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Clinical outcome and humoral immune responses of β-thalassemia major patients with severe iron overload to SARS-CoV-2 infection and vaccination: a prospective cohort study

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Summary

Background COVID-19 has raised special concern for patients with β-thalassemia major (β-TM) due to frequent comorbidities, regular blood transfusions, and iron overload. However, the exact implications of COVID-19 for patients with β-TM remain uncertain. We aimed to explore the COVID-19 incidence and severity, and the serological response to SARS-CoV-2 infection and vaccination in patients with β-TM.

Methods Patients with β-TM (n = 105) and age-matched healthy controls, all individuals of all control groups were health care workers of the hospital, were prospectively enrolled at the haematology department of Al-Shifa hospital in the Gaza Strip from January 1st, 2021 to December 31st, 2021. Data on COVID-19 incidence and severity were analysed, with Alpha, Beta, and Delta SARS-CoV-2 variants dominating at that time. Anti-SARS-CoV-2 IgG antibody levels were measured and compared between study groups.

Findings Patients with β-TM showed a higher incidence of SARS-CoV-2 infection than the general population (61.9% vs. 7.1%, p < 0.0001). Most patients with β-TM had asymptomatic (70.8%) or mild disease (26.1%), with no fatalities recorded. COVID-19 illness was more severe among female than male patients with β-TM. Anti-SARS-CoV-2 IgG antibodies were significantly higher in symptomatic patients with β-TM than controls post-infection (geometric mean ÷ geometric standard deviation 1299.0 ÷ 3.3 vs. 555.7 ÷ 2.4 AU/mL, p = 0.009) and post-vaccination (8404.0 ÷ 3.9 vs. 2785.6 ÷ 5.0 AU/mL, p = 0.015). Similar responses were observed when comparing splenectomised to non-splenectomised (both asymptomatic and symptomatic) patients with β-TM post-infection (595.4 ÷ 3.9 vs. 280.7 ÷ 3.5 AU/mL, p = 0.005) and post-vaccination (13,778.2 ÷ 3.2 vs. 4961.8 ÷ 4.1 AU/mL, p = 0.045).

Interpretation This distinctive β-TM cohort exhibited a high susceptibility to SARS-CoV-2 infection but mild disease course. Our findings support favourable serological responses to SARS-CoV-2 infection and to vaccination in patients with β-TM, indicating a potential interplay between iron availability and COVID-19-related immunity.

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Research in context

Evidence before this study

Previous studies indicated that patients with β-thalassemia major (β-TM) are potentially spared from developing a severe course of coronavirus disease 2019 (COVID-19), which could be related to differences in their severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific immune responses. We performed a literature search on the 22nd of December 2022, on PubMed for studies published during the COVID-19 pandemic that described the humoral immune response to SARS-CoV-2 in individuals with thalassemia. We used the following MeSH search terms: "thalassemia", "COVID-19", and "adaptive immunity". We identified two studies examining antibody responses to COVID-19 vaccination in individuals with thalassemia. No studies, however, were identified that examined antibody responses after SARS-CoV-2 infection, or related that to differences in disease incidence and severity observed among patients with β-TM.

Added value of this study

To our knowledge, this prospective cohort study is the first to demonstrate that the humoral immune response in patients with β-TM with severe iron overload and poor chelation therapy is favourable compared to the general population, both after SARS-CoV-2 infection and vaccination. Our findings may potentially explain the relatively mild COVID-19 disease progression as observed in patients with β-TM. We also confirmed the remarkable finding that splenectomised patients with β-TM have higher antibody titres after COVID-19 vaccination compared to those without a splenectomy history, which was also observed after SARS-CoV-2 infection. Finally, to the best of our knowledge, this is the first report which evaluates long COVID in this distinctive thalassemia population.

Implications of all the available evidence

Our study sheds new light on the implications of COVID-19 for patients with β-TM, demonstrating an achievement of good anti-SARS-CoV-2 antibody titres in patients with β-TM, both after infection and vaccination. Our finding supports the hypothesis that patients with β-TM are immunologically protected against SARS-CoV-2, in which iron may play a potential role. This could have implications for management and vaccination strategies in patients with β-TM with COVID-19. Future prospective studies are warranted to further investigate the serological response to SARS-CoV-2 in patients with β-TM.

Introduction

Patients with β-thalassemia major (β-TM), not receiving iron chelation therapy, suffer from several comorbidities related to anaemia and transfusional iron overload, including cardiac, endocrine, and hepatic insufficiency as well as pulmonary hypertension and increased risk for infections. These are attributable to iron overload and/or to an acquired immune deficiency.[1,2] Based on those features, it could be assumed that iron overloaded patients with β-TM are likely to be more susceptible to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and ensuing severe courses of coronavirus disease 2019 (COVID-19).[3] This notion is based on the facts that a high abundance of iron may promote viral replication,[4] and that hyperferritinaemia in COVID-19 was associated with a poor outcome and hyperinflammation.[5] Although hyperferritinaemia prevails in patients with transfusional iron overloaded β-TM, it cannot be associated with the severe outcome of COVID-19 infection accompanied by a rise in plasma ferritin, as the latter is part of the acute phase inflammatory response to the infection per se.[6] High circulatory iron availability and elevated transferrin saturation can promote the catalysation of toxic radical formation and thus contribute to tissue damage in the setting of inflammation associated with SARS-CoV-2 infection.[7] Importantly, recent *in vitro* and *in vivo* studies have indicated that the amount of bioavailable iron is also central for an adequate immune response, whether innate or adaptive, and that iron deprivation or iron loading evokes immune dysfunctions.[8,9]

Previous data have been reported on the implications of COVID-19 for individuals with thalassemia. For instance, data derived from the first wave of the pandemic in Italy showed a lower-than-expected number of infected patients with thalassemia, which was suggested to result from more vigorous self-isolation procedures compared to the general population.[10] Another study designed by the "Italian Society for Thalassemia and Hemoglobinopathies" reported that patients with β-TM exhibited a 5-fold increase in age-standardized lethality compared to the general population.[11] However, the exact implications of COVID-19 for patients with β-TM remains to be elucidated.

