

Scientific Letter

# Epidemiological Surveillance of SARSCov2 in β-Thalassemia Patients in the Last Two Years: Reinfection Rate, Insights and Future Challenges

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### To the editor.

Coronavirus disease 2019 (COVID-19), the highly contagious viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a catastrophic effect on the world's demographics, emerging as the most consequential global health crisis.<sup>1</sup>

RNA viruses constantly evolve through the emergence of new variants, acquiring a selective advantage with greater transmissibility and a different severity, circumventing immunity previously acquired either by natural infection or vaccination.

The clinical course of SARS-Cov2 might be asymptomatic or vary from a typical presentation like fever, cough, or respiratory symptoms to atypical presentations such as gastrointestinal symptoms or peculiar symptoms like loss of smell (anosmia), taste (ageusia), or a change in taste (dysgeusia). Data indicate that COVID-19 has a wide range of presentations, and its severity varies from asymptomatic disease to lifethreatening complications.

At the beginning of the pandemic, a study on SARSCov2 infected monkeys seemed to rule out the reinfection risk.<sup>2</sup> Nevertheless, cases in humans have demonstrated this possibility.<sup>3-4</sup>

The prevalence of SARSCoV-2 infection among  $\beta$ thalassemia patients seems to be lower than in the general population; however, associated comorbidities confer the risk of more severe disease with a poorer prognosis. Regular transfusion therapy leads to a deficit in immune response and, thus, to higher susceptibility to infectious events.<sup>5</sup>

Patients with B-thalassemia show a 5-fold increase in age-standardized lethality due to SARSCov2, representing a high-risk population compared with age and sex-matched healthy subjects.<sup>6</sup>

A long-lived protective immunity after primary infection/immunization seems unlikely, and the immune response generated to earlier variants may not cover newer ones. We conducted a retrospective cohort epidemiological investigation at our Center of all documented cases of SARSCov2 reinfection among hemoglobinopathic patients already studied,<sup>7</sup> in chronic transfusional support from February 2020 to the present.

A total of 162 hemoglobinopathic patients were followed; among them, 127 suffered from B-thalassemia (114 with major and 13 with intermediate thalassemia) and 35 with sickle cell disease. Forty-five thalassemic and five sickle cell disease patients were infected. The first infection was documented by a positive reversetranscriptase polymerase chain reaction (RT-PCR) test; and/or a baseline positive serology of SARSCov2 IgG/IgM antibodies (Ab).

Four thalassemic patients had symptomatic reinfection, as demonstrated by a time elapse of >90 days between the first and second positive COVID RT-PCR test, with > 1 intermediate negative swab between the two positive tests, according to Center Disease Control guidelines.

## **Case-Descriptions and Results.**

Patient 1. A 54-year-old female patient, with intermediate B-Thalassemia double heterozygosity Hb Lepore and an IVS II and alpha 3.7 genotype profile, was found to be positive for SARSCov2 in November 2020. During this first episode, she developed arthralgias and asthenia with intermittent cough. Her symptoms did not require hospitalization or activation of home transfusional therapy. Afterward, she was vaccinated with two Pfizer-BioNTech COVID-19 doses in May and November 2021. Antibody titers measured three months after the first vaccination appeared to be protective (IgG spike S 1415 AU/ml, BAU 201 /ml) in accordance with FDA guidelines regarding convalescent plasma use demonstrating protective values of more than 160 BAU/ml.<sup>8,9</sup> In January 2022, she developed a mild sore throat and headache. A nasal swab RT-PCR confirmed the infection, and her paucisymptomatic

clinical course was managed at home as mild COVID-19.

