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CASE REPORT

Persistent SARS-CoV-2 infection with multiple clinical relapses in two patients with follicular lymphoma treated with bendamustine and obinutuzumab or rituximab

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Abstract

Purpose People with hematologic malignancies have a significantly higher risk of developing severe and protracted forms of SARS-CoV-2 infection compared to immunocompetent patients, regardless of vaccination status.

Results We describe two cases of prolonged SARS-CoV-2 infection with multiple relapses of COVID-19 pneumonia in patients with follicular lymphoma treated with bendamustine and obinutuzumab or rituximab. The aim is to highlight the complexity of SARS-CoV-2 infection in this fragile group of patients and the necessity of evidence-based strategies to treat them properly.

Conclusions Patients with hematological malignancies treated with bendamustine and anti-CD20 antibodies had a significant risk of prolonged and relapsing course of COVID-19. Specific preventive and therapeutic strategies should be developed for this group of patients.

Keywords COVID-19 · Immunosuppressed patient · Lymphoma · Anti-CD20 · Bendamustine · Obinutuzumab · Rituximab · SARS-CoV-2

Background

Immunosuppression is a well-known risk factor for severe illness in patients with SARS-CoV-2 infection [1]. Patients with hematological malignancies have a significantly higher risk of developing a severe form of SARS-CoV-2 infection because of their immunological condition and specific therapies, regardless of vaccination status [2]. Moreover, the progression of COVID-19 is different if compared to immunocompetent patients [3]. Symptom onset may be delayed [3], the persistence of illness protracted [4], and patients may result positive for SARS-CoV-2 virus far longer than immunocompetent patients.

Vaccination against SARS-CoV-2 has completely changed disease clinical manifestation and prognosis in the vast majority of the population, with the exception of oncohematological patients who rarely develop an efficient response to SARS-CoV-2 vaccination [5, 6].

We provide a descriptive analysis of two cases of prolonged SARS-CoV-2 infection with multiple relapses of COVID-19 pneumonia in patients with follicular lymphoma treated with bendamustine and obinutuzumab or rituximab. Bendamustine is an alkylating agent that decreases CD4 + T-cells count, producing prolonged lymphocytopenia [7]. Obinutuzumab is a novel anti-CD20 monoclonal antibody (mAB) directed mainly to B cells, while rituximab is

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a chimeric (human/murine) anti-CD20 mAB causing rapid and persistent depletion of CD20 + B cells [8, 9].

Case 1

A 75-year-old woman with hypertension, dyslipidaemia and follicular lymphoma was treated with 6 s-line cycles of bendamustine between July and December 2020 and received maintenance therapy with 6 cycles of obinutuzumab between February and December 2021. She completed 3 doses BNT 162b2 in November 2021 and on January 21st, 2022 she tested positive at qualitative real-time polymerase chain reaction (RT-PCR) on nasopharyngeal swab (NPS) for SARS-CoV-2, with a Cycle threshold (Ct) value compatible with medium viral load (VL) (24–23 Ct). She was first admitted to hospital in February for fever and cough. Pneumonia was diagnosed at chest X-Ray, broncho-alveolar lavage (BAL) detected only SARS-CoV-2 and serological immune response for SARS-CoV-2 was negative (< 34 BAU/ml). Thus, sotrovimab 500 mg was administered. She required oxygen therapy up to Venturi mask (VM) FiO2 60%. After 28 days, she was discharged with no oxygen and a still positive RT-PCR on NPS with Ct value compatible with high VL (19 Ct, higher than at admission).

Her second hospitalization occurred in May 2022 due to interstitial pneumonia relapse with fever, cough and dyspnea. SARS-CoV-2 RT-PCR on NPS had a Ct value compatible with high VL (15 Ct). The patient had oxygen therapy up to 4 l/min nasal cannula (NC) and remdesivir for 5 days (200 mg day 1, 100 mg days 2–5). Symptoms completely remitted and she was discharged after 7 days; SARS-CoV-2 antigenic NPS was negative. Her third hospitalization was in July 2022 with interstitial pneumonia, asthenia, dyspnea and fever. SARS-CoV-2 RT-PCR on NPS showed again a Ct value compatible with high VL (18 Ct). She was treated with oxygen therapy up to 4 l/min NC and with both nirmatrelvir/ritonavir (300 mg/100 mg BID for 5 days) and a new administration of sotrovimab (500 mg ev). Once again, she was discharged without symptoms nor oxygen therapy.

