Case report

Detection of viable SARS-CoV-2 in deep respiratory specimens despite negative nasopharyngeal SARS-CoV-2 RT-PCR: Occult COVID-19 as an unsuspected cause of pulmonary infiltrates in immunocompromised patients

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ABSTRACT

Background: Prolonged shedding/relapse of COVID-19 infection has been reported, particularly in patients who received anti-CD20 agents (eg. rituximab). However, cases of occult COVID-19, in which SARS-CoV-2 persistence in lung parenchyma is diagnosed despite clearance from nasopharyngeal (NP) specimens, are uncommon. Case summary: We describe two cases of occult COVID-19 in immunocompromised patients. Both patients had received rituximab previously. Both cases initially presented as ground-glass infiltrates on lung imaging; the diagnosis was originally not suspected due to repeated demonstration of negative SARS-CoV-2 from NP specimens, and alternative etiologies were originally considered. Persistence of SARS-CoV-2 in lung parenchyma, however, was demonstrated on bronchoalveolar lavage (BAL) specimens; additionally, isolation of viable SARS-CoV-2 virus and detection of SARS-CoV-2 nucleocapsid and spike-protein antigen in lung tissue on immunohistochemistry close to 3-months from primary infection strongly suggested ongoing viral persistence and replication as a driver of the lung parenchymal changes, which resolved after antiviral treatment. Discussion: Occult COVID-19 can be a cause of unexplained ground-glass infiltrates on lung imaging; negative NP samples do not rule out SARS-CoV-2 persistence and invasive sampling must be considered. The unsuspected presence of viable virus on BAL, however, highlights that procedurists performing aerosol-generating-procedures during an ongoing pandemic wave must also practise appropriate infection-prevention precautions to limit potential exposure.

Introduction

Immunocompromised patients are at-risk for severe coronavirus disease 2019 (COVID-19). In particular, patients with secondary hypogammaglobulinemia after receipt of anti-CD20 treatment (eg. rituximab) tend to have more severe COVID-19 manifestations \cite{1-3}. Amongst COVID-19 cases receiving concurrent rituximab, prolonged shedding or relapse has been encountered \cite{4,5}. Cases of occult COVID-19, in which SARS-CoV-2 relapse in lung parenchyma is diagnosed despite repeated demonstration of negative PCR from nasopharyngeal (NP) specimens,
have been rarely reported in the literature [5]. We describe two patients who received rituximab and presented with ground-glass opacities (GGO) on lung imaging; COVID-19 relapse was originally not suspected due to negative NP swabs and diagnosed only after invasive sampling.

**Methodology**

**Definitions**

A COVID-19 relapse was defined as a clinical episode of symptoms consistent with acute COVID-19, accompanied by re-positive/persisting polymerase-chain-reaction (PCR) test for SARS-CoV-2 in respiratory samples, within 90 days of initial infection [4]. Occult COVID-19 was defined as COVID-19 relapse with negative PCR results on NP swab [5], with positive PCR on deeper respiratory specimens (endotracheal-aspirates [ETA] or bronchoalveolar lavage [BAL]).

**SARS-CoV-2 testing**

At our institution, a tertiary hospital housing Singapore’s largest cancer centre, Cepheid Xpert Xpress-SARS-CoV-2 RT-PCR assay (targeting E/N2-gene targets) is used for diagnostic testing of respiratory samples whilst the TaqPath-Combo PCR kit (Thermo Fisher Scientific) is utilised to detect S/N/ORF1-gene targets in blood/tissue samples. Whole-genome-sequencing (WGS) was performed according to previously published protocols on a MinION MK1b system (Oxford Nanopore Technologies).
Technologies, Oxford, UK) in accordance with the ARTIC Network protocol v3 [6]. Serology test for SARS-CoV-2 IgG (RBD) is performed using the chemiluminescent-microparticle-immunoassay (CMIA) on the Architect i2000SR system (Abbott Laboratories) with ≥ 50 IU/ml defined as a positive result. Immunohistochemistry (IHC) staining for SARS-CoV-2 antigen is performed on formalin-fixed paraffin-embedded (FFPE) transbronchial-lung-biopsy (TBLB) samples according to previously published protocols [7]. FFPE tissue sections (4-μm thick) are labelled with antibodies targeting the SARS-CoV-2 nucleocapsid (Novus Biologicals, Cat# NB100-56576, Polyclonal) and spike-protein (GenTex, Cat# GTX632604, 1A9). Respiratory viral culture is performed by inoculating samples into HeLa, HEP2, HEL, LLC-MK2 and MDCK cell cultures; cultures were observed for up to 21 days for cytopathic effect (CPE). Upon observation of CPE suspected for SARS-CoV-2, this was confirmed via PCR by an external BSL-3 laboratory.