Herein, we analysed not only the incidence and severity of SARS-CoV-2 infection in patients with β-TM but also evaluated the humoral immune response following infection and vaccination of patients with β-TM in comparison to age-matched controls.

Methods

Study design

This prospective cohort study was conducted at Al-Shifa hospital (Gaza), from January 1st, 2021 until December 31st, 2021. Alpha, Beta and Delta SARS-CoV-2 variants were dominating at that time during the COVID-19 pandemic. Approval was obtained from the national ethical committee in the Gaza strip (PHRC/HC/1015/21) and conducted according to the guidelines of the declaration of Helsinki. Informed consent was obtained from all study participants.

Study participants and data collection

The present study has included 105 young patients with transfusion-dependent β-TM with age ≥12 years with confirmed genetic β-thalassemia mutation.[12] Patients with other hemoglobinopathies or those younger than the age of 12 were excluded from the study. All patients with β-TM were physically examined, treated, and followed up bimonthly at the Haematology clinic of Al-Shifa hospital. Baseline clinical, laboratory, and radiological data have been collected from the patients' medical records. The baseline information of these patients is given in Table 1. Of note, since iron chelation therapies is insufficiently available in Gaza, patients were either not or poorly iron chelated. For the analysis of the humoral immune response two subsets of β-TM cohort were used. The first subset included patients with β-TM with symptomatic COVID-19 (n = 24 out of the 65 total infected cases) for the measurement of post-infection antibody levels. The second subset included previously uninfected patients with β-TM who received the COVID-19 vaccine (n = 31 out of 40 uninfected individuals) for the measurement of post-vaccination antibody levels. As control groups we included age-matched individuals without β-TM with no documented health comorbidities, which were all health care workers that were evaluated and followed up at Al-Shifa hospital (Table 2). Specifically, control group A included 41 health care workers of Al-Shifa hospital with qRT-PCR-confirmed SARS-CoV-2 infection, who were followed up for measurement of anti-SARS-CoV-2 IgG antibody titres around 3 months of the documented infection. Control group B included 23 healthcare workers with no prior documented history of COVID-19 infection and with a negative qRT-PCR test result for COVID-19 at the time of vaccination with the Pfizer-BioNTech mRNA vaccine. The SARS-CoV-2 IgG Antibody titres were measured at 3 months after the second vaccine dose. All individuals in the β-TM cohort and control cohort A and B were instructed to perform nasopharyngeal sample swabs to detect SARS-CoV-2 infection by qRT-PCR in case of any COVID-19 related symptoms or after being in close contact with a confirmed SARS-CoV-2 case. Furthermore, regular qRT-PCR testing for SARS-CoV-2 were performed for patients with β-TM prior to their bimonthly hospital visits. Moreover, in parallel to measurement of SARS-CoV-2 IgG antibody titres, qRT-PCR tests were performed in both vaccinated and infected individuals (both β-TM and controls) to rule out acute or recent infection.

Additionally, to examine a potential relation of iron-deficiency anaemia to COVID-19 course, an extra group consisting of 230 non-β-TM individuals with symptomatic qRT-PCR-confirmed SARS-CoV-2 infection requiring hospitalization were analysed. The patients were classified according to the haemoglobin level and iron profile—taken on the first day of admission—into non-β-TM (–) (n = 179), which included non-anaemic patients with normal iron status, and non-β-TM (+) (n = 51), which comprised patients with iron deficiency anaemia (Supplementary Table S1).

SARS-CoV-2 qRT-PCR-confirmed cases were classified according to the clinical National Institute of Health (NIH) disease severity criteria as asymptomatic, mildly, moderately, severely or critically ill.[13]

COVID-19 vaccination and the anti-SARS-CoV-2 IgG antibody response

The humoral immune response was quantified by measuring anti-SARS-CoV-2 antibodies using the SARS-CoV-2 IgG II Abbott Architect assay,[14] which detects IgG antibodies to the receptor binding domain of the S1 subunit of the SARS-CoV-2 spike protein. For the post-infection analysis, this assay was performed in symptomatic patients with β-TM (n = 24) and control group A (n = 41) around 3 months after qRT-PCR-confirmed COVID-19. As for the post-vaccination analysis, IgG antibody titres were measured in vaccinated patients with β-TM (n = 31) and in participants of control group B (n = 23) at 3 months after the second vaccine shot. All participants received an mRNA vaccine, either the Pfizer/BioNTech or the Moderna vaccine. The 23 participants from control group B received 2 doses of Pfizer/BioNTech vaccine on days 1 and 21, while among the 31 patients with β-TM, 26 (83.9%) received the Pfizer/BioNTech vaccine on days 1 and 21 and the other 5 (16.1%) patients with β-TM received the Moderna vaccine on days 1 and 28 in accordance to the manufacturer's guidelines.

