Clinical Infectious Diseases

BRIEF REPORT

Real-time whole genome sequencing to guide patient-tailored therapy of SARS-cov-2 infection.

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The management of COVID-19 has become more complex due to the expansion of available therapies. The presence of SARS-CoV-2 variants and mutations further complicate treatment due to their differing susceptibilities to therapies. Here we outline the use of real-time whole genome sequencing to characterise infections and guide treatment decisions.

Key words: whole genome sequencing, COVID-19, SARS-CoV-2

INTRODUCTION

COVID-19 treatments fall broadly into two categories: pre-emptive therapy in those at high risk of deterioration and treatments for hospitalised patients with acute illness[1]. The emergence of new variants and mutations can alter susceptibilities to these treatments, seen frequently with neutralising monoclonal antibody (nmab) therapies[1]. Previously we have used SARS-CoV-2

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whole genome sequencing (WGS) to guide nosocomial outbreak investigation[2] and public health interventions[3]. We are now applying real-time WGS to characterise SARS-CoV-2 infection to guide patient-tailored treatment decisions. The workflow takes 24 hours from sample receipt to results, allowing expedited treatment decisions. In this brief communication we present 6 cases where real-time WGS showed utility in guiding treatment.

METHODS

WGS was performed on extracted nucleic acid from respiratory samples, using ARTIC v3.0 lab protocol with updated primer sets. After amplification, products underwent library preparation using SQK-LSK109, or SQK-RBK004 and run singleplex, on R.9.4.1 flow cells (Oxford Nanopore Technologies). Sequencing data was analysed to call consensus sequences using the ARTIC bioinformatic protocol. Lineages were called using pangolin v2.0. To characterise mutations, genome annotation was performed using Systematic ProtEin AnnotatoR (SPEAR) v1.0. Analysis of variant viral populations using VarScan v2.0, as previously described[4] Treatment was decided by a multidisciplinary team of virologists, infectious disease specialists and pharmacists. Decisions outside national licensing and commissioning policies were authorised by the local Drugs and Therapeutics Committee. Note that in the UK COVID-19 therapies are centrally commissioned by government agencies. Case details and sequence results are summarised in Supplementary Table 1.

WGS TO DISTINGUISH CHRONIC INFECTION FROM RE-INFECTION.

Pre-emptive therapy is advised for immunocompromised patients with acute infection at high risk of developing severe disease[1]. Chronic infection with SARS-CoV-2 can also occur in immunocompromised patients[5] and the limited evidence around its management suggests patients may benefit from combination therapy and/or longer courses[6]. WGS can distinguish chronic infection from reinfection, by offering determining both the lineage assignment and non-lineage defining single nucleotide polymorphisms (SNP)[7]. Highly related genomes from longitudinal samples from one individual likely represent chronic infection rather than re-infection[7].

A 50 year old woman (case 1) with a type BA thymoma had symptomatic COVID-19 confirmed by PCR and lateral flow device (LFD) in February 2022, requiring admission and treatment with remdesivir and dexamethasone. She was discharged with a need for ambulatory oxygen, diagnosed as post-COVID-19 lung fibrosis with compatible imaging and lung function tests. She tested positive again in early April 2022; felt to represent shedding of non-viable RNA as it was within 60 days of symptoms. No WGS was available from samples taken between February and April 2022, as they were tested in another hospital. In June 2022 during work-up for surgical resection of tumour at our hospital she was LFD positive again, and reinfection was considered.

However, WGS of this June 2022 sample identified the Omicron BA.1.1 sublineage which had been eliminated from the UK for several months[8], strongly suggesting chronic infection continuing since initial illness in February 2022. As a result the patient was treated with dual agents: sotrovimab and Paxlovid (nirmatrelvir/ritonavir). The patient cleared infection with PCR-negative throat swabs for SARS-CoV-2 and underwent surgical resection of her tumour, and need for ambulatory oxygen resolved.

One 59 year old male outpatient (case 2) with kidney transplant and previous anti-CD20 treatment tested PCR-positive for SARS-CoV-2 in December 2020, with WGS confirming lineage B.1.177.18. In February 2021 he tested positive again with WGS showing the same lineage B.1.177.18. The patient next returned in January 2022 and WGS again demonstrated SARS-CoV-2 B.1.177.18 lineage, differing by 18 SNPs from his original sample 13 months prior. Phylogeny of longitudinal sequence data are presented in Figure 1. This is consistent with chronic infection given the rate of mutation of SARS-CoV-2 is around 1 nucleotide every two weeks[9]. With ongoing paucisymptomatic, persistent infection the patient did not meet criteria for pre-emptive treatment, nor the treatment of hospitalised, acutely unwell patients. However, treatment was advised with REGN-COV2 (casirivimab/imdevimab) due to the presence of chronic infection with susceptible lineage. The patient cleared infection with PCR-negative throat swabs for SARS-CoV-2..

