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Pathology Deterioration in a Pure β^0 Thalassemia Heterozygote After mRNA COVID-19 Vaccination: A Case Report and Literature Review

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Abstract

Background: β-thalassemia heterozygotes produce sensitive levels of fetal hemoglobin and hemoglobin A2 to remain asymptomatic for life compared to β-thalassemia intermedia and β-thalassemia major patients. The asymptomatic β^0 thalassemia minor individuals rarely deteriorate to the point of requiring a blood transfusion.

Case report: An asymptomatic individual with a pure β^0-thalassemia trait, after his first and only Pfizer modified mRNA COVID-19 injection, immediately developed cardiological, neurological, and other clinically important symptoms. The patient's severe physical impairments resembled a presyncope (about to feint) syndrome. Multiple hematological tests prior to and after the Pfizer injection revealed that the patient sustained a medically important rise in fetal hemoglobin and concurrently a remarkable drop of his hemoglobin A2 levels, compared to prior to mRNA injection. Moreover, the alterations in his life sustaining fetal hemoglobin and hemoglobin A2 levels was accompanied by a clinically significant lowering of blood hemoglobin concentration that required blood transfusion. The patient's antibody response to the spike protein remains very high (> 10,000 AU/ml) even almost three years after the Pfizer injection. Furthermore, the elevated levels of C-reactive protein — through May 2024 — after the mRNA injection, apart from pointing to multi-organ systemic inflammation, are consistent with his elevated levels of anti-p53 autoantibodies.

Conclusions: The simultaneous decrease of patient blood hemoglobin levels was consistent with the hematological readings of mean corpuscular volume, hematocrit, ferritin, folate, and zinc level deteriorations soon after the mRNA injection, which, apart from his double digit rise in C-reactive protein, resemble overall the pathological manifestations of a β-thalassemia worsening condition. By performing an investigative literature review we conclude that an autoimmune hematological disorder contributed to the patient's severe hematological stress.

Keywords: *SARS-CoV-2 mRNA vaccine; β^0-thalassaemia deterioration; pre-syncope syndrome; hematological stress; autophagy inhibition; chronic inflammation*

Introduction

β-thalassemia is a hereditary disorder characterized by a genetic deficiency in the synthesis of beta-globin chains, leading to impaired hemoglobin function. Symptomatically, the condition can range from having no symptoms at all to having severe and life-threatening consequences, depending upon the mutations involved and homozygous versus heterozygous inheritance. The terminology refers to thalassemia minor, thalassemia intermedia, and thalassemia major, to distinguish among the degrees of severity. Complications include microcytic anemia, iron overload cardiomyopathy and arrhythmias, among many other symptoms (Sanchez-Villalobos et al., 2009).

It is likely that people with β-thalassemia traits could have an increased potential to develop anemia following mRNA injection, due to impaired hemoglobin production during erythropoiesis (Rivella, 2009). The SARS-CoV-2 spike protein inhibits fetal hemoglobin in erythroid precursor cells of β-thalassemia patients, as well as inducing γ-globulin mRNA accumulation. Moreover, in *in silico* studies, it was demonstrated that the spike protein exhibits high affinity to fetal hemoglobin, comparable to the affinity with angiotensin-converting enzyme 2, ACE2 (Cosenza et al., 2024). The spike protein upregulates nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ϰB) expression, and NF-ϰB is known to suppress the expression of erythroid genes, including globin genes (Liu et al., 2003). It is becoming increasingly apparent that the modifications to the mRNA in the injectables resulted in its persistence long after it would normally have been cleared, and the double-proline modification in the mRNA spike protein coding sequence may also have significantly lengthened the lifespan of the spike protein itself (Boros et al., 2024).

Furthermore, β-thalassemia trait individuals are vulnerable to immune abnormalities that reflect their immune system imbalance of homeostasis, which results in an impaired innate immune response (Gluba-Brzózka et al., 2021). A study involving a patient with a β-thalassemia trait who received mRNA COVID-19 injection showed that, although the patient was also receiving an immunosuppressant agent to increase fetal hemoglobin levels, the patient, surprisingly, had a high innate immune response to the mRNA injectable — indicated by the production of high levels of SARS-CoV-2 spike protein neutralizing antibodies — a scenario which was not supposed to happen (Gamberini et al., 2023). The immunosuppressant, sirolimus, that the patient was receiving inhibits mToR, the mechanistic target of rapamycin, activity, and thus suppresses its related pathway. Through this suppression, the innate immune response is downregulated. Activated mTOR suppresses autophagy, and impaired autophagy directly causes hyperactivation of the innate immune response, which can lead to neurotoxicity due to severe inflammation (Shukla et al., 2019).

Impaired autophagy and increased mTOR activation are distinctive features of SARS-CoV-2 infection (Zambalde et al., 2022). Both of these features contribute to increased severity of disease during infection. Specifically, in the study by Gawaz et al. (2024), it was shown that SARS-CoV-2 spike protein accumulates within autophagosomes to promote inflammation and endothelial damage. Moreover, the spike protein promotes an autophagic inflammatory-apoptotic (cell death) process via upregulated reactive oxygen species (Li et al, 2021). The accumulation of spike proteins in phagosomes can induce the impairment caused by accumulation of other cellular proteins in autophagosomes which otherwise were destined for degradation. The degradation of unwanted proteins is vital for the cell to survive (Yang & Klionsky, 2009). In addition, another study reveals that SARS-CoV-2 spike protein, on its own, interferes with and downregulates normal autophagic processes (Halma et al., 2024). This is especially evident due to upregulation of the cytokine IL-18 by the SARS-CoV-2 spike protein, which causes a downregulation of a special form of autophagy called mitophagy. Through this process, reduced mitophagy due to the SARS-COV-2 spike protein, is believed to induce cardiopulmonary inflammation (Liang et al., 2023). When mitophagy is reduced, the clearance of damaged mitochondria through autophagosomes becomes dysfunctional, and dysregulates normal apoptotic processes within the cell (for a detailed review see Ding & Yin, 2012). Importantly, when autophagy is impaired, this cellular dysfunction can lead to several autoimmune disorders (Z. Yang et al., 2015).

Autoimmune disease is a known category of adverse reactions to the mRNA injectables, and a possible cause is molecular mimicry, whereby an amino acid sequence in the spike protein is sufficiently similar to an amino acid sequence in a human protein that it is disabled by the antibodies produced in response to the spike protein (Halma et al., 2024; Safary et al., 2023). One particularly alarming possibility is an immune attack on the C-terminal globular domain of

the prion protein, due to the resemblance of the sequence YQRGS in the prion protein to the sequence YQAGS in the receptor binding domain of the spike protein (Seneff et al., 2023). Antibodies targeting the globular domain of prion protein are neurotoxic, even though they do not trigger prion replication (Frontzek et al., 2016). The SARS-CoV-2 mRNA vaccines have also been linked to cardiac arrhythmias, including atrial tachyarrhythmias, bradyarrhythmia, ventricular arrhythmias, sudden cardiac death, and the frequently occurring myocarditis (Patone et al., 2022; Pari et al., 2023).