Case description

Patient A
A was a 70-year-old female with diffuse large-b-cell-lymphoma in remission and currently on two-monthly maintenance rituximab (last administered 2-months prior to presentation). She had previously received 3 doses of BNT162b2 mRNA vaccine. The patient presented with a 2-week history of increased breathlessness and reduced effort tolerance, requiring supplemental oxygen on admission; initial computed-tomography-scan (CT-scan) of the thorax showed unilateral ground-glass changes (Fig. 1a). COVID-19 was not suspected initially as NP SARS-CoV-2 PCR on admission was repeatedly negative, and an alternative diagnosis of rituximab-induced pneumonitis was considered. The patient underwent BAL and biopsy a week after admission; BAL was SARS-CoV-2 PCR positive, confirming the diagnosis of occult COVID-19; viral culture was not performed. BAL and lung tissue were also SARS-CoV-2 PCR positive, with atypical lymphocytes seen on BAL fluid. Omicron variant was confirmed on WGS. Histopathology results showed a chronic interstitial pneumonitis-like pattern with positive immunohistochemistry staining for SARS-CoV-2 antigen (Fig. 1bi,ii). On directed questioning, the patient subsequently provided a history of having self-tested positive for SARS-CoV-2 at home using rapid-antigen-testing (RAT) 52 days prior to admission; the patient had not sought medical care previously as initial symptoms were mild (Fig. 1c). Post-bronchoscopy, the patient deteriorated and required intubation. Given persistence of SARS-CoV-2 in lung parenchyma, intravenous remdesivir was administered for 10 d; sotrovimab was also given as SARS-CoV-2 IgG (RBD) levels were < 50 IU/ml at D59. The patient was subsequently extubated a week later; however, as fever persisted, SARS-CoV-2 PCR was performed on blood, which was positive (Fig. 1c). Viremia persisted from D65-D72 even after 10 d of IV remdesivir, and only cleared after an additional 5 d of nirmatrelvir/ritonavir. Repeat CT-thorax (D70) showed resolution of initial ground-glass changes in the right lung (Fig. 1a) and she was discharged well at D80.

Patient B
B was a 42-year-old female with systemic-sclerosis on prednisolone 10 mg daily and received rituximab 3-months prior to presentation. She had previously received 1 dose of BNT162b2 mRNA vaccine. The patient presented with a 2-week history of increased breathlessness, reduced effort tolerance, and intermittent fever; initial CT-thorax showed bilateral ground-glass changes (Fig. 1a). Again, COVID-19 was not suspected initially as NP SARS-CoV-2 PCR on admission was repeatedly negative; given that blood PCR for cytomegalovirus (CMV) was positive at low levels, a diagnosis of CMV pneumonitis was considered. She underwent BAL a week after admission; BAL was SARS-CoV-2 PCR positive,
despite intensive post-exposure surveillance and twice-weekly routes of monoclonal antibodies directed against COVID-19; this was in [12]. Both our cases made a full recovery after administration of antivirals and were unable to produce neutralising antibodies to SARS-CoV-2 [5, 7] which occurred in individuals who received anti-CD20 treatment [1].

Discussion

We present two cases of occult COVID-19 in immunocompromised hosts presenting as GGO on lung imaging, in which the diagnosis was originally not suspected due to repeated demonstration of negative SARS-CoV-2 from NP specimens, and alternative etiologies were originally considered. Persistence of SARS-CoV-2 in lung parenchyma, however, was demonstrated on BAL specimens; additionally, isolation of viable SARS-CoV-2 virus and detection of SARS-CoV-2 nucleocapsid and spike-protein antigen in lung tissue on immunohistochemistry close to 3-months from primary infection [7] strongly suggested ongoing viral persistence and replication as a driver of the lung parenchymal changes, which resolved after antiviral treatment. The hitherto unsuspected presence of viable virus on BAL also raised infection-prevention concerns, given the potential aerosol-generating nature of the procedure. At our institution, universal usage of N95 respirators was practised during the COVID-19 pandemic [8]; there was no evidence of onward transmission to potentially exposed healthcare workers post-procedure, despite intensive post-exposure surveillance and twice-weekly routine-rosed-testing for SARS-CoV-2 amongst HCWs [9]. Both patients did not seek medical care from the outset as their initial symptoms were mild and only self-tested for COVID-19 due to prevailing public health messaging strongly encouraging COVID-19 home testing for cases of mild flu-like illnesses; the only clue to the aetiology of their GGO, a history of positive RAT for SARS-CoV-2 in the preceding 2 months, could thus have been easily missed. While both cases had received BNT162b2 mRNA vaccine prior to infection, none produced detectable antibodies against SARS-CoV-2, possibly contributing to viral persistence. Patients treated with rituximab may have discordant humoral and cellular responses to COVID-19 vaccination; immunogenic non-response to mRNA vaccination is not uncommon in this patient population [10, 11]. Seroconversion rates of 17.5–40 % have been reported in the literature [10,11]. Similarly, other cases of occult COVID-19 reported in the literature occurred in individuals who received anti-CD20 treatment and were unable to produce neutralising antibodies to SARS-CoV-2 [5,12].

Both our cases made a full recovery after administration of antivirals and monoclonal antibodies directed against COVID-19; this was in keeping with emerging data suggesting good clinical outcomes in B-cell-depleted patients treated with anti-spike monoclonal antibodies [13]. Clinicians caring for such immunocompromised patients need to remain vigilant for occult COVID-19 in cases with unexplained ground-glass infiltrates on lung imaging and consider treatment with COVID-19 therapeutic. Negative NP samples do not rule out SARS-CoV-2 persistence and invasive sampling must be considered. The unsuspected presence of viable virus on BAL, however, highlights that procedures performing aerosol-generating-procedures during an ongoing pandemic wave must also practise appropriate infection-prevention precautions to limit potential exposure.

CRediT authorship contribution statement

WLE: study design, data collections, data analysis, Writing – original draft, Writing – review & editing. TJY: data collection, Writing – original draft, Writing – review & editing. KKKK, WWY, DCML, LLEO, ATG, JPSY, AMTP, TKHL, EPC, IV: data collection. LW, TTT: Writing – original draft, Writing – review & editing, Supervision.

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Ethical approval

Ethics approval was not required for case reports under our institutional review board guidelines.

Conflict of interest

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