Virological and serological characterization of critically ill patients with COVID-19 in the UK: Interactions of viral load, antibody status and B.1.1.7 variant infection

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40 word summary: Patients enrolled in a trial for convalescent plasma treatment were highly heterogeneous in terms of viral loads, infecting SARS-CoV-2 types and antibody status, each potentially influencing treatment outcomes. B.1.1.7 infections were associated with higher viral loads and reduced clearance post-seroconversion.

FOOTNOTE PAGE

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ABSTRACT

Background. Convalescent plasma containing neutralising antibody to SARS-CoV-2 is under investigation for COVID-19 treatment. We report diverse virological characteristics of UK intensive care patients enrolled in the Immunoglobulin Domain of the REMAP-CAP randomised controlled trial that potentially influence treatment outcomes.

Methods. SARS-CoV-2 RNA in nasopharyngeal swabs collected pre-treatment was quantified by PCR. Antibody status was determined by spike-protein ELISA. B.1.1.7 was differentiated from other SARS-CoV-2 strains using allele-specific probes or restriction site polymorphism (*Sfc*I) targeting D1118H.

Results. Of 1274 subjects, 90% were PCR-positive with viral loads 118-1.7x10^{11} IU/ml. Median viral loads were 40-fold higher in those seronegative for IgG antibodies (n=354; 28%) compared to seropositives (n=939; 72%). Frequencies of B.1.1.7 increased from <1% in early November, 2020 to 82% of subjects in January 2021. Seronegative individuals with wild-type SARS-CoV-2 had significantly higher viral loads than seropositives (medians 5.8x10^{6} and 2.0 x10^{5} IU/ml respectively; p=2x10^{-15}). However, viral load distributions were elevated in both seronegative and seropositive subjects infected with B.1.1.7 (4.0x10^{6} and 1.6x10^{6} IU/ml respectively).

Conclusions. High viral loads in seropositive B.1.1.7-infected subjects and resistance to seroconversion indicate less effective clearance by innate and adaptive immune responses. SARS-CoV-2 strain, viral loads and antibody status define subgroups for analysis of treatment efficacy.

Key words: SARS-CoV-2 COVID-19 Coronavirus Polymerase Chain Reaction Diagnostics Variant of Concern Clade B.1.1.7 ELISA Viral load Randomised clinical trial Convalescent plasma

INTRODUCTION

The catastrophic zoonotic emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the human population in China at the end of 2019, and its subsequent pandemic spread has caused global devastation[1]. To date, only limited treatment regimens exist for COVID-19 patients [2-4], and their management is primarily supportive, with case-fatality rates remaining at 1% - 2% in Western countries including the UK [5]. In the search of alternative methods to treat COVID-19, convalescent plasma therapy boosting levels of neutralising antibody has been considered as a potential means to reduce morbidity and mortality [6, 7], particularly if given in early stages of infection [8]. RECOVERY and REMAP-CAP are randomized controlled trials investigating the efficacy of convalescent plasma therapy conducted in the UK with REMAP-CAP also recruiting participants globally [8, 9]. In the REMAP-CAP trial [9] patients with severe COVID-19, restricted to those in intensive care units (ICUs), are transfused with one to two plasma units collected from donors with previous documented SARS-CoV-2 infection and confirmed to have high plasma titres of neutralising antibody [10].

While final statistical analysis is awaited, both trials have been closed as an initial analysis did not show a significant benefit of treatment across the overall study population in terms of either COVID-19 associated mortality or number of organ-support free days [11, 12]. However, analysis of the data will need to account for patient variables that may influence treatment efficacy, and address patient outcomes other than death. For example, the potential efficacy of convalescent plasma therapy may be influenced by disease stage at trial enrolment. Severe COVID-19 disease may be driven by either the direct effects of virus replication in the respiratory tract, or secondarily from damage associated with the often intense inflammatory antiviral response that usually leads to clearance of SARS-CoV-2. Treatment with neutralising antibody may primarily influence outcomes during the early stages of active virus replication-

induced disease but could be less relevant in those patients who have already seroconverted for anti-SARS-CoV-2 antibody. Therefore, we have determined the pre-treatment viral loads in respiratory samples and serological status of study subjects.

Another variable influencing treatment outcomes may originate from effects of strain variation of SARS-CoV-2. A variant of SARS-CoV-2 with a D614G mutation in the spike protein has been described [13, 14] along with more recent mutants such as B.1.1.7 in the UK, B.1.351 in South Africa [15] and P.1 in Brazil [16], all potentially more transmissible [17-19]; furthermore, B.1.1.7 may result in a higher mortality than the original pandemic strain [20, 21]. As regards B.1.351 (South Africa), clustered amino acid changes in the spike protein gene may render this mutant partially antigenically distinct from the prototype strain [22]. These virological differences, including the possibility of antigenic escape from neutralising antibodies in convalescent plasma collected from individuals with earlier strains of SARS-CoV-2, may individually or collectively affect the efficacy of convalescent plasma therapy. To identify SARS-CoV-2 strains, we developed a real-time PCR targeting the D1118H polymorphism in the spike gene that is characteristic of the B.1.1.7 strain. This assay was found to be faster and more effective than high throughput sequencing for low viral load samples. A simpler agarose gel-based method was also developed and evaluated to enable B.1.1.7 identification in resource-limited settings.