In the case study we present here, the Caucasian male patient, who is a carrier of a β^0-thalassemia trait, has sustained life threatening cardiological, neurological, respiratory, hematological and many other disease manifestations that appeared soon after his single mRNA COVID-19 injection and unfortunately were still occurring through May 2024.

Detailed Case Description

In this report, we describe a unique case of an mRNA COVID-19 injectable (Pfizer) injury regarding a 37-year-old male patient with a pure β^0-thalassemia trait, who had received a single COVID-19 mRNA injectable and developed severe anemia-related pathology. The mixture of cardiological, respiratory, neurological and muscular defects of this are numerous and have persisted since his single COVID-19 mRNA injection back in July 2021, up until the present time (May 2024). The patient gave a written and signed consent form allowing us to publish this case study. The patient, prior to the mRNA vaccination, was a healthy individual with Body Mass Index (BMI) up to 29.32, and asymptomatic from his β-thalassaemia trait. The patient was a long-distance walker and exercised regularly. He was never found in need to conduct an obstruction sleep apnea (OSA) evaluation.

Cardiological Complications

Immediately after the COVID-19 injection, the patient developed palpitations which were subsequently confirmed by EKG monitoring in the ED as a supraventricular tachycardia of 200 bpm. This first episode lasted 1 hour followed by a period of 3 hours where the rate persisted at 150 bpm and then gradually reduced to 100 bpm for a further 3 hours before returning to normal. The patient experienced recurrent episodes of tachycardia over the next 7 months especially during the night, each lasting approximately 40 minutes. He also experienced prolonged paradoxical episodes of bradycardia dropping to a rate of 45 bpm. The patient re-presented a number of times to the emergency services but did not receive medications, nor was he given a firm cardiological diagnosis.

The episodes of high heart rhythm abnormalities were accompanied by high blood pressure (episodes reaching 200mmHg /150 mmHg). The hypertensive episodes lasted for 50 minutes on average for each episode, and then the blood pressure dropped gradually to normal limits after one hour. During the high blood pressure episodes, no arrythmias were detected, e.g., as in its 24-hour Holter evaluation described in the following section of differential diagnosis.

However, Holter evaluations performed on 26.07.2022 revealed tachycardia events (127 pulses per minute), as shown in Figure 1. A much later Holter examination conducted on 16.01.2024 revealed that the patient still suffered from sinus tachycardia episodes, with maximum heart rate up to 151 beats/minute (data not shown).

Moreover, the patient had a structurally normal heart, as revealed by his echocardiographic evaluation that was conducted during the time period of the heart rhythm abnormalities. After the hypertensive episodes that lasted for almost a year after the mRNA injection, the patient developed hypotension with a blood pressure of 103/55 mmHg during a rate of 40-50 bpm, that

lasted for the next three months. Similar hypertensive and hypotensive episodes are described after the mRNA injections (Simonini et al., 2022).

THE HEMATOLOGICAL COMPLICATIONS

Prior to the mRNA injection, our patient was an asymptomatic individual with a pure β-thalassemia trait of Greek origin. However, for the sake of this description, as detailed in later sections, the patient underwent thorough genetic screening to identify the globin gene mutations responsible for his thalassemia condition.

The patient's hematocrit, serum ferritin, free blood iron, and folate were within normal limits prior to the mRNA injection. However, according to his β-thalassemia trait of Greek origin, he had elevated levels of fetal hemoglobin and hemoglobin A2 which can be considered typical of this condition (Kattamis et al., 1979). His red blood cell count and distribution width were slightly raised, as expected. Also as expected in individuals with asymptomatic β-thalassemia traits, he had a slightly lower mean corpuscular hemoglobin and mean corpuscular volume than normal (Roth et al., 2018). Our patient, in a short time interval after the mRNA injection (days), experienced a lowering of his hematocrit value, below the normal limits that he had had prior to injection, a further lowering of his mean corpuscular volume value, a further rise in his red cell distribution width, a lowering in his hemoglobin estimated concentration in blood, and a significant rise of his fetal hemoglobin (+ 211.11%) and a drop of his hemoglobin A2 (- 27.45 %) levels, as summarised in Table 1. Moreover, our patient, after the mRNA injection, had a significant decrease (-25 %) in his low folate levels, which were originally (prior to the mRNA injection) within normal borderline limits (measurements after the mRNA vaccine were made several months and 2.5 years after; see Table 1). Also, the patient has a continuous clinically significant rise of his serum ferritin levels, which has persisted for the entire 2-plus years after the mRNA injection. In addition, ever since his mRNA injection (see Table 1), the patient has shown repeatedly (through May 2024) a persistent leukocytosis (Mank et al., 2024).

THE RESPIRATORY COMPLICATIONS

During the first 4 months of his deterioration post-injection, the patient experienced profound episodes of hypoxia, often during the periods of tachycardia described. These were confirmed by home oxygen saturation monitoring reaching as low as 80%. These were accompanied by dyspnea, weakness of the respiratory muscles, insomnia and severe headaches. These episodes lasted for several days. A sleep study almost a year after the injection confirmed multiple episodes of sleep apnoea with the lowest reading of 86%. Later, in April 2022, the patient reported that he felt he was having a stroke-like episode harming the whole left side of his body. The patient said that he felt that his left side "broke "from head to foot and he noticed in the next few days a slight left side facial palsy. These effects, however, were not feasible for the patient to record, as he was alone at home during that time.

After the extreme episode of low oxygen saturation, the patient himself decided that he required the help of a BiPAP — bilevel positive airway pressure — non-invasive ventilation (face mask) — especially during sleep, to help withstand a drop measured by the pulse oximeter into the 80%-85% range of oxygen saturation. The patient required BiPAP oxygen supplementation for the next four consecutive months. On two further occasions, i.e., July 2023, and May 2024, the patient again sustained similar severe drops (80-85%) in oxygen saturation and, as prescribed by a pneumologist this time, the patient was advised to begin to use the BiPAP oxygen supplementation as needed. The oxygen supplementation on these later occasions lasted for approximately a month.