Statistical analysis

The IBM SPSS Statistics software version 25 was used for data analysis and Python programming language (v.3.9.0, Python Software Foundation) with the *pandas* (v.1.3.3), *matplotlib* (v.3.4.3), and *seaborn* (v.0.11.2) packages for graphic representations. Continuous numerical (quantitative) variables were presented as mean ± standard deviation. Skewed data (SARS-CoV-2 IgG) were presented as geometric mean ÷ geometric standard deviation after log 10 transformation. Categorical data were presented as proportions n with corresponding percentages (%). Assessment of normality was performed both visually using histograms and statistically using the Shapiro–Wilk test. Associations of

	β-TM patients (*n* = 105)
Age (years)	22 ± 6.1
Female sex, *n* (%)	52 (49.5)
BMI (kg/m2)	20.5 ± 2.5
β-TM mutation types in descending order	IVS1-1/IVS1-1, IVS 1–110/IVS1-110, IVS1-6/IVS1-6, N37/N37, N39/39
History of splenectomy	
Yes, *n* (%)	59 (56.2)
No, *n* (%)	46 (43.8)
β-TM-associated complications	
Cardiomyopathy, *n* (%)	15 (14.3)
Endocrinopathy, *n* (%)	38 (36.2)
Transfusion-related characteristics	
Transfusion frequency	Once every 2–3 weeks
PRBC units per year	21 ± 4.1
Blood group	
O, *n* (%)	49 (46.7)
A, *n* (%)	34 (32.3)
B, *n* (%)	16 (15.3)
AB, *n* (%)	6 (5.7)
Laboratory measurements	
PtHb (g/dl)–SP	7.2 ± 0.8
PtHb (g/dl)–NSP	6.8 ± 1.0
TSAT (%)	90.8 ± 6.8
Ferritin (ng/ml)	5577 ± 3015
Iron (μg/dl)	195 ± 510
CRP (mg/dl)	0.8 ± 0.8
Normal Laboratory References	
Hb (g/dl)	Female ≥12/Male ≥13
TSAT (%)	20.0–45.0
Ferritin (ng/ml)	24.0–336.0
Iron (μg/dl)	60.0–170.0
CRP (mg/dl)	≤0.6
SARS-CoV-2 IgG Antibody titers (AU/ml)	≥50

Data are presented as means ± SD or proportions *n* with corresponding percentages (%). For some variables, the [normal range] was added to the table. Abbreviations: BMI, body mass index; β-TM, β-thalassemia major; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; Hb, haemoglobin; NSP, non-splenectomised; PRBC, packed red blood cells; PtHb, pretransfusion haemoglobin; SP, splenectomised; TSAT, transferrin saturation.

Table 1: **β-TM patient characteristics.**

disease severity (ordinal data) with sex, splenectomy history and anaemia (binary categorical data) were examined by performing the chi-squared tests for trend. SARS-CoV-2 IgG titres were non-normally distributed and log-transformed, after which normality was assessed. As most distributions remained non-normally distributed, we employed nonparametric testing for the comparison of IgG antibody titres between two groups. Univariable and multivariable analyses, using backward selection (P_{OUT}>0.05) were performed to identify clinical factors that were associated with both post-infection and post-vaccination (log-transformed) anti-S1 SARS-CoV-2 IgG antibody titres. Standardized beta (β) coefficients and corresponding p-values were reported to represent the magnitude and statistical significance of the associations. Standardized β-coefficients represented the difference in post-vaccination or post-infection anti-S1 SARS-CoV-2 IgG antibody titres per 1-SD increment or decrement. Assumptions of residual variance normality and homoscedasticity for linear regression analysis were satisfied. Finally, for the comparison of post-infection IgG antibody titres between the four ABO blood groups (with a non-normal distribution), the Kruskal–Wallis test with post-hoc correction was performed. A two-tailed p-value of less than 0.05 was considered statistically significant.

Role of the funding source

This study was funded by Mr. Hosam and Wasim s. El Helou. The funding source had no role in study design, in the collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication. No external funding from a pharmaceutical company or other agency was involved

	Control group A (*n* = 41)	Control group B (*n* = 23)
Age (years)	28.6 ± 6.3	29.0 ± 5.7
Female, *n* (%)	18 (43.9)	10 (43.5)
BMI (kg/m2)	21.2 ± 1.7	22.1 ± 1.8
Medical comorbidities	None	None
Laboratory measurements		
Hb (g/dL) [normal: female ≥12/male ≥13]	14.7 ± 0.7	13.9 ± 0.5
TSAT (%) [normal: 20.0–45.0]	32.7 ± 3.5	31.7 ± 2.7
COVID-19-related characteristics		
Prior COVID-19 infection (reported)	Yes	No
COVID-19 vaccination	No	Yes[a]
COVID-19 illness severity		
Mild, *n* (%)	41 (100)	–
Moderate, *n* (%)	0 (0)	–
Severe, *n* (%)	0 (0)	–
Critical, *n* (%)	0 (0)	–
Death, *n* (%)	0 (0)	–

Data are presented as means ± SD or proportions *n* with corresponding percentages (%). For some variables, the [normal range] was added to the table. Abbreviations: BMI, body mass index; COVID-19, coronavirus disease 2019; Hb, haemoglobin; TSAT, transferrin saturation. [a]Individuals were vaccinated with 2 doses of the Pfizer-BioNTech vaccine.

Table 2: **Control group characteristics.**

in writing the manuscript. H.G, H.Z, H.J., L.E.v.E., and G.W. directly accessed and verified the underlying data reported in the manuscript. All authors contributed to the review of the final article and accept responsibility for the decision to submit for publication.

Results

Higher incidence but milder course of SARS-CoV-2 infection in patients with β-TM

Our study demonstrated that the majority of patients with β-TM in our cohort (61.9%) experienced infection with SARS-CoV-2 in the year 2021. This was much higher than the incidence of infection in the general population which was—according to data from Ministry of Health in Gaza strip—at average 7.1% during 2021.[15] This suggests a significantly higher relative incidence of SARS-CoV-2 infection among patients with β-TM compared to the general population.

It must be mentioned, however, that patients with β-TM were frequently PCR-tested for SARS-CoV-2 mostly upon regular hospital visits, which resulted also in the detection of asymptomatic cases and thus in a higher incidence of infections as the compared to that reported for the general population.