We report substantial variability in pre-treatment viral loads, variability in serological status and an increasing detection of B.1.1.7 during the enrolment period of UK participants in the international REMAP-CAP trial [9]. Measurement of these variables will be of considerable value in analysing effects of convalescent plasma therapy and potentially identifying sub-groups of patients for whom this treatment may be effective.

MATERIALS AND METHODS

Patient Recruitment and Sample Collection. All subjects were enrolled in the UK and had a laboratory confirmed diagnosis of SARS-CoV-2 infection with concomitant severe pneumonia requiring ICU admission. Patients were not eligible if more than 48 hours had elapsed since their ICU admission, if they had already received treatment with any other non-trial prescribed antibody therapy (monoclonal antibody, hyperimmune immunoglobulin, or convalescent plasma) or if more than 14 days had elapsed since hospital admission. A total of 122 hospitals have recruited patients in this trial in the UK [23]. Nasopharyngeal or oropharyngeal swabs and serum samples for baseline virological testing were collected from patients at enrolment and frozen at -80°C.

Ethical statement. The study was conducted according to the principles of the latest version of the Declaration of Helsinki (version Fortaleza 2013), and in accordance with regulatory and legal requirements (EudraCT number: 2015-002340-14). The study was approved by London-Surrey Borders Research Ethics Committee London Centre (18/LO/0660). Written or verbal informed consent, in accordance with regional legislation, is obtained from all patients or their surrogates.

Nucleic Acid Extraction. Viral RNA was extracted from patient samples using the QIAamp Viral RNA Mini Kit according to the manufacturer's protocol (QIAGEN). 140 µL of respiratory sample was mixed with 560 µL of Buffer AVL containing 20 µg/mL of linear polyacrylamide (ThermoFisher Scientific) and eluted into 60 µL of buffer AVE. Dry swabs were resuspended in 2 mL of PBS and incubated at room temperature for 20 minutes prior to extraction. NIBSC reagent 19/304 (https://nibsc.org/products/brm_product_catalogue/detail_page.aspx?catid=20/146) was extracted using the QIAamp Viral RNA Mini Kit and serially diluted in RNA storage solution (Thermo Fisher Scientific;

1 mM sodium citrate, pH 6.4) containing herring sperm carrier RNA (50 µg/mL) and RNasin (New England BioLabs UK, 100 U/mL). All samples were subject to a single freeze-thaw cycle between extraction and RT-PCR quantification.

Real-time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Viral RNA was detected and quantified by RT-PCR using the Quantitect Probe RT-PCR kit (QIAGEN) with CDC N1 primers (Table S1; Suppl. Data: https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html). RT-PCR was carried out on an Applied Biosystems StepOnePlus Real-Time PCR system (ThermoFisher Scientific) with the following settings: 50°C for 30m, 95°C for 15m, and 40 cycles of 94°C for 15s and 60°C for 1m. Intra-assay variation was standardized through use of a standard curve of NIBSC RNA control 19/304 serially diluted from 10,000 copies/reaction to 100 copies/reaction. Ct values were converted to to international units/mL (IU/ml) by the conversion rate provided by NIBSC.

Typing assays to identify B.1.1.7 SARS-CoV-2 strains. An RT-PCR using allele-specific probes (ASP) and nested PCR Restriction Fragment Length Polymorphism for the G→C polymorphism at position 24814 (D1118H) (Table S1; Suppl. Data) were used to differentiate wild-type and B.1.1.7 strains. Detailed methods are available as Protocols A and B (Suppl. Data).

Nucleotide sequencing. Whole virus genomes were sequenced using the virus-specific PCR-free sequencing method veSEQ. The full method description is provided in Protocol C (Suppl. Data).

Serology. Baseline blood samples were collected from all patients to determine the presence of SARS-CoV-2 IgG antibodies against spike protein at the time of transfusion. These were determined using the 386-well plate ELISA coupled with an automated liquid handler [24].

Statistical analysis. All analyses of association between demographic, clinical and virological variables were performed using SPSS version 26. Categorical variables analysed were patient sex, ethnic background, invasive ventilation, pre-existing chronic conditions (asthma, other respiratory disease, liver disease, immunosuppression, cardiovascular disease and diabetes) and infecting SARC-CoV-2 type (WT or B.1.1.7). Continuous variables were patient age (years), viral load (VL), body mass index (BMI) and Acute Physiology and Chronic Health Evaluation II (APACHE II) score. Analysis of categorial variables associated with VL differences were performed using the Mann-Whitney or Kruskall-Wallis non-parametric test. Continuous variables were analysed by linear regression. For multivariate analysis, categorical variables were numerically recoded and analysed with continuous variables using ANOVA (*p* IN=0.05; *p* OUT=0.1). Type association with patient variables were estimated using Pearson Chi-square (categorial variables), Mann-Whitney U test (continuous variables) and by binary logistic regression.