Table 1
Selective Blood Test Findings Prior to and Throughout the Time After Patient Received an mRNA COVID-19 Pfizer Injection

Day.Month.Year, or Interval (in column 6) when blood/serum parameter(s) or units were collected as reported here	One month before mRNA injection	Measures or Units after the mRNA injection						
	28.09.20	**14.04.22**	**31.07.21**	**15.02.22**	**18.3.22-14.04.22**	**21.04.22**	**04.01.24**	**25.01.24**
Red Blood Cell count [M (10^{12})/L)] Normal: 4.5-5.5 M/L	6.97	7.17	6.41	6.96	6.83			6.88
****Mean corpuscular volume [fL] Normal: 83-101 fL**	61.3	**60.7**	**58.2**	**58.3**	**59.6**			**59.6**
Mean corpuscular hemoglobin [pg] per cell Normal: 25-33 pg	18.6	18.7	19.2	18.8	18.6			19.3
Mean corpuscular hemoglobin concentration [g/L] Normal: 315-345 g/L	304	308	330	302	312			324
Percent Red blood cell distribution width Normal: 11-16%	16.3	**18.6**	**21.2**	**20**	**18.9**			**21.4**
Percent hemoglobin F Normal: 0.00-1.00%	**1.8**				**3.3**		**3.7**	**3.8**
Percent hemoglobin A2 Normal: 1.80-3.30%	**5.1**				**4**		**3.7**	**3.7**
Percent hemoglobin A Normal: 95-98%	82.9				82.7		90	92.5
Percent hematocrit Normal: 40-50%	42.8		**37.3**	**37**			**38.1**	**39.01**
Hemoglobin estimation [g/L] Normal: 130-170 g/L	130		**125**	**121**	**112**	**127**		**125**
White Blood cells [10^9/L] Normal: 4-10 x 10^9/L	9.9		**10.7**	**10.9**		**10.8**		**11.4**
Percent lymphocyte count Normal: 19-48%	24	22.8	29.3	25	28			18.9
Percent monocyte count Normal: 2-10%	5	7.5	6.1	7	7.2			9.8
Percent neutrophil count Normal: 40-80%	68	75.1	62.4	64	58			68.7
Percent eosinophil count Normal: 0-0.5%	2	0.06	1.9	0.2	0.2			2.4
Percent basophil count Normal: <5mg/L	1	0.06	0.3	0.1	0.1			0.5

Percent platelets [10^9/L] Normal: 150-410 x 10^9/L	370	**461**				**465**		**463**
C-Reactive protein [mg/L] Normal: <5 mg/L	5		**10.07**	**10**	**12**	**13.01**	**20.01**	**16**
Zinc Umol/L Normal: 11-19 Umol/L	11.5	**10.4**						**10.1**
Urea [%mg] Normal: 17-49 mg%		23						30
Creatinine [%mg] Normal for adults: 0.70-1.30%mg		0.99				0.9		0.95
Lactic acid (Plasma) [mg/dl] Normal values 4.5-19.8 mg/dl	19.01					**22**	**23.1**	**24.2**
Creatine phosphokinase (CPK) [U/L] Normal: <190	60	69						**258**
Serum albumin [gr/dl] Normal: 3.5-5.2g/dl		4.5						5
Serum transaminase ALT (SGPT) [U/L] Normal for adults: <41 U/L	35		**63**		**45**			28
Anti-p53 autoantibodies [U/mL] Normal: 0.0-120 U/mL								**188.3**
Anti-double stranded DNA [U/mL] Negative: <30 U/mL								0.6
****SARS-CoV-2 IgG spike protein antibodies [AU/ml] Negative: <50 Positive: >50**				60.5				*****11117.80**
SARS-CoV-2 nucleocapsid antibodies [S/CO] Negative: <1.40 Positive: >1.40								0.71
Anti-angiotensin-2 (ACE-2) autoantibodies [U/ml] Negative: <26.1 U/ml Positive: >26.1 U/ml								17.1
Immunoglobulin G4 [mg/dl] Normal for adults: 3.0-201 mg/dl								**1.1**
Serum Ferritin [ng/ml] Adult males normal: 6-323 ng/ml	250	**438**			**581**	**629**		**392**
Folate [nd/ml] Normal: 3.1-24 ng/ml	4				**3**			**3**
Blood iron [µg/dl] Normal: 65-175 µg/dl	70		**56**		**58**			77

*Clinically important numbers appear in bold.
** Result obtained April 8, 2024. Repeated twice in the same laboratory.
***Same testing was conducted in yet another diagnostic laboratory and the result showed 137.80 AU/ml, negative < 1 AU/ml, concentration of SARS-CoV-2 IgG spike protein antibodies.

Neurological, Muscular, and Other Clinical Complications

The patient, soon after the day of mRNA injection, developed distortion of sense of taste, which is characteristic of neural gustatory pathway inflammation (Heckmann & Lang, 2006). These episodes (occurring daily or twice per day) were accompanied by a feeling of weakness, extreme fatigue and dizziness resembling pre-syncope rather than vertigo - threatening loss of consciousness whilst walking.

During that same period, the patient felt that his muscles were progressively wasting. He also experienced difficulty breathing and felt it was respiratory-muscle-related. Therefore, he underwent maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) measurements to evaluate for respiratory muscle weakness. The MIP/MEP evaluation revealed a MIP measurement at 51 cmH_20 with normal > 57 cmH_20, and a MEP measurement at 49 cmH_20 with normal > 88 cmH_20. A second MIP/MEP evaluation, performed days after the already described evaluation, revealed even more alarming results. The second evaluation revealed a MIP measurement at 41 cmH_20 with normal > 57 cmH_20 and a MEP measurement at 18 cmH_20 with normal > 88 cmH_20. Both MIP/MEP evaluation results were consistent with intense respiratory neuromuscular abnormalities leading to respiratory failure (Rodríguez-Núñez et al., 2020 The patient noticed that his tongue had become swollen, and ulcers appeared in his mouth and underneath his tongue. See Photo 1, in the Image Gallery appearing in Figure 2.

During the seventh month after the mRNA injection, the patient began to experience episodes of trigeminal neuralgia and facial palsy on the left side. The pain was especially severe and mostly located in both the ophthalmic regions. Also, at that time, the patient began to periodically hear a bass-like sound in his left ear, his eyes became dry and sensitive to sunlight, he started to see in a slow-motion vision pattern during eye-movements from one focal point to another. Blurring can describe this condition. The patient started to hear voices louder than normal, indicative of cranial nerve inflammation. Similar ocular symptoms have been described after mRNA COVID-19 injection by Ng et al. (2021). For our own patient, we ruled out the possibility of a brain tumor by performing multiple MRIs during that period (data not shown). Severe tinnitus accompanied these events.

During the episodes of hypoxia and weakness, the patient also experienced pain and pins and needles in his hands and feet suggestive of peripheral neuropathy. These episodes were accompanied by severe constipation indicative of autonomic neuropathy, specifically affecting the vagus nerve. (National Institute of Neurological Disorders and Stroke, 2024). The patient reported that he started to feel that his mouth, gums, tonsils and nostrils were shrinking (see Photo 2, Figure 2). Moreover, the patient had an almost complete loss of appetite. Although our patient had been obese (150 kg), during his illness he lost 25 kg, and later weighed only 117 kg. All this occurred over a period of only two months. His stools contained undigested food, indicating malabsorption (see Photo 3, Figure 2). A similar case of constipation association with celiac disease has been described in a thalassemia patient and indicated for folate deficiency (Gavidel, 2019).