Fig. 1A demonstrates a classification of SARS-CoV-2 disease severity of patients with β-TM according to National Institute of Health (NIH) criteria indicating that the majority of those with qRT-PCR-confirmed SARS-CoV-2 infection remained asymptomatic (70.8%), whereas 26.1% of patients with β-TM experienced mild and only 3.1% moderately severe infection. Interestingly, none of the patients with β-TM developed severe or critical COVID-19. Of note, 45.5% of the SARS-CoV-2-infected patients with β-TM in the asymptomatic β-TM category, 17.7% in the mild category, and 0% in the moderate category suffered from cardiac and endocrine comorbidities. Remarkably, COVID-19 severity significantly differed between females and males in the β-TM cohort ($p = 0.013$). While the male sex was predominantly present in the asymptomatic COVID-19 category, the female patients mainly comprised the mild COVID-19 category. Furthermore, the moderate category included only female patients with β-TM (Fig. 1A).

No significant difference of COVID 19 illness severity was found between splenectomised (69.4% asymptomatic, 27.8% mild, and 2.8% moderate disease) and non-splenectomised patients with β-TM (72.4% asymptomatic, 24.1% mild, and 3.4% moderate disease) ($p = 0.863$), as shown in Fig. 1B. Finally, none of the patients with β-TM experienced symptoms 3 months after initial SARS-CoV-2 infection (such as fatigue, dyspnoea, cognitive dysfunction, among others) that could indicate long COVID.

COVID-19 severity in non-β-TM individuals

Supplementary Table S1 indicates the distribution of COVID-19 illness severity of the groups non-β-TM (-anaemia) and non-β-TM (+anaemia). When comparing hospitalised COVID-19 patients without β-TM according to the presence or absence of anaemia upon hospital admission, we found no significant association between anaemic individuals and disease severity ($p = 0.077$) (Supplementary Figure S1). The causes of death in both control groups were related to COVID-19-induced respiratory failure.

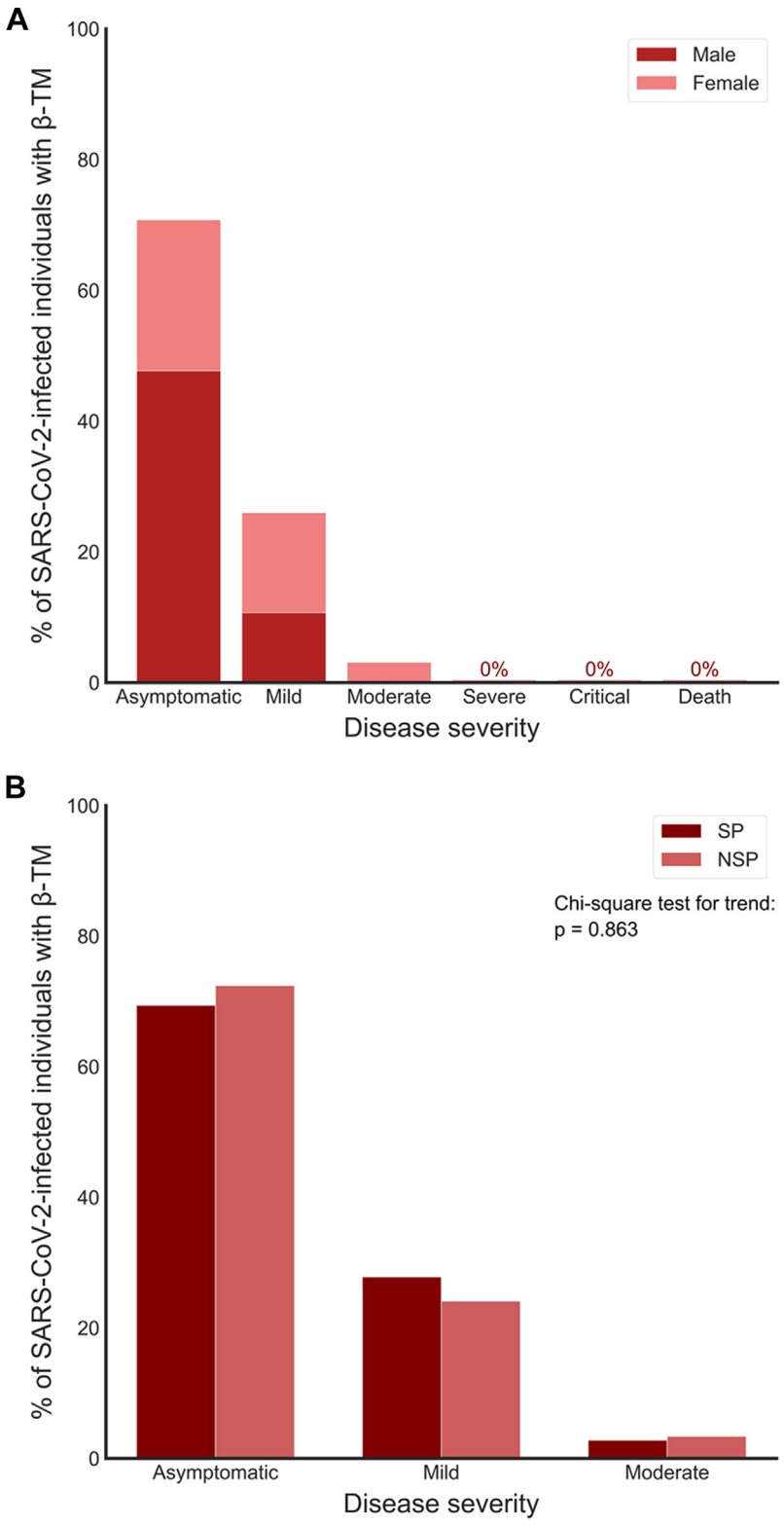

Fig. 1: **COVID-19 illness severity in β-TM categorised by sex (panel A) and splenectomy history (panel B).** (A) COVID-19 illness severity in β-TM cohort (*n* = 105). Most of the patients (70.8%) remained asymptomatic, while 26.1% experienced mild symptoms, and only 3.1%

Humoral immune response after infection or vaccination

Fig. 2A displays a comparison of the humoral immune response between symptomatic patients from the β-TM cohort and control group A, assessed by the serum anti-SARS-CoV-2 IgG titre level after PCR-confirmed SARS-CoV-2 infection, measured at (mean ± SD) 94.8 days ± 2.6 vs. 93.5 days ± 1.9, respectively. The results showed a significantly higher post-infection IgG antibody titre (geometric mean [GM] ÷ geometric standard deviation [GSD] 1299.1 ÷ 3.3 AU/ml) in the β-TM cohort than in the control group (555.7 ÷ 2.4 AU/ml, p = 0.009).