Patient 2. The second case was an unvaccinated 48year-old female with B-thalassemia (CD39/IVS1-110) in chronic transfusional support. She suffered from a severe first COVID-19 episode in August 2021, with life-threatening bilateral pneumonia and acute respiratory distress syndrome requiring high-flow oxygen therapy and in-patient hospital care. Genomic analysis of the SARSCov2 variant of this first infection revealed the presence of the Omicron (B.1.1.529) variant. Transfusion therapy was required during hospitalization, together with intravenous antibiotic therapy to treat gram-negative sepsis due to a centralvenous-catheter-infection. Post-infection antibody titers were not available. Seven months later, she developed diffuse arthralgias and flu-like symptoms, with confirmation of SARSCov2 infection by a nasal swab RT-PCR. Molecular characterization this time was unavailable, and she was managed at home without transfusion. She was never vaccinated because of personal choice and is currently tested with nasal swabs before Day Hospital access.

**Patient 3.** A 45-year-old female suffering from intermediate B-thalassemia (Cod39-homozygosis/Alfa 3.7 type -1-heterozygosis) was found to have COVID-19 twice, confirmed by RT-PCR.

On March 8, 2020, at the beginning of the pandemic, she developed a fever, cough, asthenia, and multiple arthralgias. At this time, it was not easy to be tested, if not in selected hospitals; thus, the patient was not investigated regarding the COVID-19 test. Three months later, however, first contact with the virus was determined once with a serological examination, showing previous SARSCov2 infection with positive IgG antibodies. She was vaccinated with 2 BNT162b2-Pfizer doses in March 2021. Antibody values three months after the first vaccination appeared protective (IgG spike S of 13460 AU/ml, BAU 1911/ ml).

The second COVID-19 episode occurred in February 2022, with a four-day paucisymptomatic flu-like course. Due to worsening anemia, she received a transfusion of filtered red blood cells in the COVID-19 area of the Emergency Department.

**Patient 4.** A 59-year-old female with Major Bthalassemia (Codon 39 B homozygosity) suffered from a first infection in January 2020, presenting with asthenia and headache. A positive serological test for SARSCov2 IgM and IgG antibodies identified COVID-19.

She was vaccinated with 2 BNT162b2-Pfizer doses in February 2020, 15 days after the first mild infection. She also received a BNT162b2-Pfizer third dose in November 2021. Antibody values three months after the last vaccination were present at the high title (IgG spike S of 7688 AU/ml, BAU 1091/ml). The second episode occurred in June 2022, with a paucisymptomatic flu-like course, confirmed by an RT-PCR positive swab.

The patient was transfused in a COVID-19 Emergency Department.

Detailed features of all patients can be found in **Tables 1-2-3**.

**Discussion.** We performed a retrospective cohort study in 127 hemoglobinopathic patients to estimate the incidence of SARSCov2 reinfection over two years and their clinical outcomes.

The rate of reinfection in COVID-19 patients who have recovered and had a long-lasting negative RT-PCR test is an emerging topic.

After the first documented case in August 2020 in Hong Kong, many studies have reported SARSCov2 reinfection after a primary episode.

Up to now, except for a single case report, this is the first cohort study screening thalassemic patients for SARSCov2 reinfection and confirms that patients with thalassemia who have recovered from COVID-19 can be reinfected.<sup>10</sup> All reinfections were less severe than primary and with a shorter duration of the second

Patient N.	Sex	Diagnosis	Age	Date of RT- PCR first positivity	Category of COVID-19	Date of PCR negativity	Date of second RT-PCR positivity	Category of COVID-19	Date of PCR negativity	Days to second RT-PCR positivity
1	F	β-thalassemia	54	15 November 2020	Mild	6 December 2020	25 January 2022	Mild	29 January 2022	430
2	F	β-thalassemia	48	8 August 2021	Severe	23 August 2021	12 April 2022	Mild	17 April 2022	215
3	F	β-thalassemia	45	Not performed, diagnosis based on serological tests	Intermediate	Not performed	4 February 2022	Mild	8 February 2022	690
4	F	β-thalassemia	59	Not performed, diagnosis based on serological tests	Mild	Not performed	28 June 2022	Mild	5 July 2022	540

**Table 1.** Clinical and laboratory characteristics of SARS-Cov2 infection in patients with  $\beta$ -thalassemia (first and second infection).