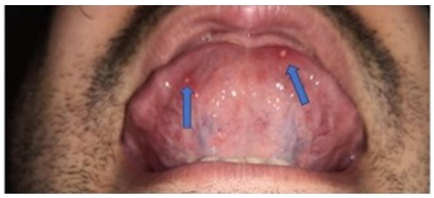

Photo 1. Painful tongue ulcers

Photo 2. Painful shrinking gums after the mRNA Covid-19 vaccination

Photo 3. Faeces of characteristic morphology and silver-yellow colour

Photo 4. Patient reports pull-bar muscle loss, cramps, weakness and pain.

Photo 5. Tongue muscle weakness. Styloglossus muscles lack capacity to elevate tongue's lateral margins.

Figure 1. This is Image Gallery 1 with numbered and labelled photos showing the patient's multiple disease related symptoms.

Photo 6. Lack of sole muscle contractions leading to pain and difficulty in walking.

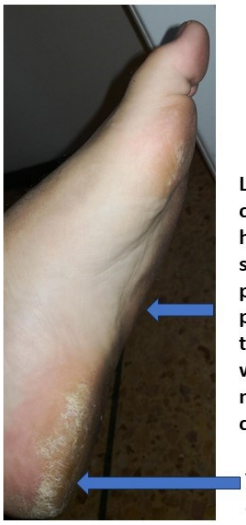

Photo 7. Painful and hard contracting regions of patient's sole, making walking difficult.

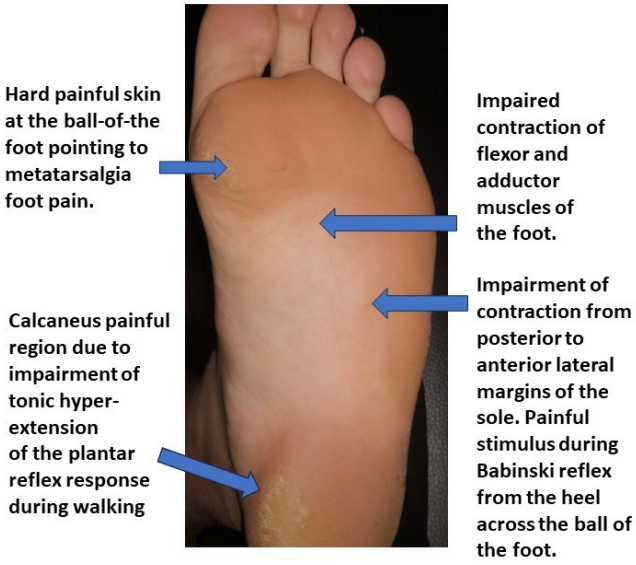

Figure 2. Here in Image Gallery 2 are two more labeled photos, these showing additional abnormalities causing pain and making it difficult for the patient to walk.

Up until the present time, the patient has severe muscle weakness and extreme fatigue. He reports muscle loss, cramps and weakness, especially in the pull-bar muscles (predominantly the Latissimus dorsi muscles) and pain in these areas (see Photo 4, Figure 2). Tinnitus, sensitivity to light, vision abnormalities and hearing low voices as loud persist. He still has paraesthesias in hands and feet, difficulty concentrating, brain fog and a feeling of continuous pressure in the head. He often bites his tongue during sleep, he has skin dryness, and body and mouth odours. Finally, he has sustained a degree of muscle weakness in the tongue (see Photo 5, Figure 2) and hardening of the foot palm, making walking difficult (see Photos 6 and 7, Figure 3). Moreover, and as the patient's oxygen saturation remains low (below 93%), as advised by a pneumonologist, he regularly requires the BiPAP plus oxygen supplementation to overcome episodes of intense

confusion and brain fog during his daily activities. These episodes are sporadically recurrent and continue to this day.

IMPAIRMENT OF PATIENT'S HEALTH-RELATED QUALITY OF LIFE

After the mRNA injection, our patient developed health-related effects that profoundly affected his quality of life. The main symptoms were a) impending loss of consciousness (pre-syncope), b) general weakness, c) orthostatic tachycardia (heart rhythm abnormality characterized by increased palpitations upon waking up), and d) confusion. These symptoms resembled those of a patient who suffers from "presyncope syndrome," and his symptoms were consistent with neurological autonomic dysfunction (Hockin et al., 2022).

In order to further evaluate whether the patient suffered from a pre-syncope or a near-syncope syndrome, a Syncope Functional Status Questionnaire (SFSQ) was used to evaluate the severity of these symptoms (van Dijk et al., 2007; Whitledge et al., 2023). Our patient gave affirmative answers to 9 of the 11 questions on the SFSQ test (see Table 2). Since our patient gave many affirmative answers on the SFSQ questions, he was further evaluated by the Impact of Syncope on Quality of Life (ISQL) test by Rose et al. (2009). Notably, in this test also, our patient gave affirmative answers to 9 out the 12 questions of the ISQL test (see Table 3). Based on the Rose et al. (2009) study, we inserted into Table 2 the frequency (which is the proportion of syncope patients responding affirmatively to the particular question), the severity or importance (which was based on a mean score of patients responding affirmatively to the particular question), and the impact of this particular question, which is the product of frequency and importance reported in the Rose et al. study. This enabled the construction of an ISQL/SFSQ graphical presentation for our patient similar to that in the Rose et al. study. The affirmative answers that the patient gave, and the ISQL/SFSQ graphical presentation in Figure 4, show that our patient has suffered from a severe deterioration of quality of life comparable to syncope patients (Rose et al., 2009). The end result unfortunately was for him to lose his job because he did not have a clinical evaluation and diagnosis that clearly defined his presyncope or near-syncope health-related physical impairments when his debilitating symptoms overtook him.

Table 2

The Syncope Functional Status Questionnaire (SFSQ) with Answers Provided by the Patient Revealin Multiple Daily Physical Impairments Pairing and Indicating a Diagnosis of Presyncope or Near-Syncope Syndrome

Items	No	Yes
Interfering with my life or routine		X
Preventing or causing me to avoid driving a vehicle		X
Reducing the amount of walking I do each day		X
Interfering with my use of public transportation		X
Interfering with my performing errands		X
Interfering with my physical activities		X
Affecting my ability to work at my job		X
Affecting my relations with my spouse/girlfriend/boyfriend	X	
Affecting my relationship with my family	X	
Affecting my relationship with my friends		X
Affecting my sexual functioning	X	

Table 3

The Impact of Syncope on Quality-of-Life Questionnaire (ISQL), with Answers Our Patient Gave, Along with Frequency of Endorsement, Severity and Impact Scores Reproduced According to Rose et al. (2009)