Fig. 2B compares post-infection humoral immune response between the splenectomised and the non-splenectomised patients with β-TM (asymptomatic and symptomatic), measured at (mean ± SD) 90.6 days ± 1.6 for asplenic patients and 89.0 days ± 2.3 for non-splenectomised patients. This time, both asymptomatic and symptomatic individuals were included—in contrast to Fig. 2A – to increase sample size and because no differences in disease severity were observed between the groups (Fig. 1B). This indicates a significantly higher post-infection IgG antibody titre level in the splenectomised (GM ÷ GSD 595.4 ÷ 3.9 AU/ml) compared to the non-splenectomised patients (280.5 ÷ 3.5 AU/ml, p = 0.005).

Furthermore, we studied the humoral immune response to SARS-CoV-2 vaccination in previously uninfected patients with β-TM and control group B. After a mean duration of three months after receiving the second vaccine dose, β-TM individuals presented with significantly higher IgG antibody titres (GM ÷ GSD 8404.0 ÷ 3.9 AU/ml) as compared to age-matched controls (2785.6 ÷ 5.0 AU/ml) (p = 0.015) (Fig. 2C). When analysing the post-vaccination humoral immune response between splenectomised and non-splenectomised patients with β-TM, we found significantly higher anti-SARS-CoV2 IgG titres in the splenectomised patients (GM ÷ GSD 13778.2 ÷ 3.2 AU/mL) as compared to the non-splenectomised group (4961.8 ÷ 4.1 AU/mL, p = 0.045) although with a high variation of results (Fig. 2D).

To identify clinical factors associated with anti-S1 SARS-CoV-2 antibodies, linear regression analyses were performed. Univariable linear regression in infected β-TM individuals demonstrated that splenectomy history (St.β 0.279, p = 0.041), symptomatic COVID-19 (St.β 0.560, p < 0.001), and creatinine (St.β 0.311, p = 0.031) were significantly positively associated with post-infection anti-S1 SARS-CoV-2 IgG titres, whereas alanine transaminase (ALT) (St.β –0.346, p = 0.016) showed an inverse association. The significance of the association with a history of splenectomy was, however, not sustained after adjustment for creatinine, ALT, and symptomatic COVID-19 (St.β 0.236, p = 0.058) (Supplementary Table S3). As for vaccination, splenectomy history was the only clinical factor significantly associated with post-vaccination anti-S1 SARS-CoV-2 IgG titres (Supplementary Table S6).

No notable difference in the antibody immune response was found between the vaccine type (Pfizer BioNTech or Moderna) (p = 0.358). Of interest, we found significant differences in post-infection anti-SARS-CoV-2 IgG titres in patients with β-TM with both asymptomatic and symptomatic COVID-19, according to their underlying blood group antigens (p = 0.013), with highest titres in individuals with blood group O (GM ÷ GSD 684.9 ÷ 3.2 AU/ml), followed by blood group AB (397.2 ÷ 2.6 AU/ml), blood group A (366.4 ÷ 4.7 AU/ml), and blood group B (162.2 ÷ 2.2 AU/ml). Post-hoc analysis indicated that patients of blood group O and blood group B were significantly different regarding their post-infection antibody levels (p = 0.012) (Fig. 3). Overall, there was no significant association between blood group types, infection severity (p = 0.771), and the post-vaccination titre level in the β-TM cohort.

Discussion

Our study presents a distinctive cohort of patients with transfusion-dependent, poorly iron-chelated β-TM of whom more than half underwent splenectomy. All patients showed evidence of severe iron overload, sometimes reflected by the presence of iron related complications, such as cardiac, hepatic, and endocrine dysfunction. Our results indicated that patients with β-TM tested positive for SARS-CoV-2 more frequently as compared to the general population. Even though patients with β-TM had more co-morbidities, infections were mostly asymptomatic or mild and no severe or fatal courses of COVID-19 were recorded. Interestingly, none of the patients with β-TM developed long COVID.

The finding of a higher incidence of SARS-CoV-2 infection among patients with β-TM compared to the general population is contradictive of the results of a lower COVID-19 incidence rate among patients with

developed a moderate infection. None of the infected patients with β-TM fell into the severe, critical, or death categories. A significant difference in COVID-19 illness severity between infected male (*n* = 38) and female (*n* = 27) patients with β-TM was found (p = 0.013). While the male infected patients with β-TM were predominantly included in the asymptomatic category, the mild category included mostly female patients, and the moderate category was comprised only of female patients. (B) No significant difference in COVID-19 illness severity was found between splenectomised (*n* = 36) and non-splenectomised patients with β-TM (*n* = 29) (p = 0.863). Abbreviations: β-TM, β-thalassemia major; COVID-19, coronavirus disease 2019.