The fourth hospitalization occurred in July due to cough, dyspnea and fever. RT-PCR for SARS-CoV-2 on NPS had a Ct value compatible with high VL (21–23 Ct). She needed oxygen up to VM FiO2 50% and received therapy with 5-day remdesivir and tixagevimab/cilgavimab (300 mg/300 mg IM). AmpC Beta-lactamases producing *Klebsiella pneumoniae* was isolated from urine and a 7-day course of cefepime was administered. She recovered and she was discharged after 15 days, still positive at RT-PCR for SARS-CoV-2 on NPS with a Ct value compatible with medium VL (25 Ct).

A new relapse occurred in September (fever, asthenia, exertional dyspnea, lack of appetite), one more time RT-PCR for SARS-CoV-2 on NPS had a Ct value compatible with high VL (18 Ct), but the patient was treated early at home with nirmatrelvir/ritonavir (300 mg/100 mg BID for 5 days) and she showed a fast clinical improvement, avoiding a new hospitalization.

There was no occurrence of new clinical relapses onward. All molecular typing analyses carried out by whole genome sequencing showed that the virus belonged, in all cases, to the variant Omicron, sub-lineage BA.1.1.

Beyond SARS-CoV-2, no other respiratory pathogens were ever detected throughout her hospitalizations. In every single episode of hospitalization, blood cultures, serum β-d-glucan, galactomannan, and CMV-DNA were negative such as PCR test for the principal respiratory viruses, bacteria and *Pneumocystis jirovecii* on respiratory specimens and PCR test for herpes viruses on plasma. During the first and the last hospitalization the patient was also treated with empiric antibiotic therapy with no improvement. Furthermore, repeated CT scan excluded lymphoma progression.

Case 2

A 61-year-old man with follicular lymphoma and hypertension was treated with 3 first-line cycles of bendamustine and rituximab between September and November 2021. In the same period, he completed 3 doses of BNT 162b2. He tested positive for SARS-CoV-2 on December 11th, 2021 (RT-PCR for SARS-CoV-2 on NPS, Ct not available) with mild symptoms, he had a negative serologic test (< 34 BAU/ml) and bamlanivimab/etesevimab (700 mg/1400 mg) was administered as an outpatient.

He was hospitalized on January 10th, 2022 due to a worsening of symptoms (fever, cough, asthenia and dyspnea). RT-PCR for SARS-CoV-2 on NPS showed a Ct value compatible with high VL (19–21 Ct) and interstitial-parenchymal pneumonia was detected at chest X-Ray. Oxygen therapy up to VM FiO2 40% and a 3-day course of remdesivir (200 mg day 1, 100 mg days 2 and 3) were administered. He rapidly recovered with progressive weaning of oxygen up to discharge.

His second hospitalization occurred in February 2022 for asthenia and low-grade fever. RT-PCR for SARS-CoV-2 on NPS had a Ct value compatible with medium VL (31 Ct). The patient did not show respiratory failure but CT scan documented radiographic signs of interstitial lung injury. No antiviral or mAB was administered and after clinical improvement, he was discharged.

In March, he tested negative for SARS-CoV-2 at RT-PCR NPS and therefore he received a fourth cycle of bendamustine and rituximab. At the end of this chemotherapy cycle, fever and asthenia reappeared; he was treated at home with levofloxacin without benefit. SARS-CoV-2 infection was confirmed with RT-PCR NPS (Ct value compatible with

medium VL, 25–26 Ct) and the patient was admitted to hospital. Despite microbiological and instrumental investigations, no other pathogens were found. He was then treated with remdesivir for 3 days (200 mg day 1, 100 mg days 2 and 3) with clinical benefit. At discharge, antigenic NPS was still positive.

Ten days after, in April, he was hospitalized again due to a relapse of pneumonia with ground glass opacities at chest CT, with dyspnea, fever and cough. RT-PCR for SARS-CoV-2 on NPS was positive with a Ct value compatible with medium VL (25–26 Ct). He was treated with oxygen therapy up to 6 l/min NC and with Nirmatrelvir/Ritonavir (300 mg/100 mg BID), with rapid symptom remission. Antigenic NPS was finally negative. During the last hospitalization, the patient was also treated with an empiric 7-day course of cefepime without clinical response.

There were no new relapses onward. Throughout this period, every NPS variant typing carried out by RT-PCR result was confirmed as a Delta variant. Whole genome sequencing was performed only on a NPS collected during the last hospitalization in April, showing the belonging to variant Delta, sub-lineage AY.126. Furthermore, on April 4th, a national surveillance prevalence was conducted, showing that Omicron variant was the only circulating variant in Italy.