RESULTS

Trial enrolment. The study group was derived from patients enrolled in the REMAP-CAP trial in the UK from 25th May 2020 through 7th January 2021, with the main demographic and clinical variables listed in Table 2. Viral loads and anti-SARS-CoV-2 IgG antibody status were determined in respiratory and/or serum samples collected from patients prior to convalescent plasma treatment or standard care.

Pre-treatment viral loads. RNA extracted from respiratory samples was amplified by real-time RT-PCR targeting the nucleoprotein (N1) gene (Fig. 1A), and viral loads calculated as IU/ml. The assay sensitivity threshold was set at 100 copies/ml. A total of 1141 from 1274 samples (90%) were SARS-CoV-2 RNA positive. Viral loads ranged from <100 IU/ml to 1.7 x 10^{11} IU/ml, a remarkable >2 billion-fold range in levels of secreted virus.

Serology. The serological status was determined by spike ELISA. Results were expressed as positive and negative although the degree of antibody reactivity could not be quantified by the nature of the assay. Antibody status has been determined in 1293 patients included in this study to date, of whom 804 were determined as seropositive (62%). The median viral load of seronegative individuals was 36x higher than seropositives with markedly different distributions of viral loads between the two groups (Fig. 1A). The proportion of seropositive patients varied systematically with viral load ranges, being highest (85%) in those with the lowest viral load (< through to 48% in those with viral loads >10^8 IU/ml (Fig. 1B).

Effects of patient variables and anti-SARS-CoV-2 antibody serostatus on viral loads were investigated (Table 2). Negative SARS-CoV-2 serostatus, increased age and higher APACHE II score (reflecting disease severity) were each significantly associated with increased viral loads; those with diabetes, immunosuppression and requiring invasive ventilation additionally showed significantly raised viral loads. Independent variables associated with increased viral loads on multivariate linear regression were age, invasive ventilation (Fig. 1C) and immunosuppression (Fig. 1D) in addition to antibody status.

SARS-CoV-2 strain identification. As infections with the emerging B.1.1.7 strain may respond differently to treatment compared to previously circulating strains of SARS-CoV-2, two different typing assays were developed to identify B.1.1.7 in trial patients. We first analysed the specificity and predictive value of individual polymorphic sites in the spike gene of B.1.1.7 (H69/V70del., Y144del., N501Y, A570D, P681H, T716I, S982A, D1118H) that differentiated them from other SARS-CoV-2 strains (Table S2; Suppl., Data). All but one of the defined polymorphisms differentiated B.1.1.7 with very high sensitivity and specificity from previously circulating strains in the UK and from emerging strains in South Africa and Brazil (B.1.351 and B.1.1.28.1). The exception was the H69/V70 del site which showed increasing frequencies with time in multiple lineages of wild-type strains (Table S2; Suppl. Data). As the G->C change associated with

D1118H also led to the loss of a restriction enzyme site (*Sfc*I), amplicons from this region were used in typing assays with allele-specific probes (ASP; Fig. S1A; Suppl. Data) and nested-PCR RFLP assays (Fig. S1B; Suppl. data).

For baseline genomic characterisation, the first 284 respiratory samples collected in November 2020 from the trial patents were sequenced by HTS using SARS-CoV-2-baited target enrichment. Variable coverage of the genome was achieved, associated with viral load (Fig. S2; Suppl. Data). All sequences with coverage over codon 164 of the spike gene showed the D614G mutation. Phylogenetic analysis (Fig. S3; Suppl. Data) of those samples showing >65% sequence coverage demonstrated that the majority fell into the lineage GV (according to its designation in GISAID). Of the sequences obtained, 13 of the 173 sequences with sufficient sequence in the spike gene to enable assignment were identifiable as B.1.1.7. No sequences corresponded to emerging strains B.1.351 (South Africa) or P.1 (Brazil).

A subset of 40 samples sequenced across the spike gene were used for evaluation of the typing assays. These produced highly concordant results with 100% specificity, 97.5% sensitivity for the ASP assay, and 97.5% specificity, 93.5% sensitivity for the RFLP assay (Table S3; Suppl. Data). Study samples were typed by a combination of the three methods with 808 (527 WT; 281 B.1.1.7) from 1119 tested (72%) successfully typed; >98% of samples with viral loads >10^6 RNA copies/ml successfully typed by ASP and RFLP assays (Fig. S4; Suppl. Data).