Items	Yes/No	Frequency	Severity	Impact
As a result of your fainting or light headed spells, how often in the last month have you felt				
Tired and worn out?	YES	0.79	2.73	2.156
Frustrated?	YES	0.73	2.31	1.686
As a result of your fainting or lightheaded spells, how often in the last month have you been				
Worried about fainting?	YES	0.71	2.75	1.952
Frightened of fainting?	YES	0.70	2.66	1,862
Limited in the type of work you could do?	YES	0.54	2.76	1,490
How often during the last month has your fainting or lightheaded spells				
Interfered with performing vigorous physical activities?	YES	0.54	2.95	1,593
Think back over the last month and indicate how often you have avoided				
Driving a vehicle	YES	0.59	3.04	1.793
Standing for long periods of time (longer than 5 min)	NO	-	-	-
Being in warm or hot environments in case you faint	NO			
Think back over the last month and indicate how much you agree with the following statements				
No one understands the effect that fainting has on my life	NO	-	-	-
Because of my fainting, I accomplish less than I would like	YES	0.53	3.19	1,690
My fainting leaves me feeling confused	YES	0.60	3.20	1.92

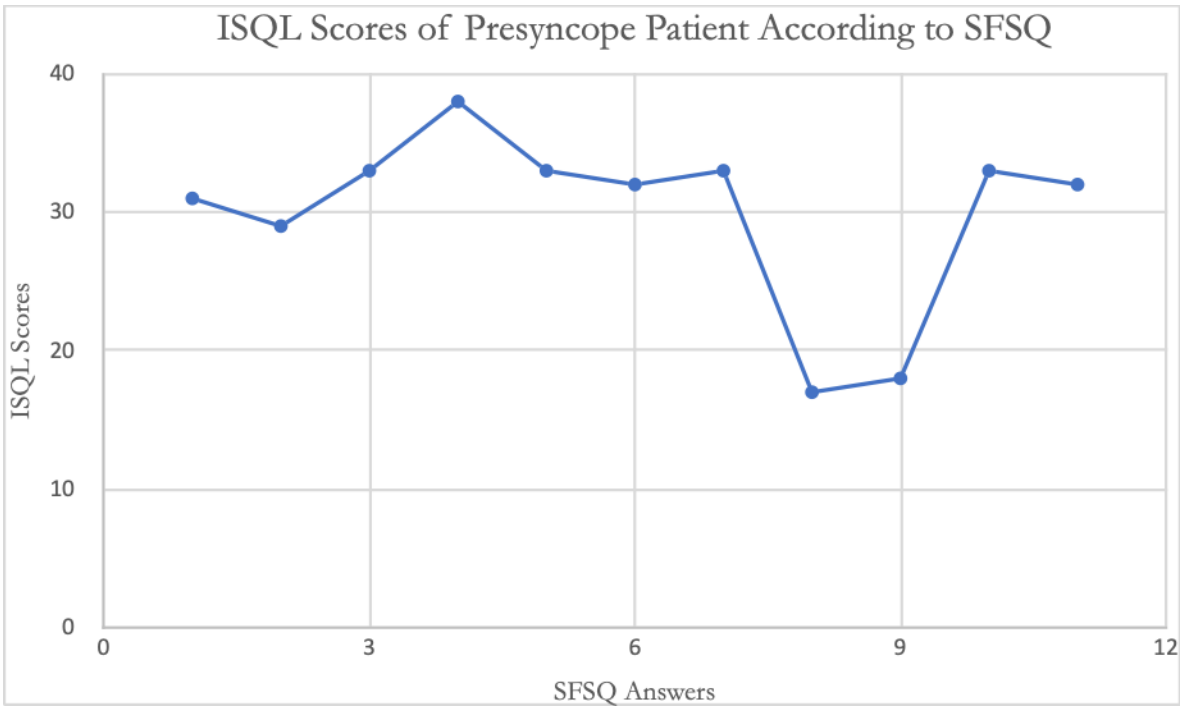

Figure 3. According to the affirmative SFSQ answers, the ISQL scores were high, affecting the patient's physical activities and ability to work.

The Differential Diagnosis

The 24-hour Holter test, performed a year after the mRNA injection, revealed sinus tachycardia of 127 pulses per minute. The recent 24-hour Holter test (two years and six months after the mRNA injection) revealed that the patient still had a sinus tachycardia of 151 pulses per minute. The patient at the time of initial Holter evaluation (two weeks after the mRNA injection) had troponin levels: 0.008 ng/ml, negative <0.015 ng/ml, indicating no myocardial damage. ~~However,~~ Also, the echocardiogram showed a normal ejection fraction of 60-65% with no structural heart abnormalities (data not shown).

Heart rhythm disorders are frequent adverse reactions of mRNA COVID-19 injection. A series of cases similar to our patient are methodically described by Cocco et al. (2023). Moreover, our patient had an anatomically normal heart with normal ejection fraction as shown by an ultrasound examination. A case similar to ours was described by Aiba et al. (2021) in which the patient suffered from arrythmias after the mRNA injection. That individual had an implantable cardioverter defibrillator to correct acquired "long QT syndrome" — a condition in which the relaxation phase after a completed heartbeat is abnormally extended before the next heartbeat even begins. The length of the complete heartbeat as measured on an electrocardiogram is divided into segments that are labelled alphabetically Q, R, S, and T from start to finish. When the interval from one heartbeat to the next is too long, this kind of heart irregularity can potentially cause the person to feel weak and about to pass out. It is a potentially fatal condition. In the Aiba et al. case, as contrasted with our own patient, the ventricular tachycardia — that is, life-threatening irregularity of the heartbeat — lasted for only a short period of time (8 days after the second mRNA injection). For our patient, it is sinus tachycardia that lasted for 8 consecutive months after one mRNA vaccination and was found to still occur at a later Holter examination, conducted 20 months after the mRNA vaccination. Earlier ECGs that the patient conducted as routine testing for his work, months before the mRNA vaccination, indicate that our patient was free from sinus tachycardia (data upon request). Additionally, during the initial period following the appearance of mRNA-injection associated cardiological and neurological adverse effects, the

patient we describe had elevated levels of C-reactive-protein — where the "C" stands for complement" as in the "complement cascade" immune reaction. When this protein is elevated, it shows that there is significant inflammation in the patient's body. Raised levels of that protein in ordinary blood tests have been found previously in patients with myocarditis after one or more mRNA injections (Matta et al., 2021).

The elevated levels of C-reactive-protein reached values from 10 to 20 mg/ml — whereas the normal range for an individual such as our patient is expected to be between 1 and 3 mg/ml (Pearson et al., 2003). These irregular measures, and other symptoms were still evident in our patient through May 2024 (see Table 1). They lasted more-or-less throughout the whole period (2.5 years) after the mRNA injection. Clearly, our patient had blood tests that were not normal, especially the hematological parameters, either prior to or after the mRNA COVID-19 injection.