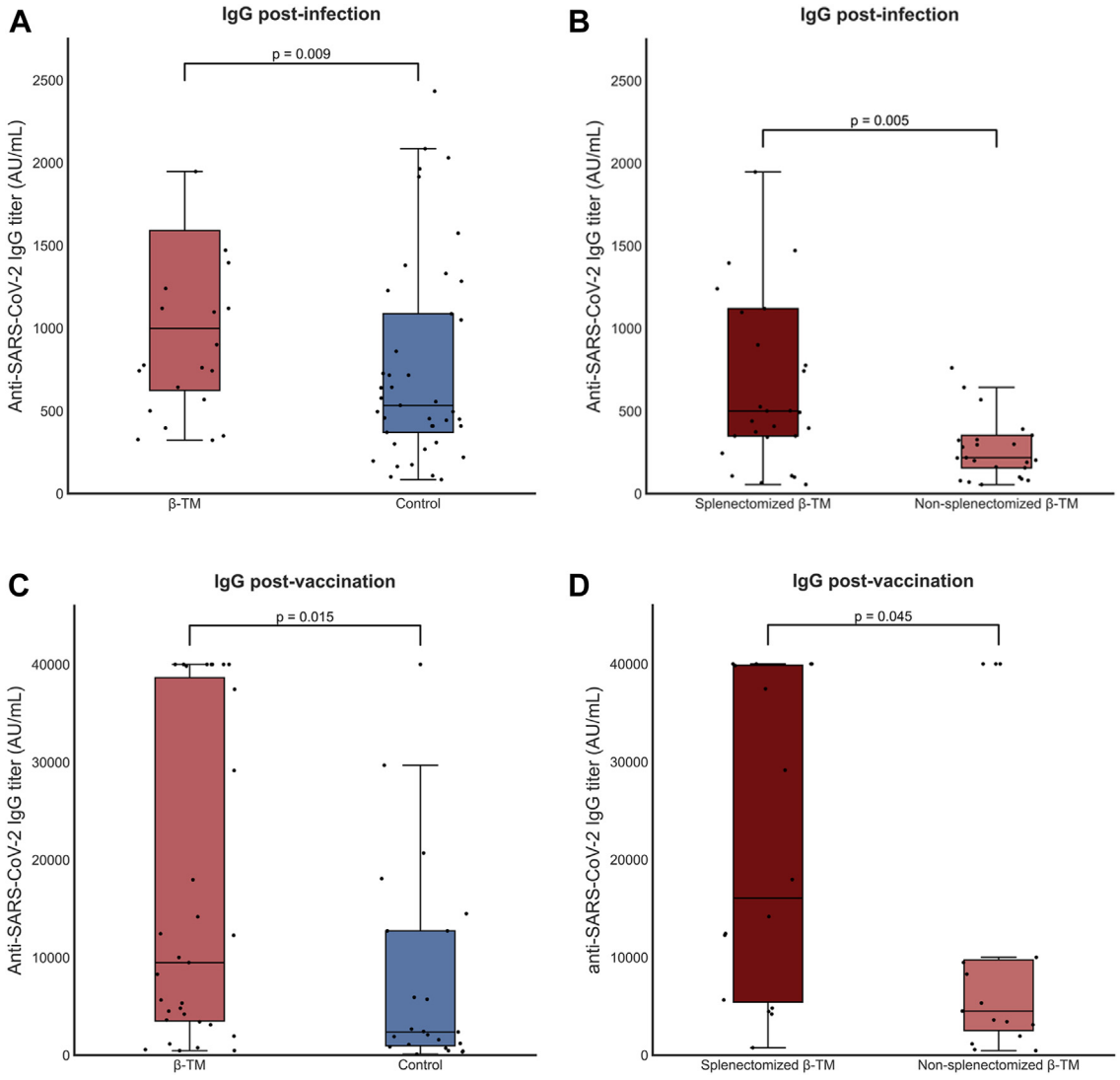

Fig. 2: **Serum anti-SARS-CoV-2 IgG antibody titres in patients with β-TM and age-matched control groups both 3 months post-infection (Panels A & B) and 3 months post-vaccination (Panels C & D).** (A) Symptomatic β-TM (*n* = 24) showed significantly higher anti-SARS-CoV-2 IgG antibody titres compared to control group A (*n* = 41) after infection (GM ÷ GSD 1299.0 ÷ 3.3 vs. 555.7 ÷ 2.4 AU/ml, p = 0.009). (B) Post-infection anti-SARS-CoV-2 IgG antibody titres were significantly higher in (asymptomatic and symptomatic) splenectomised (*n* = 29) compared to non-splenectomised patients with β-TM (*n* = 25) (GM ÷ GSD 595.4 ÷ 3.9 vs. 280.7 ÷ 3.5 AU/ml, p = 0.005). (C) In comparison to age-matched individuals of control group B (*n* = 23), previously uninfected vaccinated patients with β-TM (*n* = 31) had significantly higher anti-SARS-CoV-2 IgG antibody titres (GM ÷ GSD 8404.0 ÷ 3.9 vs. 2785.6 ÷ 5.0 AU/mL, p = 0.015). (D) Among the vaccinated patients with β-TM, splenectomised individuals (*n* = 16) had a significantly higher anti-SARS-CoV2 IgG titre than non-splenectomised individuals (*n* = 15) (GM ÷ GSD 13778.2 ÷ 3.2 vs. 4961.8 ÷ 4.1 AU/mL, p = 0.045). Abbreviations: β-TM, β-thalassemia major; GM, geometric mean; GSD, geometric standard deviation; IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

β-TM described in a recent large meta-analysis.[16] Our results may in part be explained by more frequent testing of patients with β-TM, who require at least bimonthly blood transfusions and PCR screening tests prior to entering a hospital. Furthermore, most patients did not follow the internationally advised precautions and hygienic measurements to prevent SARS-CoV-2 infection, which can be attributed to several causes, including their poor socioeconomic status in addition to the psychosocial and emotional implications caused by the distressing treatments and physical deformities due to the disease.[17] We do not have enough information regarding the preventive measures of the non-β-TM population; however, according to statistical data derived from the Palestinian Central Bureau of Statistics, almost 53% of the population in Gaza strip suffers from poverty