Emergence and virological associations of SARS-CoV-2 strains. Infections with the B.1.1.7 strain were first detected among enrolled patients in early November 2020 and rapidly increased in frequency through December and early January, representing over 80% of infections in week 1, 2021 (Fig. 2). Frequencies of B.1.1.7 infections matched or indeed occurred ahead of their emergence in the general UK population

based on analysis of SARS-CoV-2 sequences deposited on GISAID in the corresponding study weeks. Geographically, B.1.1.7 infections occurred at greatest frequency in subjects enrolled in South East England (including London) and in East England (approximately 50%; Fig. S5; Suppl. data). Cohort enrolment from different regions of England closely matched the proportions of COVID-19 diagnoses reported to PHE over the study period; B.1.1.7-infected subjects showed comparable demographics (age range, gender distribution, ethnicity and BMIs) as those infected with WT virus (Table 2).

However, viral loads ranges from subjects infected with the B.1.1.7 strain differed substantially from those with wild-type SARS-CoV-2, with medians of 2,424,129 (n=273) and 656,801 (n=512) IU/ml respectively ($p = 1.4 \times 10^{-4}$). Remarkably, significant differences in viral loads were only apparent among the 528 subjects who were seropositive for SARS-CoV-2 antibody (Fig. 3), with an approximately 8-fold difference between medians of B.1.1.7 and wild-type infections. Contrastingly, viral loads varied by less than 1.5-fold between those with B.1.1.7 and wild-type infections in the 257 seronegative subjects ($p = 0.94$). Seropositivity had marginal effect on B.1.1.7 viral loads with viral load distributions elevated in both seronegative (n=85) and seropositive (n=188) subjects infected with B.1.1.7 (4.0×10^6 and 1.6×10^6 IU/ml, respectively).

Among the previously listed demographic or clinical variables, only age (medians of 63 and 60 years for WT and B.1.1.7 respectively) and BMI score (31.8 and 30.1) varied significantly between those infected with different SARS-CoV-2 types (Table S4; Suppl. data), although BMI was not independently associated on multivariate analysis. Such differences were only manifest in those who were anti-SARS-CoV-2 antibody positive. For metrics of infection severity, rates of invasive ventilation were comparable between WT and B.1.1.7 infections (Table 3). Contrastingly, APACHE II scores were higher in the WT infections (median 15 compared to 11; p = 5.6E-05) and remained significantly different on multivariate analysis (Table 3, last column).

DISCUSSION

The motivation for this study was the need to characterise the virological variables, viral loads, anti-SARS-CoV-2 serology status and SARS-CoV-2 genotype that may individually or collectively influence the observed treatment efficacy of convalescent plasma. For example, emerging SARS-CoV-2 variants have been shown to display reduced neutralisation capacity [13, 22, 25] and greater pathogenicity [21, 26], and potential greater resistance to immunotherapy. Such analyses have been rarely performed in previous or ongoing COVID-19 treatment trials, even though the extensive variability in these parameters are likely to exert potent effects on patient response. In this respect, the calibration of viral loads into international units using the NIBSC standard will be important in enabling better standardisation of quantitative data and more effective cross-trial comparisons. The widely reported uncalibrated Ct values provide only an indirect metric of viral load, differing for example through varying efficiencies of primer/probe combinations [27] that do not make quantitative results directly comparable across studies.

Our most striking observation was an approximately billion-fold variation in SARS-CoV-2 viral loads. While the predictive value of viral loads for clinical outcomes or treatment response is unclear, with several studies reporting significant [28-33], minimal [34, 35] or no [36-38] associations with increased severity or mortality from COVID-19, we report significant independent associations of pre-treatment variables such as invasive ventilation and immunosuppression with higher viral loads in our cohort that may subsequently influence their response to treatment (Table 2A; Fig. 1C). The range of viral loads we observed is consistent with the kinetics of SARS-CoV-2 secretion in the respiratory tract, where rapid decline following the acute infection stage were observed [30, 32, 36, 37]. Indeed, the observation that RNA levels were <100 IU/ml in 10% of subjects on PCR screening (Fig. 1A) despite severe COVID-19 leading to ICU admission suggests that a substantial element in the disease mechanisms of this latter group is inflammatory or cytokine-related, rather than being directly virally induced. The higher frequency of

seropositivity for SARS-CoV-2 in subjects who were classed as PCR-negative or showed lower viral loads in those infected with the wild-type genotype (Fig. 1B) provides further evidence for differences in the timings of severe disease presentations relative to the evolution of SARS-CoV-2 infection. Overall, 62% of patients included in this study were shown to have SARS-CoV-2 antibodies.

Prospectively, viral load measurements and serological status provides potentially valuable information for patient stratification in treatment selection for COVID-19. Antiviral effect of neutralising antibodies in convalescent plasma may be most apparent in seronegative patients where respiratory tract pathology is primarily virus driven. Conversely, those presenting late post-seroconversion may therefore not be helped by infusion of additional neutralising antibodies where disease mechanisms may primarily derive from inflammation-mediated damage and who may better respond to interventions that modulate host response (corticosteroids, IL-6 receptor antagonists [2, 39-41]). The importance of identifying those at early stages of infection is demonstrated by the efficacy of convalescent plasma given within 3 days from disease onset [8] whereas no benefit was seen when plasma was used in later stage infections (median 8 days from onset [42]).