Patient N.	Diagnosis	Genotype	Splenectomy	Comorbidity	Iron Chelation Regimen	Obesity	Vaccination	Kind Of Vaccinatior	Endocrine Complications	Protective Antibody Title After Vaccination	Admission To The Hospital
1	Intermediate BThalassemia	IVSII and alpha 3,7	YES	Pulmonary microembolism, Recurrent thrombophlebitis HCV related liver disease	DFX	NO	YES	Pfizer BNT162b2	Osteoporosis hypovitaminosis D	Yes antiSARS Cov2 IgG Spike S 1415 AU/ml BAU 201/ml	NO
2	Intermediate BThalassemia	IVS1-110 CD3	YES	Kind II diabetes mellitus, HCV- related liver disease, paroxysmal atrial fibrillation	Sequential Regimen DFX and DFO	YES	NO	NONE	Osteoporosis, hypovitaminosis D, hypothyroidism hypogonadism	NOT AVAILABLE	YES
3	Major BThalassemia	CD39 homozygosity alpha 3,7 heterozygosity	NO	Gastritis, HCV- related liver disease.	DFX	NO	YES	Pfizer BNT162b2	Osteoporosis hypovitaminosis D, GH deficiency	Yes antiSARS Cov2 IgG Spike S 13460 AU/ml BAU 1911	NO
4	Major BThalassemia	BETA 39 mutation, homozygosity	YES	HCV-related liver disease, hypothyroidism, adrenal insuficiency	DFX	NO	YES	Pfizer BNT162b2	Osteoporosis hypovitaminosis D	Yes antiSARS Cov2 IgG Spike S 7688 AU/ml BAU 1091	NO

Table 3. Comparison among the first presentation and the recurrence.

Patient	Source of infection	Presenting symptoms	Chest x ray	ECG	COVID19 specific treatments	Blood trasfusion	Isolation	Clinical course	<b>Iron overload</b> transfer saturation index > 42%
1 F.P.	Sick contact	Arthromyalgias, asthenia, cough	Negative	Normal	No	Yes	Yes	Mild	Yes
2 F.P.	Sick contact	Fever, Cough, Dyspnoea	Positive for bilateral massive infiltrates	Normal	Yes (cortisone, low molecular weight heparin)	Yes	Yes	Severe requiring oxigen supplementation	Yes
3 F.P.	Sick contact	Fever, cough, asthenia	Normal	Normal	No	Yes	Yes	Intermediate	Yes
1 R.	Sick contact								
2 R.	Sick contact	Flu syndrome	Not performed	Not performed	No	No	Yes	Mild	Yes
3 R.	Sick contact	Asymptomatic	Normal	Normal	No	Yes	Yes	Mild	Yes
4 F.P.	Sick contact	Asthenia	Not performed	Not performed	No	No	Yes	Mild	Yes
4 R.	Sick contact	Flu syndrome	Negative	Normal	No	Yes	Yes	Mild	Yes

Legend: F.P. First presentation, R. reinfection.

episode. 3/4 were diagnosed incidentally through random and routine testing. Unfortunately, we were only able to chacarterize the viral genotype in one case. The reinfection incidence is relatively rare, accounting for 0,088% (45/127 infected patients and four reinfections) and rapid virus clearance.

These findings are consistent with other studies in the general population (incidence variable from 0.061% to 0.66%), showing reinfection prevalence among female, unvaccinated patients and omicron variant.<sup>11-12</sup>

According to Thalassemia-International-Federationguidelines (TIF), patients with hemoglobinopathies are frail and prone to COVID-19 reinfection due to multiple factors such as periodic blood transfusion, splenectomy, and iron chelation therapy.<sup>13</sup>

The outcome at reinfection may reflect the balance between friability and immune response. However, the exact role played by the various adaptive immune responses in previously infected/immunized patients during reinfection is unclear, and very little is known about their mechanisms.