The blood test results summarized in Table 1 characterize an individual with a pure β-thalassemia trait that, prior to the mRNA injection, remained asymptomatic — without severe anemia — throughout his life, based on his reported medical history and the normal levels of fetal hemoglobin and hemoglobin A2 (see Table 1) for persons of Greek origin (Fessas et al., 1964). The levels of fetal hemoglobin and hemoglobin A2, but especially those of fetal hemoglobin, however, have shown considerable variability, when measured several months after the mRNA injection. They remain considerably altered up to the time of this writing (see Table 1). The fluctuations, especially of fetal hemoglobin, mean that our patient has gone from having asymptomatic heterozygote pure β-thalassemia to full-blown anemia related symptoms of fatigue (Musallam et al., 2022), loss of muscle strength at different body locations (Ismail et al., 2018), peripheral neural inflammation (Sawaya et al., 2006), and muscle wasting (Shapira et al., 1990). The latter symptoms are frequently described in patients with β-thalassemia intermedia and β-thalassemia major conditions. As far as the cardiological and neuromuscular symptoms are concerned, the pathological changes were initiated soon after the mRNA COVID-19 injection. The electromyograph (EMG) examinations conducted at a time when all symptoms were prominent (several months after the mRNA injection) prove that there was no evident muscle or electrical activity impairment in response to neural stimulation of muscles in our patient's neuromuscular system (data not shown).

According to the mean corpuscular volume measured before the mRNA injection, he was categorized as a carrier of pure β^0-thalassemia trait (with only one mutation in the β-globin gene), whereas the β+ -thalassemia traits (with several mutations in the β-globin gene) have mean corpuscular volume values well above the lower normal cutoff point (Shapira et al., 1990). What is worrying about our patient however is that his mean corpuscular volume decreased after the mRNA injection and continued to be low right through May 2024. The lowering of mean corpuscular volume in individuals with pure β^0-thalassemia traits may specify a clinically worsening progression to a β-thalassemia intermedia phenotype (Galanello et al., 2010). The enlargement of liver and spleen (splenomegaly, with 14.5 cm diameter) of our patient recorded several months after the mRNA injection inclines more to this medical conclusion. Furthermore, his chronic dizziness and presyncope syndrome conditions are also described as symptoms of β-thalassemia major (Akiki et al., 2023). The elevated levels of C-reactive-protein (Moftah et al., 2020) and anti-p53 autoantibodies are also described in β-thalassemia major and correlate with high levels of ferritin in these patients (Athiyarath et al., 2012). Also, the continuous raised C-reactive-protein, on its own, accounts for estimating that our patient suffers from chronic systemic inflammation almost certainly involving many organs (Karadag et al., 2008).

Furthermore, the latest blood count revealed mild leucocytosis, and the patient's platelet count has been continuously elevated after the mRNA injection (see Table 1). Similar mild leucocytosis and raised platelet count have been described in another case study of a patient, soon after the mRNA injection, suggesting an autoimmune hematological disorder (Gaignard et al., 2021). Lactic acid measurements reveal that the patient suffers from hyperlactatemia, a condition that was not apparent before the mRNA injection (see Table 1). This indicates the inability of the

patient's mitochondria to regulate pyruvate metabolism (Gray et al., 2014). The patient's admission of muscle weakness in bulbar muscles and tongue and the weakening of respiratory muscles revealed from the MIP/MEP evaluation is suggestive of mitochondrial myopathy. As seen in Table 1, the patient's elevated levels of creatinine phosphokinase are especially concordant with mitochondrial myopathy (Finsterer, 2009). The below-normal low levels of IgG4 antibodies measured in the patient's blood predict immunodeficiency and are associated with autoimmune disorders (Heiner, 1988).

Moreover, the elevated levels of ferritin and the decrease in folate concentration are prominent in our patient's multiple hematological testing after the mRNA injection (Table 1). Although folate is reduced in individuals with pure β-thalassemia traits that require blood transfusion (Soontornpanawet et al., 2023), our patient prior to the mRNA had levels of folate within normal limits. The reduced, below to lower normal limits of folate concentrations would mean that the patient's body is consuming more folate than prior to the mRNA injection, due to the decrease in erythropoiesis. Therefore, a prominent folate deficiency symptom such as hemolytic anemia should be a serious consideration for our patient's condition (Zhang & Chen, 2020; Khan & Jialal, 2024), and this closely matches the constipation period our patient experienced after the mRNA injection and the peculiar morphology of his stools (see Photo 3, Figure 2) indicating maldigestion. Similar studies on individuals with β-thalassemia intermedia in Italy show that the increased ferritin and the low concentrations are related to increased morbidity incidents (Musallam et al., 2012). Fortunately, the patient presented in this study has experienced an increase in fetal hemoglobin, and this may have helped him to remain alive.

Another marker worthy of comment is the mild deficiency of zinc the patient developed soon after the mRNA injection (see Table 1). The relevant blood testing that the patient underwent at that time excludes the possibility of hypoalbuminemia with serum albumin (4.5 g/dl with normal limits 3.5-5 g/dl) as seen in Table 1. Moreover, the patient had never in his life received any copper supplementation according to his medical history report. The patient's normal kidney functioning markers, urea 23 mg/dl (normal: 17-24 mg/dl), creatinine 0.99 mg/dl (normal for adult males: 0.70 -1.35 mg/dl) led us to exclude kidney disease. The patient also had a negative test for traceable protein in urine and normal serum Na^{+}, Ca^{2+}, phosphate, and magnesium (data not shown).

A relevant study by Mashhadi et al., (2014) on 333 patients with β-thalassemia major found that all of them suffered from zinc deficiency, and all but five of them (1.5%) had severe zinc deficiency. These observations led us to suggest mild to moderate zinc deficiency. The zinc deficiency strengthens the diagnosis of the patient's thalassemia condition deteriorating as seen also in the worsening of his hematological profile (see Table 1). His pathological symptoms began to develop soon after the mRNA injection and were still present after 2.5 years.

Probably one of the most intriguing laboratory results are the extremely high concentrations of SARS-CoV-2 spike protein neutralizing antibodies (11117.80 AU/ml). The patient was still producing these more than two years after his single mRNA injection (see Table 1). Supporting this suggestion, according to Swadźba et al. (2024), high levels of SARS-CoV-2 spike protein antibodies (median 7440 AU/ml) were detected after 360 days in mRNA vaccinees that received booster doses. These high levels were attributed in part to repeated exposure to the virus though overt symptoms were absent. To the best of our knowledge, our patient's SARS-CoV-2 spike protein neutralizing antibody concentration so many days after a single mRNA COVD-19 injection is the highest ever referred to in the literature so far. A recently published case study of a patient with a β-thalassemia trait shows that the patient had an unexpectedly abnormal upregulation of SARS-CoV-2 spike protein neutralizing antibodies (Gamberini et al., 2023). We return to this finding in a later section.

Hematological Considerations

For the sake of this investigation, our patient has been genetically screened for his thalassemia related mutations. The patient was found to be a pure β-thalassemia heterozygous carrier of the HBB: c.315+1G>A(IVSII-1 G>A) beta zero mutation. The alteration of his hemoglobin A2 and fetal hemoglobin levels after the mRNA injection, however, cannot be attributed to extra mutations in the globin gene, as he was found to be free from any abnormalities in the α and δ globin genes.