The effects of mutations in the spike gene on the transmissibility, viral loads, disease severity and potential antigenic variation is currently an area of substantial concern for COVID-19 pandemic management, public health measures and immunisation programmes. The development of practical methods developed here for rapid and large-scale identification of B.1.1.7 strains is of paramount importance for monitoring its spread worldwide, given its greater propensity to transmit [19]. The RFLP assay furthermore enables typing to be performed in resource-limited diagnostic facilities.

SARS-CoV-2 B.1.1.7 strains became increasingly prevalent in the UK population whilst the REMAP-CAP convalescent plasma trial patients were drawn. The study subjects showed a substantial representation of B.1.1.7 infections, particularly those recruited towards the end of the study period (Fig. 2), mirroring the appearance of this variant in the wider UK population over this period among primarily non-hospitalised individuals. In terms of the potential effects of their emergence on trial outcomes, several spike mutations are associated with reductions in susceptibility to neutralisation by antibodies elicited by infection or immunisation with previously circulating variants of SARS-CoV-2 [25, 43]. However, whether this affects their susceptibility to convalescent plasma treatment is unclear - less than 10-fold reductions in titres are typically observed in *in vitro* neutralisation or pseudotype assays and there was little or no effect on detection of anti-spike binding antibodies by ELISA [25, 44, 45].

In terms of potential pathogenicity differences, recent investigations have shown B.1.1.7 infections to lead to a 1.7-fold higher hazard of death within 28 day of diagnosis [26]. These findings are consistent with some, but not all, ongoing studies (summarised in [21]), including higher rates of hospitalisation and intensive care admission [46], higher relative case fatality ratios between B.1.1.7 and WT of 1.29-1.36 (Imperial College London) and mortality hazard ratios of 1.7 (PHE) and 1.7 (University of Exeter). The observation that B.1.1.7 patients prior to the seroconversion for anti-SARS-CoV-2 antibody were significantly more likely to be ventilated (Table 2B) is consistent with a possible greater severity of B.1.1.7 infections (Table 2). However, contrasting with these conclusions is the recent study of hospitalised COVID-19 patients found no association between B.1.1.7 infections and disease severity or mortality [47].

The observation that viral loads were higher in B.1.1.7 infected individuals than those infected with wild-type, but only in those who had already seroconverted for anti-SARS-CoV-2 antibody (x40-fold; Fig. 3), are consistent with replication capacity and/or a less effective cellular or humoral host immune response to

contain B.1.1.7 replication than wild-type. This is supported by the previous observation of higher viral loads in individuals infected with B.1.1.7 [47, 48]. A prolonged duration of respiratory virus shedding in subjects infected with B.1.1.7 compared to other strains (mean values of 13.3 and 8.2 days respectively) but equal peak viral loads [49] supports our evidence for the potentially extended trajectory of B.1.1.7 infections and delayed clearance manifested in Fig. 3. This contrasts with the G614 spike mutant that possesses greater *in vitro* fitness and replicative capacity compared to wild-type strains with D614 [50, 51].

Virus strain characterisation contributes substantially to the virological characterisation of the REMAP-CAP cohort and its association with higher viral loads may potently influence treatment response. While current indications from the REMAP-CAP trial demonstrate no overall effect on the treated cohort compared to untreated controls [12], data obtained in the current results will form the basis for further analyses of treatment efficacy in subgroups defined by antibody status, viral load band and by infecting strain. This *post hoc* analysis may identify specific patient groups who will benefit from convalescent plasma and related immunotherapies in the future.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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TABLE 1

SPECIFICITY AND SENSITVITY OF THE EIGHT POLYMORHISMS IN B.1.1.7 FOR STRAIN IDENTIFICATION

		H69/V70 deletion[1]		Y144 deletion		N501Y		A570D	
Strain	**Source**	**WT**	**Del**	**WT**	**Del**	**A**	**U**	**C**	**A**
B.1.1.7	UK[2]	0	26,328	0	26,328	0	26,328	2	26,359
B.1.1.28	All[3]	374	0	374	0	294	80	374	0
B.1.351	All[4]	834	0	834	0	0	834	833	0
WT	UK[4]	36,577	470	35,708	46	36,868	177	37,597	0
Specificity/Sensitivity[5]		98.73% / 100%		99.87% / 100%		99.52% / 100%		99.98% / 99.99%	

		P681H		T716I		S982A		D1118H	
Strain	**Source**	**C**	**A**	**C**	**U**	**U**	**G**	**G**	**C**
B.1.1.7	UK	0	26,351	2	26,351	0	26,360	0	26,358
B.1.1.28	All	374	0	374	0	374	0	374	0
B.1.351	All	832	1	832	1	831	0	834	0
WT	UK	37,442	34	37,528	23	37,563	0	37,579	8
Specificity/Sensitivity		99.69% / 99.97%		99.94% / 99.99%		100% / 100%		99.98% / 99.97%	

[1]For each mutation, mutations associated with B.1.1.7 shown in right hand column