Discussion

As shown in several studies, patients with hematological malignancies are the most vulnerable population, with higher risks of hospitalization and mortality following infection with SARS-CoV-2 [10–12]. A dysregulated immune response was demonstrated in B-cell-depleted lymphoma patients with COVID-19 [3]. Furthermore, patients who received anti-CD20 mAB within 12 months may fail to develop a protective antibody response against SARS-CoV-2, despite completing a full cycle of mRNA vaccination [13]. In this regard, a recent study reported that the administration of anti-CD20 treatment severely impairs humoral response to BNT162b2 vaccine in patients with non-Hodgkin lymphoma and, even more, that the seroconversion in these patients is dependent on the anti-CD20 treatment timing, becoming present from the 3rd month after the last administration of anti-CD20 [14].

In our cases, patients were treated with Bendamustine associated with an anti-CD20 agent, obinutuzumab or rituximab. Bendamustine decreases CD4 + T-cells count, producing prolonged lymphocytopenia, while anti-CD20 mAB deplete B lymphocytes, impairing humoral immunity. Indeed, Rituximab induces poor responses to various types of vaccinations and provokes viral reactivations as a consequence of rapid and persistent depletion of B cells. Obinutuzumab, a novel type 2 anti-CD20 mAB, enhances antibody-dependent cell mediated cytotoxicity, inducing higher levels of direct cell death than rituximab [8, 9].

Our case reports seem to confirm such findings, as a poor or absent immune response anti-SARS-CoV-2 has been detected by serological tests in both cases, following treatment with anti-CD20 within 3 months (68 and 26 days, respectively).

More specifically, these case reports focus on the persistence of positive SARS-CoV-2 RT-PCR associated with severe and recurrent COVID-19 in hematological patients actively treated with immunosuppressive therapy. We describe two cases of persistence of SARS-CoV-2 RT-PCR-positive NPS for longer than 9 and 5 months respectively, with multiple COVID-19 symptomatic relapses. The number of hospitalizations due to SARS-CoV-2-related pneumonia had been 4 throughout 7 months for patient 1 and 4 throughout 5 months for patient 2, with respectively overall 56 and 43 days of hospitalization. In both cases patients had relevant symptoms at every relapse episode, with severe impairment of health status and quality of life.

It is worth noting that repeated tests always showed the same SARS-CoV-2 sub-lineage. This finding supports the fact that the recurrences over time were driven by persistence of the virus, rather than new infections. This is also in line with the biological characteristics of SARS-CoV-2, a plus-strand RNA virus that does not undergo latency, while it remains persistently replicating, at levels that may also be very low, with sudden relapses. This situation is similar to plus-strand RNA viruses, and can be exacerbated by immunosuppression, that makes the body unable to clear the virus.

The management of hematological patients with SARS-CoV-2 is complex and expensive in terms of hospital resources, demand of acute care and impact on life quality. In addition, differential diagnosis is time-consuming; in our cases, treatments of suspected co-infections had no significant clinical benefit. On the other hand, the administration of antiviral therapy and anti-SARS-CoV-2 mAB was associated with clinical remission during each hospitalization but was not effective to clear SARS-CoV-2 infection.

With this regard, it is relevant to consider that every treatment currently available against SARS-CoV-2 was studied in the general population [15, 16] and not specifically in immunocompromised patients. As argued by several studies [17, 18], immunosuppressed patients may benefit from new therapeutic strategies such as administration of known therapies for a longer period or at an increased dosage or in combination. Furthermore, the use of anti-SARS-CoV-2 therapy as prophylaxis of clinical relapses for these patients should be investigated and we reckon that preventive therapies strategies with tixagevimab/cilgavimab should be implemented in the groups of patients with greater risk of severe COVID-19, particularly those receiving anti-CD20

mAB. Another critical issue, considering the lack of benefit in terms of overall survival, is whether the maintenance treatment with obinutuzumab or rituximab should be withheld in those patients with persistence of positive SARS-CoV2 RT-PCR [19].

Due to the low incidence of SARS-CoV-2 interstitial pneumonia in immunocompetent vaccinated patients, more trials and studies, focusing on a tailored treatment for immunocompromised patients with COVID-19, should be carried out. According to this new set of evidences, it could then be advisable to draw updated guidelines for clinicians regarding clinical management, therapy and prophylaxis of clinical relapses in patients with COVID-19 and hematological malignancies, as attempted in the first phases of the COVID-19 pandemics [20].

In conclusion, the two clinical cases we described highlight the prolonged and relapsing course of COVID-19 in patients with follicular lymphoma treated with bendamustine and anti-CD20 mAB, suggesting that specific preventive and therapeutic strategies should be developed for these patients.

Author contributions Study concept: EF, GD. Data collection: MP, VT, GD, GFS, AG. First draft: EF, GD. Critical revision for important intellectual content: EF, MP, VT, GD. Supervision: CFP, GG, CM. All authors reviewed the manuscript.

Data availability The data supporting this paper are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at AOU Policlinico Modena.

Declarations

Conflict of interest The authors declare no competing interests.

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