Assessment of SARSCov2 memory B and T cellmediated responses in patients exposed to the virus could help to define the risk of future SARS-Cov2 infection; however, unlike other hematological diseases, as of our knowledge, only the humoral immune response has been investigated in thalassemia.

Carsetti et al. have shown antibodies titers significantly lower than controls in patients with transfusion-dependent thalassemia 12 weeks after the second dose, reaching comparable results only after a third additional dose.<sup>14</sup> As a matter of fact, evidence of premature aging of the immune system has been demonstrated in patients with thalassemia, maybe due to multiple transfusions and circulating interleukin with a detrimental effect on immune response, resulting in an immunosenescent profile.

However, splenectomized patients seem to have higher antibodies against the viral Spike protein than non-splenectomized thalassemic patients, probably related to a compensatory mechanism of antibody production by peripheral lymphatic tissue and bone marrow. Indeed, as our patients, thalassemic are good responders to the Pfizer BNT 162b2 vaccine in terms of clinical outcome and humoral response.<sup>15</sup>

Certain immunocompromised people, such as those with primary immunodeficiency or recipients of immunosuppressive therapy,<sup>16</sup> often with an inadequate antibody response, experience months of positivity at different viral loads, alternating symptomatic to a clinical-recovery period. In contrast, efficient response with normal virus clearance in primary infection and rapid negativization at reinfection was proven in our patients.

Thus, immune memory protects from clinical symptoms and reduces viral shedding as vaccines do, but it does not seem to protect against reinfection.<sup>17</sup>

Pathophysiological mechanisms underlying the development of a second infectious episode remain not fully understood, involving both true reinfection or virus reactivation from sanctuaries due to a decreased cellular immune function.

The sensitivity and specificity of diagnostic methods should also be considered to identify true reinfections or long-lasting virus persistence.

All our patients presented with >90 days between first and second positivity, suggesting a real reinfection. Further, at least two RT-PCR swabs should be available to confirm the absence or presence of SARSCov2. False-negative results have been reported, mainly due to sampling procedures. Consequently, when asymptomatic patients are tested, it is not always easy to discriminate between the recurrence of COVID-19 infection, intermittent shedding of RNA fragments, or new onset infections. However, this is unlikely in our cases due to the long-time interval between the two infections.

With the challenges associated with developing an effective COVID-19 immunization and the probability of reinfection by SARS-CoV-2, the risk of severe disease in susceptible hosts may persist.

For this reason, several Monoclonal Antibodies (mAbs) have been developed against SARCov2 and have proven their ability in therapeutic and prophylactic fields. Most of them have indications for use in thalassemia and sickle cell disease. They represent an alternative prevention route for COVID-19, offering short-term protection to those who are not yet vaccinated or lack a proper response to vaccination.<sup>18</sup>

The mildness of reinfection in our 4 cases may suggest that severe disease manifestations are rare once some immunity against the virus has been elicited.<sup>19-20</sup>

**Conclusions.** The true prevalence of COVID-19 reinfection may be difficult to estimate as people with paucisymptomatic or asymptomatic reinfections are less likely to be identified. Variants of concern and the title decline can lead to a higher burden of reinfection in the future, because genetic flexibility may lead to escape humoral immune responses. Ongoing surveillance will be critical, and newer vaccines covering variants could help. Much remains to be learned regarding coronavirus immunity, including the maintenance of immunity against this virus and the etiology of the COVID-19 relapse. Vaccination against SARSCov2 remains crucial to reduce mortality and morbidity of frail patients.

Despite the limitations of a small study sample, the present study is one of the few works providing information on SARScov2 reinfection among frail hemoglobinopathic patients during the two epidemic waves.

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