[2]Available sequences from GISAID Nov, Dec, 2020 and Jan, 2021

[3]All available sequence worldwide

[4]Available sequences from GISAID Jan-April, July, Nov, Dec, 2020 and Jan, 2021

[5]Specificity and sensitivity for differentiation B.1.1.7 from WT SARS-CoV-2 sequences

TABLE 2

BASELINE CHARACTERISTICS OF THE STUDY GROUP

	All	**WT**	**B.1.1.7**
Total number of patients	1192	485	251
Background			
Age in years (median, IQR)	61 (52-70)	63 (55-72)	61 (51-70)
Sex (males/all)	804/1192 (67.4%)	345/485 (71.1%)	166/251 (66.1%)
Ethnicity (non-white/all)	86/302 (28.5%)	50/167 (29.9%)	7/21 (33.3%)
BMI (median, IQR)	30.8 (26.7-36.0)	30.1 (26.6-35.4)	31.8 (27.4-37.4)
Pre-existing comorbidities			
Immunocompromised	54/1190 (4.5%)	28/485 (5.8%)	14/251 (5.6%)
Diabetes	361/1190 (30.3%)	163/485 (33.6%)	74/251 (29.5%)
Respiratory disease	274/1190 (23.0%)	129/485 (26.6%)	45/251 (17.9%)
Cardiovascular disease	98/1162 (8.4%)	44/477 (9.2%)	13/245 (5.3%)
Severity			
APACHE II Score (median, IQR)	12 (8-19)	14 (9-20)	12 (7.5-18)
Invasive Ventilation	415/1192 (34.8%)	192/485 (39.6%)	96/251 (38.2%)

TABLE 3

PATIENT AND VIROLOGICAL VARIABLES ASSOCIATED WTH VIRUS LOAD AND VIRUS TYPE

560

A) V*iral load associations*

Variable	**No**	**Yes**	***P*[1]**	**MLR *p*[2]**
Categorical variables				
Diabetes	62,570 (2,526 - 3,125,789)	193,575 (4,443 - 7,863,774)	0.015	0.099
Immunosuppression	77,510 (2,790 - 3,554,804)	1,605,794 (16,884 - 18,369,106)	0.004	0.013
Invasive Ventilation	42,366 (1,661 - 1,782,723)	241,973 (13,951 - 10,369,484)	7.10E-09	5.3E-7
Anti-SARS-CoV Ab	1,289,633 (39,151 - 26,681,971)	36,264 (1,543 - 1,262,019)	<1.0E-30	1.5E-20
Continuous variables				
	R^2		***P*[3]**	**MLR *p*[2]**
Age	0.015		0.00003	0.002
APACHE II	0.025		5.70E-08	0.15

B) *Associations of disease severity with virus type*

	WT	**B.1.1.7**	***p***	**BLR *p*[4]**
All				
Viral load	718,445 (43,785 - 8,682,234)	3,536,790 (198,314 - 32,349,877)	2.7E-08	4.8E-09
APACHE II	14 (9 - 20)	12 (8 - 18)	0.026	0.005
Invasive ventilation	38.2%	39.6%	0.72	0.97
Ab-Neg				
Viral load	5,746,214 (367,928 - 48,852,455)	4,168,803 (335,710 - 57,126,270)	0.57	0.30
APACHE II	15 (11 - 21)	11 (7 - 17)	5.6E-05	0.001
Invasive ventilation	29.5%	42.8%	0.048	0.38
Ab-Pos				
Viral load	196,556 (32,219 - 2,573,817)	1,840,314 (137,041 - 22,308,100)	1.5E-10	2.3E-10
APACHE II	12 (8 - 20)	12 (8 - 19)	0.99	0.27
Invasive ventilation	42.2%	38.0%	0.39	0.50

565 [1]*p* values from Mann-Whitney U test

[2]*p* values from multivariate linear regression (MLR) with log transformed viral loads