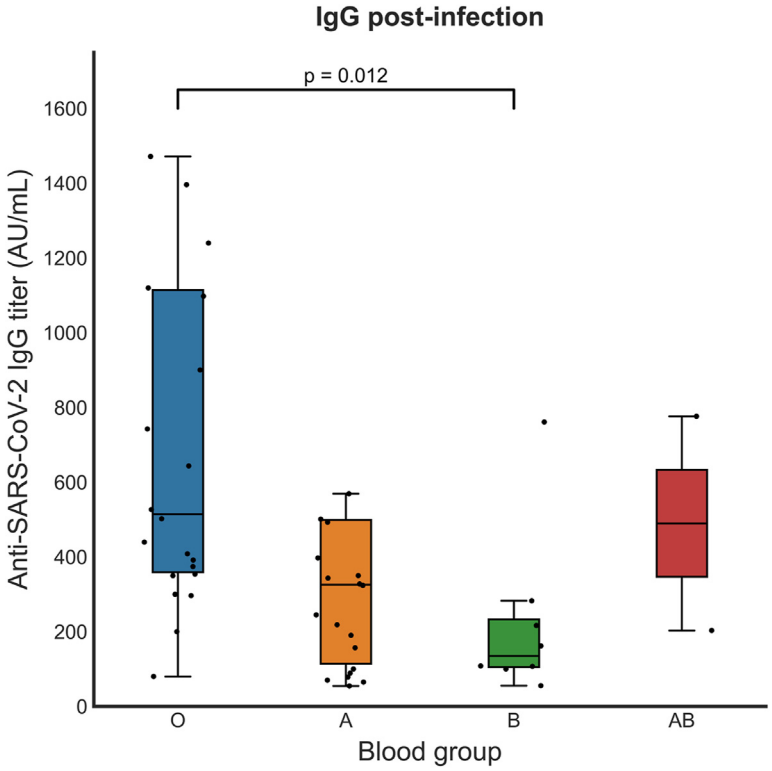

***Fig. 3*: Serum anti-SARS-CoV-2 IgG antibody titres in β-TM patients after infection, categorised by blood group**. Significant differences in post-infection anti-SARS-CoV-2 IgG titres in patients with β-TM, with both asymptomatic and symptomatic COVID-19, according to their underlying blood group types indicate lowest titres in individuals with blood group B (*n* = 8) (GM ÷ GSD 162.2 ÷ 2.2 AU/ml) and highest titres in blood group O (*n* = 22) (684.9 ÷ 3.2 AU/ml), followed by blood group AB (*n* = 2) (397.2 ÷ 2.6 AU/ml), and blood group A (*n* = 22) (366.4 ÷ 4.7 AU/ml) (p = 0.012). Abbreviations: β-TM, β-thalassemia major; GM, geometric mean; GSD, geometric standard deviation; IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

which makes it difficult for the general population to adhere to the advised COVID-19 testing policy, as well as preventive and self-isolation measures.

Although asplenic individuals are at higher risk of encapsulated bacterial sepsis,[18] studies showed no evidence that splenectomy raised the risk for COVID-19 severity,[19] which is in line with our data revealing no significant difference in COVID-19 severity between splenectomised vs. non-splenectomised patients with β-TM. Surprisingly, splenectomised patients with β-TM had higher anti-SARS-CoV-2 IgG antibody titres after infection and vaccination as compared to non-splenectomised counterparts. This is in line with a previous study demonstrating the finding of higher anti-SARS-CoV-2 antibody titres in splenectomised patients with transfusion-dependent thalassemia after COVID-19 vaccination, as compared to those without a splenectomy history.[20] The mechanism behind this interesting finding remains to be elucidated. However, one may speculate that the affected spleens in the non-splenectomised β-TM individuals filter out the antibodies in the blood, leading to lower circulating antibody concentrations compared to splenectomised patients with β-TM. Another potential reason is the administration of vaccines directed against other pathogens to prevent post-splenectomy sepsis that may boost the immune system and could possibly indirectly enhance the humoral immune response to SARS-CoV-2 in asplenic patients with β-TM.

Furthermore, we found that patients with β-TM in general had higher anti-SARS-CoV-2 IgG antibody levels following infection or vaccination as compared to the control group. Previous studies have examined the immune responses following SARS-CoV-2 vaccination in patients with hemoglobinopathy, including thalassemia,[21–23] but the current study specifically concerns a cohort of patients with severely iron-overloaded, transfusion-dependent β-TM. This is interesting, as iron availability is crucial for B and T cell differentiation,[24] and iron deficiency has been associated with reduced responses to vaccines.[25] Thus, one could speculate that the high iron availability in patients with TM promotes adequate immune responses, whereas in the general population, the high

prevalence of iron deficiency and iron deficiency anaemia of around 20% in the Gaza strip hampers efficient humoral immune responses. However, we did not find a significant difference in disease severity between hospitalized COVID-19 patients without β-TM with iron deficiency anaemia (non-β-TM (+)) and those without (non-β-TM (–)).

It may well be that a more sustained humoral immune response among patients with β-TM during infection represents an underlying protective mechanism against SARS-CoV-2 infection severity.[26,27] Of note, T-cell immune responses play essential roles for the control of SARS-CoV-2 infection and specifically for preventing severe COVID-19.[28] Thus, it will be of interest to further investigate T-cell immunity at baseline, as well as after infection and vaccination in patients with β-TM, being aware of the fact that iron availability can affect T–cell differentiation, activation and cytokine expression.[29]

This leads to the question why individuals with β-TM in our cohort developed less severe COVID-19. First, a potential explanation may be increased levels of erythropoietin (EPO). EPO is the main regulator of erythropoiesis,[30] and is mostly produced in the kidneys in response to tissue hypoxia.[31] High levels are found in association with ineffective erythropoiesis as observed in a similar β-TM cohort.[32] In addition to its erythropoietic activity, EPO exerts a variety of pleotropic effects through distinct mechanisms and plays important roles in tissue protection, tissue regeneration, anti-apoptosis, and anti-inflammation.[33,34] Moreover, EPO has been shown to inhibit pro-inflammatory immune effector pathways by inhibiting NF-kB signaling,[34] a central pathway being involved in severe COVID-19 and associated hyperinflammation.[35] Thus, it may be assumed that SARS-CoV-2-infected patients with β-TM are somewhat protected from hyperinflammation and severe COVID-19 by their high EPO levels.