Similarly, WGS has also been used to confirm re-infection instead of chronic infection. A 44 year old male (case 3) with a combined immune deficiency had was SARS-CoV-2 PCR-positive in March 2022. He was treated with sotrovimab as per national guidelines for pre-emptive therapy[10]; he remained PCR-positive on repeated testing including in May 2022 but became LFD negative. In June 2022 he had recrudescence of symptoms with fever and cough and LFD became again positive. His immunology team felt this represented recrudescence of persistent infection and proposed treatment with dual agents: sotrovimab and Paxlovid. WGS instead confirmed that episodes were caused by two separate infections with different sub-lineages of Omicron: BA.2 then reinfection with BA.5.2. As a result he received sotrovimab as pre-emptive treatment in line with commissioned guidelines, and dual therapy was not offered. The patient recovered.

EVALUATING FOR MUTATIONS CONFERRING REDUCED SUSCEPTIBILITY TO TREATMENT.

During chronic infection patients can receive several courses of treatment. Treatment COVID-19 therapies can lead to the emergence of mutations conferring resistance[1]. Establishing whether resistance mutations emerge after failed treatment can guide further rounds of therapy.

A 45 year old female (case 4) with advanced HIV had chronic, asymptomatic SARS-CoV-2 Delta AY.4 infection identified in February 2022. She was treated with REGN-COV2. WGS of a

post-treatment sample taken 19 days after treatment showed emergence of a G22989A mutation below consensus level but in the majority of reads. This confers a spike G476D substitution, known to reduce sensitivity to casirivimab by 1021-fold[11]. Similarly, T22896C was seen in a majority of reads spanning this position, conferring spike V445A and a >500-fold decrease in sensitivity to imdevimab[11]. WGS of the pre-treatment sample showed wildtype sequence at these positions. There is limited data on the neutralisation of Delta by sotrovimab[12] and some data that this treatment can lead to rapid resistance[13]. This information, along with the development of resistance to REGN-COV2, supported the decision to forgo further treatment with nmabs. Instead a course of Paxlovid was offered. In August 2022, her throat swabs became SARS-CoV2 PCR-negative after immune reconstitution.

Similarly, a 60 year old male (case 5) was immunocompromised from previous treatment with obinutuzimab for chronic lymphoid leukaemia. He developed COVID-19 in April 2022 and was later admitted with COVID-19 pneumonitis and respiratory failure. After receiving Paxlovid and double dose (1000 mg) sotrovimab in mid-July he had higher cycle threshold values in his throat swabs, showed clinical improvement, and was discharged. However, approximately six weeks after treatment he was readmitted with hypoxia and progression of pneumonitis. Other infectious aetiologies were excluded and respiratory sampling confirmed a high SARS-CoV-2 viral load. Persistent BA.2.3 infection was confirmed by comparison of longitudinal sequence, with only 7 SNPs difference over 10 weeks of infection. Sequencing of his July 2022 sample also revealed post-treatment emergence of a spike K356R substitution. This substitution can arise after treatment with sotrovimab[14] but does not affect the *in vitro* activity of sotrovimab [15]. In addition, resistance to Paxlovid was investigated by looking at the sequence of the nsp5 gene, which encodes the main protease of SARS-CoV-2 which Paxlovid targets[1]. Only the Omicron lineage-defining P132H was detected, and *in vitro* evidence suggests this does not affect inhibition by Paxlovid[16]. No mutations associated with resistance to remdesivir were identified in the RNA-dependent RNA polymerase encoded by nsp12 which is the target of remdesivir[1,17]. This information supported the decision to re-treat with Paxlovid and remdesevir. Outcome is awaited.

DETERMINATION OF LINEAGE TO DIRECT NMAB THERAPY

Sub-lineages of Omicron have varying *in vitro* susceptibility to sotrovimab[18], which is the only nmab licensed in the UK with activity against Omicron. Sotrovimab has most activity against BA.1, reduced against BA.4/5, and least activity versus BA.2[18]. Subsequently, clinical trials are evaluating double dose (1000mg) sotrovimab for Omicron sublineages such as BA.2 with decreased susceptibility[19]. We have given double dose sotrovimab to cases where WGS confirms PCR-genotyping results which suggest Omicron sublineages are present with decreased susceptibility. Notably, our PCR-based genotyping assay[20] cannot distinguish between

emerging sublineages of Omicron, meaning WGS may have increasing value for identifying resistant variants.

A 47 year old immunocompromised woman (case 6) with primary sclerosing cholangitis awaiting liver transplantation developed hospital-onset COVID-19. BA.5 was dominant in London at this time, but WGS identified the BA.2.12.1 variant. Given the sub-lineage detected, double dose sotrovimab was recommended as pre-emptive therapy - being ineligible for other commissioned therapies (Paxlovid and remdesivir) due to interactions and contraindications respectively. The patient recovered withSARS-CoV-2 PCR-negative throat swabs.