[3]*p* values from simple linear regression with log transformed viral loads

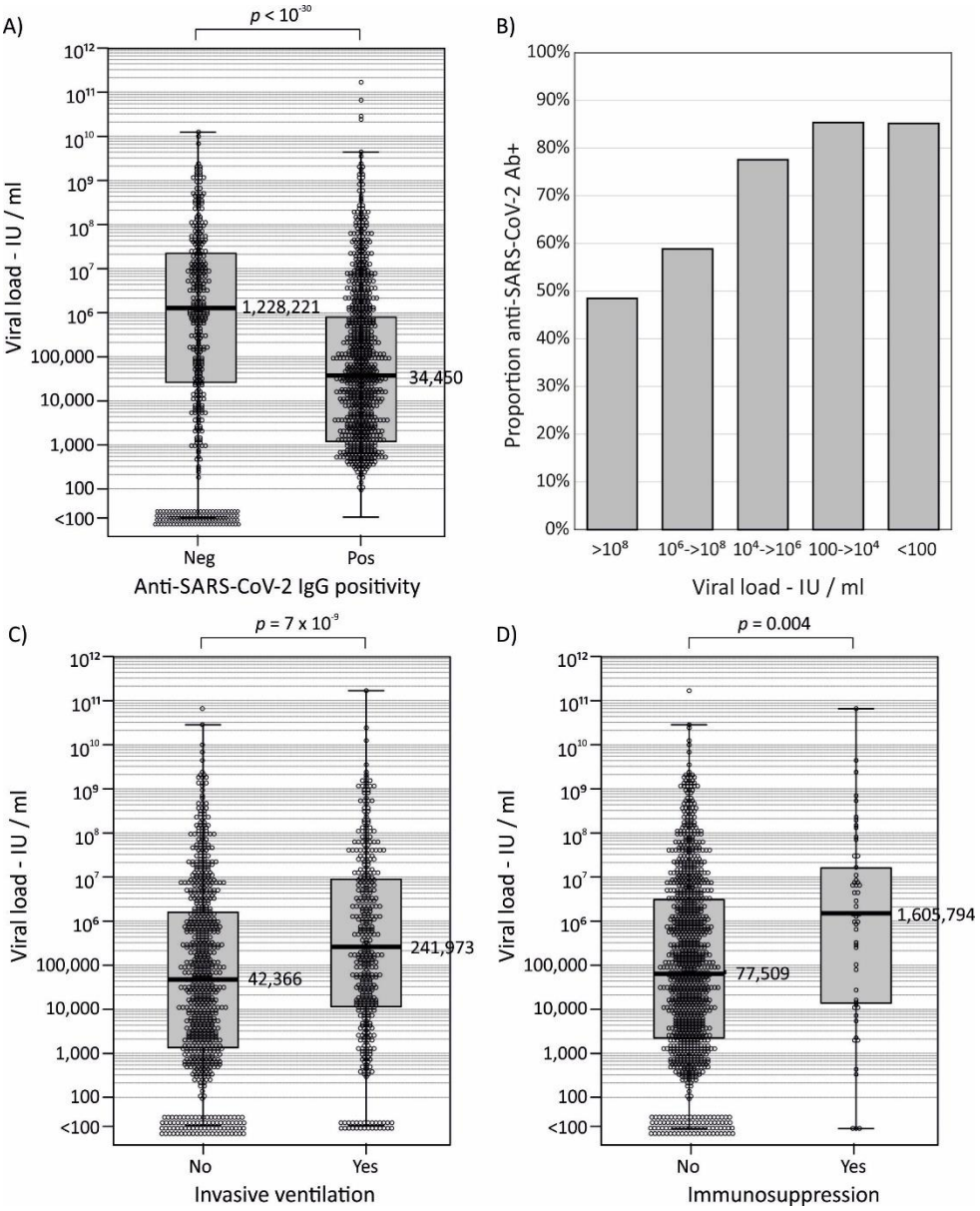

Fig. 1. Comparison of viral loads in anti-SARS-CoV-2 seropositive and seronegative subjects. (A, C, D) Associations of anti-SARS-CoV-2 antibody status, invasive ventilation and immunosuppression on viral load distributions as determined by RT-qPCR of pre-treatment respiratory samples. Median values shown to the right of Tukey box plots. Distributions were compared by Kruskall-Wallis non-parametric test. (B) Frequency of seropositivity in individuals with different viral loads quantified in respiratory samples.