Before the mRNA injection, our patient had an increased percentage of hemoglobin A2 (5%) in his blood, which is well in excess of the borderline values of hemoglobin A2 (3.0-3.8%). The excess of hemoglobin A2 levels limits the possibility that the patient could have had an α-globin gene mutation co-existing with those identified in him prior to the mRNA injection (Thilakarathne et al., 2024). Additionally, a large cohort study reveals that most β-thalassemia carriers have elevated hemoglobin A2 levels (Yamsri et al., 2015). Recent hematological examinations of the patient's father that were conducted also for the sake of this investigation, and from whom our patient has inherited the hemoglobin subunit beta-c β-thalassemia trait, reveal a similar percentage of hemoglobin A2 levels (hemoglobin A2 of 5%) to those of our patient prior to the mRNA injection. Likewise, the patient's father had low levels of fetal haemoglobin like the son, prior to his mRNA injection. The elevation of fetal hemoglobin, along with many other reasons, may indicate that the patient underwent a condition of erythropoietic stress following his mRNA injection that is not related to mutations in the α and δ globin genes (Amato et al., 2014).

The HBB:c.315+1G>A(IVSII-1 G>A is a pathogenic mutation in the first intervening sequence (IVS) 5' splice site. This mutation causes RNA splicing and transcription defects that affect the final expression of hemoglobin B (Treisman et al., 1983). However, heterozygotes for this mutation do not show an increase in the expression of fetal hemoglobin, which occurs in order to compensate for the low levels of their hemoglobin B. A rise of fetal hemoglobin occurs only in the homozygotes of this mutation (Oppenheim et al., 1990). Moreover, heterozygotes for the HBB:c.315+1G>A(IVSII-1 G>A) mutation progress to present thalassemia intermedia conditions (worsening of pathologic symptoms) when the mutation is combined with the hemoglobin B Knossos mutation (Nasouhipur et al., 2014). Our patient was genetically screened to be free from any other mutations in the hemoglobin B gene. Also, the patients that have a combination of these two mutations generally have normal hemoglobin A2 levels (Nasouhipur et al., 2014).

Our patient's drop in hemoglobin A2 levels from 5% to 4% and 3.7% after the mRNA injection, which is within the borderline trait limits of hemoglobin A2 (3.0-3.9 %), lead us to suggest that this occurred for other reasons than a genetic defect. Borderline levels of hemoglobin A2 are present in a high percentage (87%) of β-thalassemia patients that have also have another mutation defect in the α and δ genes (Colaco et al., 2022). However, as mentioned before, our patient was free from any other mutations. This indicates, in our judgment based on the findings we have reported and discussed here, that our patient incurred hematologic stress attributable to the mRNA injection that caused the rise of fetal hemoglobin expression in order for the patient to withstand the hematological pressure to saturate oxygen. The re-activation of γ-globin genes (fetal hemoglobin) due to the lack of normal hemoglobin β, leads to the an increase in β-thalassemia severity (Ruangrai et al., 2016). Hematologic stress and therefore stress in erythropoiesis (Ruangrai et al., 2016), resemble an inflammatory condition (Paulson et al., 2020). The mRNA COVID-19 injection not only could account for the patient's sharply rising fetal hemoglobin, but it could also account for the patient's tachycardia episodes (Akiki et al., 2023), the hypertension, arrythmias, weakness, and fatigue that the patient experienced (Mashhadi et al., 2014; Fraidenburg & Machado, 2016; Akiki et al., 2023). All these symptoms occur after hematologic deterioration in β-thalassemia patients (Ismail et al., 2018).

In conclusion, our patient, having 5.1% hemoglobin A2 before the mRNA injection, similar to his father from whom he inherited the trait, that has dropped to 3.7 % after the mRNA injection, and currently has a relatively high value of fetal hemoglobin (3.70 %), months after injection, most likely has sustained a hematological disturbance that has lowered his blood circulating hemoglobin to 11.2 g/dl (requiring blood transfusion; Farmakis et al., 2022) and seems to have caused the increase in his red cell distribution width to 17.6 %, soon after the mRNA injection (see Table 1). The drop in hemoglobin at that time was clinically important and should have required a blood transfusion, as suggested by the hematology team that performed the genetic screening for *β* thalassaemia mutation (Agia Sophia Hospital, Athens Greece, Balkan Centre for Thalassaemia). Unfortunately the patient was never advised to receive a blood transfusion by physicians at the time required, putting his life in danger.-A recent study indicates that mRNA injection promotes hematologically related pathology, including multiple severe anemias and blood coagulation defects (Choi et al., 2023). This relevant study, although it does not seem to have included mRNA vaccinees with β-thalassemia traits, nevertheless concludes with a cautionary note that care should be taken when administering the mRNA COVID-19 vaccines to individuals with hematologic impairments. To our knowledge, the case study we are reporting here is the first to be added to the bibliography of a hematological abnormalities occurring in a pure thalassemia trait heterozygote individual, carrying the HBB:c.315+1G>A(IVSII-1 G>A mutation, developing soon after his mRNA COVID-19 injection.

Other hematological indices of the patient, after the mRNA injection, indicate the worsening of his pathology. After the mRNA injection, the patient had lower mean corpuscular volume values than before the injection. In individuals with pure β^0-thalassemia traits, this may suggest a progression of clinical deterioration leading to a β-thalassemia intermedia phenotype (Galanello et al., 2010). Moreover, the low mean corpuscular volume observed with this patient are predictive of iron deficiency anemia in β-thalassemia trait patients (Jameel et al., 2017).

Our patient has sustained until now high levels of ferritin that were not encountered before the mRNA injection (see Table 1). After the mRNA injection, the patient has also sustained until now continuously elevated levels of complement-reactive-protein, and as measured lately, medically important raised levels of p53 autoantibodies (Kuhn et al., 1999; Suppiah & Greenman, 2013). Leucocytosis after the mRNA injection shows a predominance of systemic inflammation that may accompany the elevated levels of complement-reactive-protein, pointing to a plethora of adverse cardiac events (Gogo et al., 2005; Mank et al., 2024).

Both elevated complement-reactive-protein and p53 autoantibodies are commonly associated with elevated levels of ferritin when β- thalassemia-related pathology worsens (Moftah et al., 2020; Zhang & Chen, 2019). Elevated p53 and ferritin predict a deficiency in iron homeostasis (Zhang & Chen., 2019). Moreover, our patient, after the mRNA injection, has had low levels of folate (see Table 1). Although folate deficiency is encountered in β-thalassemia heterozygotes, the reduction below normal of folate in red blood cells, in our patient's case, suggests that the injectable has impaired erythropoiesis. Therefore, a prominent folate deficiency symptom such as hemolytic anemia is a serious consideration for our patient's condition (Khan & Jialal, 2024). Similar studies on individuals with β-thalassemia intermedia show that increased ferritin and low levels of fetal hemoglobin are related to increased morbidity risk (Musallam et al., 2012). Thankfully, our patient's fetal hemoglobin levels increased after the mRNA injection, in order to withstand the worsening of his hematology-related pathology.