CONCLUSIONS

WGS has utility in guiding treatment decisions, especially in complex cases with chronic infection. These cases illustrate the benefit of characterising emergent resistance after treatment, and in detecting SARS-CoV-2 variants below consensus frequency in the viral population. Of note, this method cannot exclude the presence of low-frequency minority variants which could confer resistant phenotypes.. To this end, workflows able to detect low-frequency, resistant minority variants may be required. Elucidation of mutations associated with resistance will be important for maximum utility of sequencing-directed therapy. In our experience, rapid, near-patient capability for WGS, analysis and interpretation was important to guide patient-tailored therapy for COVID-19. Evidence on optimal treatment of chronic infection is also needed.

NOTES

Data availability: Consensus sequences and read data are available on the Sequence Read Archive (BioProject: PRJNA881323). Individual NCBI genome accessions: OP680467-OP680475.

Ethics: Collection of surplus samples and linked clinical data was approved by South Central – Hampshire B REC (20/SC/0310). Treatment decisions outside of licence and/or commissioning policy were agreed by both the multidisciplinary team and local Drug & Therapeutics Committee.

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FIGURE LEGEND

Figure 1: Phylogenetic representation of case 2 (panel A), case 4 (B) and case 5 (C). The outgroup for each case is represented by sequences from the same lineage submitted to GISAID in the same week as the case was diagnosed. A maximum of 50 sequences are displayed; where GISAID contains more than 50 sequences, subsampling was performed with seqtk v1.3. Maximum likelihood phylogenetic trees were computed using IQTree v.1.6, nodes calculated using ultrafast bootstrap and Shimodaira–Hasegawa approximate likelihood ratio test with 1000 replicates. Trees were visualised in Figtree v1.4.4. Branch tips are labelled with the GISAID accession number or case details.

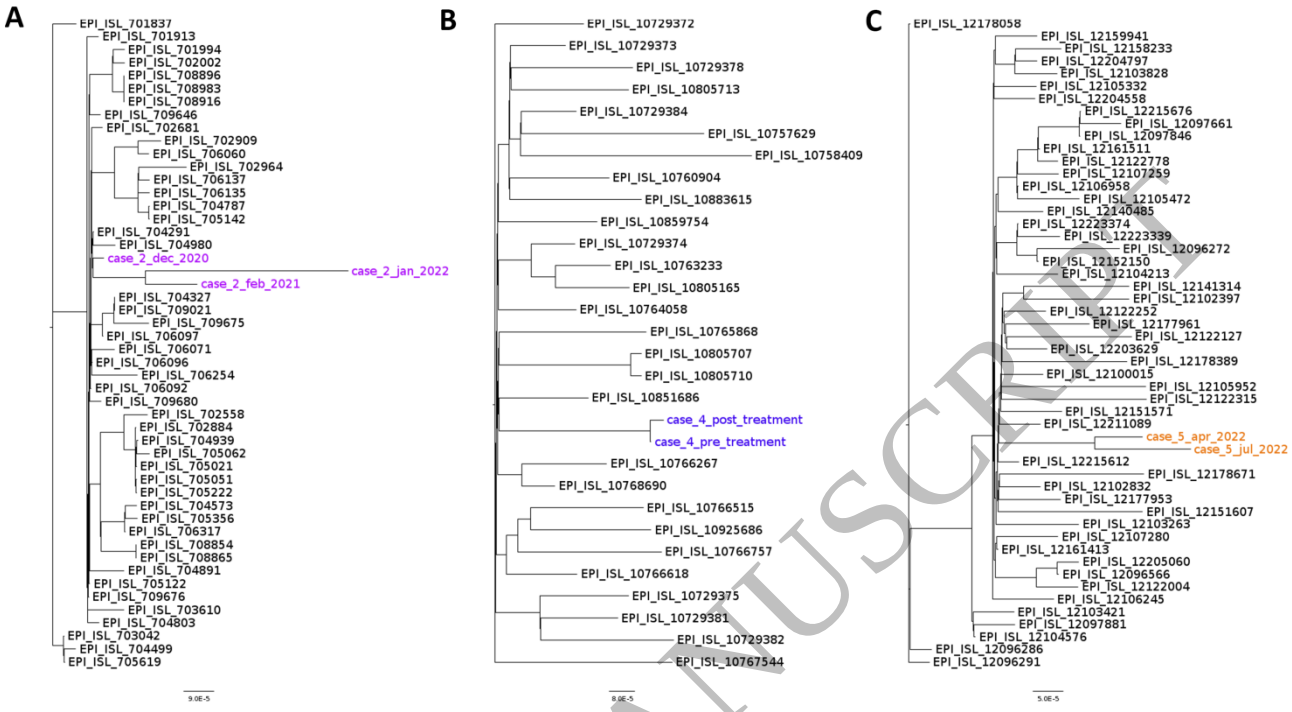

Figure 1
165x128 mm (.95 x DPI)