data).	10-11
Interpretation	19a	V	For validation, discuss the results with reference to performance in the development data, and any other validation data.	10-11
	19b	D;V	Give an overall interpretation of the results, considering objectives, limitations, results from similar studies, and other relevant evidence.	10-11
Implications	20	D;V	Discuss the potential clinical use of the model and implications for future research.	10-11, table S5
Other information				
Supplementary information	21	D;V	Provide information about the availability of supplementary resources, such as study protocol, Web calculator, and data sets.	Supplement

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TRIPOD Checklist: Prediction Model Development and Validation

				ary appen dix
Funding	22	D;V	Give the source of funding and the role of the funders for the present study.	12

*Items relevant only to the development of a prediction model are denoted by D, items relating solely to a validation of a prediction model are denoted by V, and items relating to both are denoted D;V. We recommend using the TRIPOD Checklist in conjunction with the TRIPOD Explanation and Elaboration document.