Another potential explanation to the milder COVID-19 course in patients with β-TM involves the structure of haemoglobin in β-TM. In general, haemoglobin consists of four globin subunits, 2-α and 2-β, and each subunit has an iron-bound porphyrin, known as haem. It has been suggested that the SARS-CoV-2 proteins ORF1ab and ORF3a bind to the β-globin chains, resulting in the breakdown of haem into free iron and porphyrin, leading to loss of the oxygen-carrying function of hemoglobin.[36] In β-thalassemia, the production of β-globin chains is partially reduced (β-thalassemia minor or intermedia) or completely absent as in β-TM.[1] In patients with β-TM, the oxygen binding function of HbA is taken over by the foetal Hb (HbF, α2/γ2) and HbA 2 (α2/δ2). Both of these forms of haemoglobin do not contain β-globin subunits.[1] If, as suggested, SARS-CoV-2 selectively affects β-globin chains, then patients with β-TM would be fortunately spared from the virus-related haem attacking effect. In our study none of the SARS-CoV-2-infected patients with β-TM had a decrease in Hb level or an increase in blood transfusion frequency.

Furthermore, in individuals without TM, 10–15% of the patients with COVID-19 in the pre-Omicron surges progressed into a life-threatening acute respiratory distress syndrome,[37] which became also evident in our group of hospitalized COVID-19 patients without β-TM. Interestingly, although the poorly iron-chelated patients with β-TM had severe iron-overload, none of them experienced severe, critical, or fatal COVID-19 illness. COVID-19 severity and mortality are largely related to the hyperacute cytokine storm, caused by an uncontrolled release of inflammatory cytokines and chemokines, mainly from monocytes and macrophages, as a consequence of poor control of viral replication.[38] However, iron-overload can be associated with impaired innate immune responses. Specifically, iron loading of macrophages reduces the formation of pro-inflammatory cytokines such as TNF-alpha or IL-6 while increasing the formation of anti-inflammatory cytokines such as IL-10.[39] This is in line with the observation of reduced formation of those cytokines and impaired neutrophil function in dialysis patients under treatment with iron or with iron overload.[40] We hypothesize that iron-overloaded monocytes and macrophages may be associated with lowering and slowing down the acute cytokine response in COVID-19, which may explain the low infection severity among patients with β-TM with severe iron overload. This point needs to be further explored in future studies.

Finally, different published research has demonstrated favourable COVID-19 disease courses among individuals with blood group O.[41] Although patients with β-TM with blood group O were seen to have the highest post-infection SARS-CoV-2 IgG titres, this finding was not translated into milder severity. Consistent with a previous study, we found no association of blood group type with post-vaccination IgG antibody responses.[42]

This study has several strengths and limitations warranting recognition. Most notably, this prospective cohort study provides novel evidence of favourable humoral immune responses in patients with β-TM with severe iron overload as compared to the general population, both after SARS-CoV-2 infection and vaccination. The poor chelation therapy in these patients makes this cohort unique and may reinforce discussions to which extent iron availability may be involved in COVID-19-related immunity. The availability of data on splenectomy history allowed us to make observations on quantitative humoral immune responses in splenectomised and non-splenectomised patients with β-TM separately, showing the remarkable finding that higher post-infection and post-vaccination antibody titres are present in splenectomised patients in comparison to those without splenectomy. An important limitation of

the current study includes its small sample size, both in the β-TM and control groups. As this study included such a unique cohort of patients with severely iron-overloaded, transfusion-dependent β-TM, with no comparable populations described in similar studies on COVID-19 incidence, severity, and humoral immune responses, an a priori sample size calculation could not be performed. A larger confirmatory study is warranted to validate our results. Furthermore, even with the evaluation of previous COVID-19 infection by means of medical records and PCR tests for SARS-CoV-2—prior to antibody measurements in both patients with β-TM and controls, and bimonthly at hospital visits in patients with β-TM—it cannot be ruled out with certainty that patients already had previous episodes of infection. Availability of anti-SARS-CoV-2 antibody titres prior to vaccination or infection would have been more optimal to clarify this issue.

In summary, we provide evidence for a high susceptibility to SARS-CoV-2 infection but mild clinical course of COVID-19 in poorly iron-chelated patients with transfusion-dependent β-TM. We also provide evidence for favourable humoral immune response of patients with β-TM following infection and vaccination compared to non-anaemic controls with normal iron status. The underlying factors that modulate the favourable immune response to COVID-19 infection in patients with β-TM need to be explored in the context of the last pandemic but also in the more general context of a possible adaptive protection that patients with chronic overloaded β-TM develop against different pathogens.

Contributors

H.G. contributed to conceptualization and research design. H.G. and H.Z. performed the methodological procedures. H.G. and H.Z. analysed the data. H.G., H.Z., L.E.v.E., and A.R.B. prepared the figures. H.G., H.Z. and I.G. wrote the original draft of the manuscript. H.G., L.E.v.E., and G.W. were responsible for editing and finalizing the manuscript. H.G, L.E.v.E. and G.W supervised the research. H.G, H.Z, H.J., L.E.v.E., and G.W. directly accessed and verified the underlying data reported in the manuscript. All authors contributed to the review of the final article and accept responsibility for the decision to submit for publication agreed to submission of the final version of the manuscript.

Data sharing statement

The data that support the observations in this study are available from the corresponding author, H.G. upon reasonable request and with a signed data access agreement. No identifying data will be provided.

Editor note

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Declaration of interests

There are no competing interests to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102096.

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