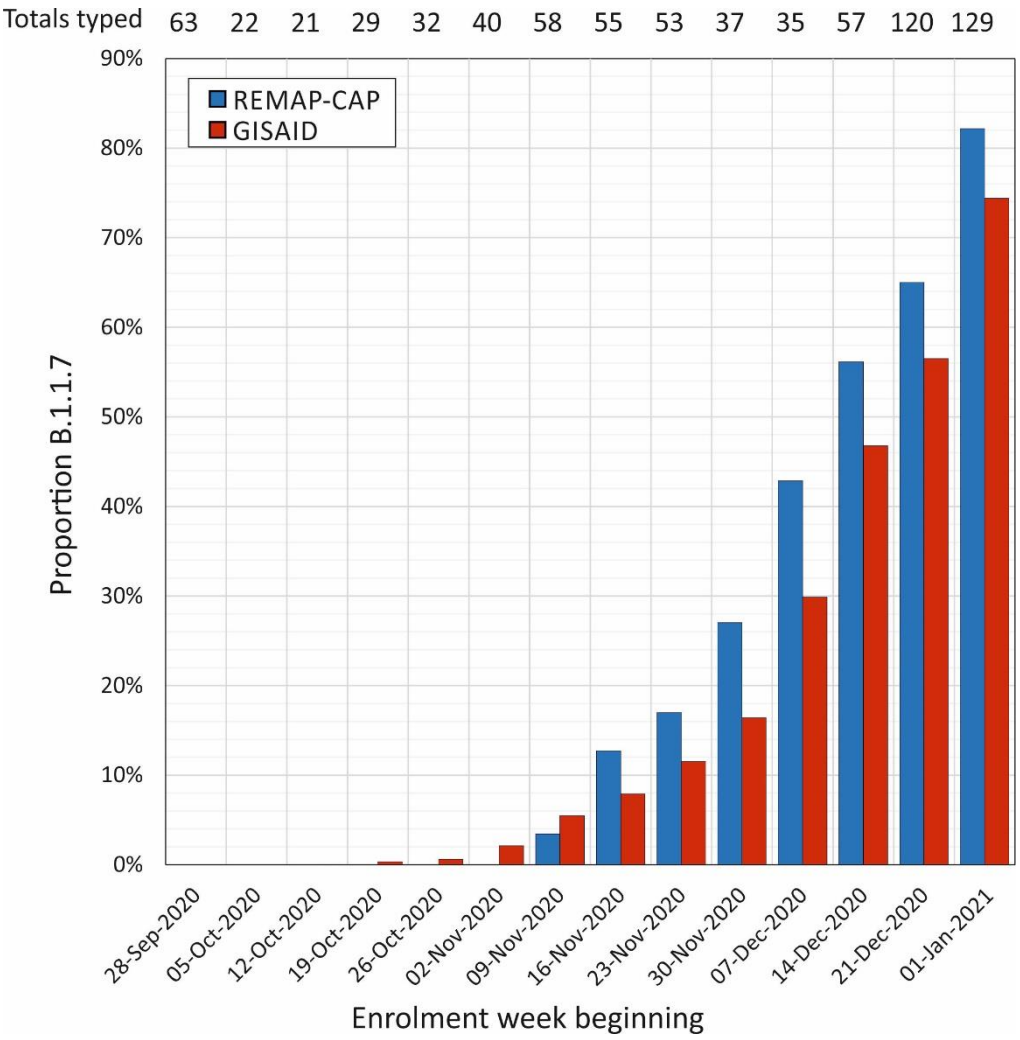

Fig. 2. Temporal emergence of the SARS-CoV-2 B.1.1.7 clade. Proportion of subjects with the B.1.1.7 clade virus enrolled to the REMAP-CAP trial in different weeks over the study period compared to proportions in the wider UK population from sequences deposited in GISAID. Numbers at the top of the graph indicate total enrolments / week.

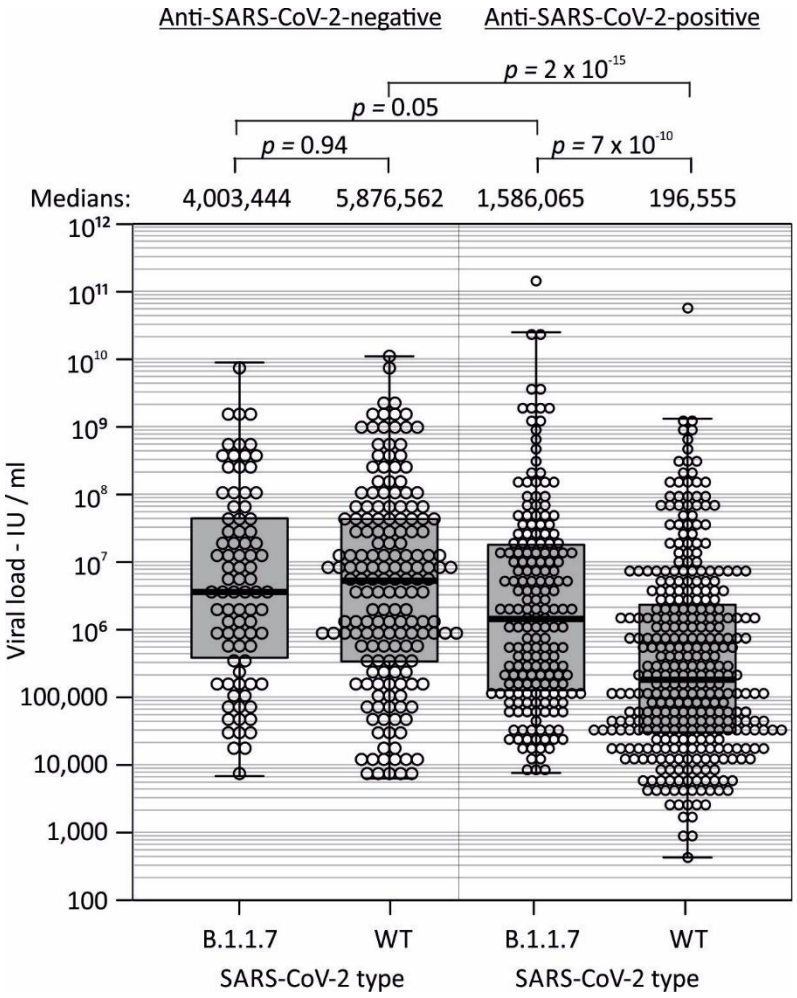

Fig. 3. Viral loads of wild type and B.1.1.7 strains in seronegative and seropositive subjects. Distributions of viral loads of in samples from patients infected with wild-type and B.1.1.7 strains, sub-divided by serostatus. Distributions were compared by Kruskall-Wallis non-parametric test.