A Potential Role for Autoantibodies and Chronic Inflammation

The idea that our patient might have been developing autoimmunity similar to that of systemic lupus erythematosus — which turns out to be a frequent event occurring after the mRNA COVID-19 vaccinations (Báez-Negrón & Vilá, 2022) — can be excluded because of his increased levels of anti-p53 autoantibodies. When we measured anti-double stranded DNA autoantibodies in his blood, we found a negative result (see Table 1) enabling us to exclude

systemic lupus erythematosus. In the study by Herkel et al. (2001), it was found that, during the development of systemic lupus erythematosus, elevated levels of anti-p53 autoantibodies, due to idiotypic autoimmunity reactions, induce the production of anti-double stranded DNA autoantibodies that target the anti-p53 autoantibody domains. This was not the case for our patient as he had diminished levels of anti-double stranded DNA autoantibodies (see Table 1).

The induction of anti-p53 autoantibodies production has, however, also been associated with carcinogenesis (Suppiah & Greenman, 2013). Nevertheless, yet another study indicates that the upper borderline elevated levels of anti-p53 autoantibodies in our patient's case can be regarded as a bio-marker of chronic systemic inflammation (Maacke et al., 1997), where p53 accumulates in the cytoplasm rather than the nucleus, and this provokes premature mitochondrial-dependent cell death (Green & Kroemer, 2009). When p53 accumulates in the cytoplasm under stress, it readily undergoes proteasomal degradation (Lavin & Gueven, 2006). Degraded fragments of p53 are thus more frequently presented to antigen presenting cells to induce B-cell production of anti-p53 autoantibodies in higher amounts, plausibly as in our patient's condition (Suppiah & Greenman, 2013). Finally, p53 is highly expressed in β-thalassemia syndromes involving pathologically ineffective erythropoiesis (Athiyarath et al., 2012). In patients with thalassemia major, the high expression of p53 correlates with high levels of ferritin circulating in blood. These two biomarkers are also remarkably elevated in the pure β^0-thallasemia condition of our patient, as we have shown in this report.

In a related study recently published by Kyriakopoulos et al. (2022), it was determined that the SARS-CoV-2 spike protein, by potently stimulating the p38 phosphokinase pathway, can enhance the transcriptional activation and thus the expression of p53 via the β-amyloid pathway. The stimulation of this molecular process by the SARS-CoV-2 spike protein can also induce the upregulation of cellular prion protein and β-amyloid proteins, thus predisposing recipients to prion or prion-like disease In that paper, we wrote: "The pathways induced by the spike protein via toll-like receptor activation induce both the upregulation of cellular prion protein (the normal isoform of the prion protein) and the expression of β amyloid. Through the spike-protein-dependent elevation of p53 levels via β amyloid metabolism, increased cellular prion protein expression can lead to prion protein misfolding and impaired autophagy, generating prion disease." In fact, a study involving 26 patients who developed severe, even fatal, Creutzfeldt-Jakob disease, where symptoms first appeared within a month of the second mRNA vaccine, provides strong evidence that the spike protein is amyloidogenic (Perez et al., 2023). Autophagy is the process by which cells sequester damaged organelles and proteins into autophagosomes and deliver them to the lysosomes for clearance (Yang & Klionsky, 2009). Autophagy plays a protective role in protein-misfolding diseases by degrading and clearing aggregate-prone proteins (Yao et al., 2013). Remarkably, the prion protein activates autophagy, mediated through α7nAChR signaling, thereby protecting cells from toxicity (Jeong & Park, 2015).

Furthermore, it has been predicted that the synthetic mRNA vaccines can abnormally activate mTOR, and the molecular mechanism was described in a study by Kyriakopoulos and McCullough (2021). Upregulation of mTOR — mammalian target of rapamycin — suppresses autophagy. By suppressing autophagy, it also suppresses protein clearance, including clearance of cellular prion protein. Elevated concentrations of cellular prion protein induce a greater risk of their misfolding of cellular prion protein into PrPSc, the toxic form that leads to prion disease. Elevated levels could also promote the development of antibodies to cellular prion protein.

What is Causing the Muscle Wasting Condition?

It is very challenging to explain the underlying cause of our patient's muscle wasting disease. While the symptoms match well with an amyotrophic lateral sclerosis diagnosis, repeated tests for metrics linked to amyotrophic lateral sclerosis came out negative (data not shown). Antibodies to nicotinic acetylcholine receptors (nAChRs) could explain muscle weakness associated with myasthenia gravis, but our patient tested negative for these as well. Very high

antibody levels to the spike protein, confirmed multiple times, are a virtually certain indicator that the mRNA injectable caused his disease, and also suggest that molecular mimicry may be causing these antibodies to attack some other as yet unidentified human protein. The only other autoantibody test that came out positive was autoantibodies to the tumor suppressor p53, and we will show in the following paragraphs that this could also explain the elevated serum lactate levels that were observed.

A Role for p53?

One of the few positive markers identified in our patient was borderline p53 autoantibodies. While we have not been able to find literature directly linking elevated p53 antibodies to overexpression of p53, studies have shown both that p53 is overexpressed in 39% of cases of metastatic cancer (Porter et al., 1992), and that elevated p53 autoantibodies predict bad outcomes in cancer, presumably due to the inactivation of p53, a tumor suppressor, by the antibodies (Sobhani et al., 2021). This suggests that antibodies may develop against p53 when it is overexpressed in the cytoplasm.

When it functions normally, p53 is a powerful tumor suppressor, in part through its action in the nucleus as a transcription factor that activates the genes for several proteins involved in DNA repair (Sobhani et al., 2021). p53 also plays important roles in the cytoplasm. Highly significant for our case, p53 has been shown to promote oxidative phosphorylation, reducing lactate production via glycolysis. This effect does not depend on target gene activation, but rather is due to the ability of cytoplasmic p53 to inhibit lactate dehydrogenase activity that converts pyruvate to lactate, which is released into the circulation (Williams & Schumacher, 2016). Our patient was found to have elevated levels of serum lactate, which could be a consequence of p53 inactivation due to autoantibodies. It is conceivable to suggest that our patient could have a defective version of p53 in the muscle cells. Overexpression of a dominant-negative p53 mutant in the skeletal muscle of rats resulted in decreased muscle mass, histological muscle damage, decreased oxidative phosphorylation, and muscle fibre atrophy (Langer et al., 2022).

It is also said that p53 is the guardian of the genome, and it has the power in part to induce apoptosis in cells with severe DNA damage — hence its involvement as a checkpoint during the growth stage 2 of mitosis and its similar function, it seems, in erythropoeisis during the manufacturing of blood cells in the marrow. A study involving patients with pancreatitis or pancreatic cancer found that p53 was overexpressed in a high percentage of both groups (59% of patients with pancreatitis and 67% of patients with pancreatic cancer). The authors determined that the overexpressed p53 in the pancreatitis group was predominantly wild type p53. It was proposed that the production of inflammation-induced tumor-necrosis-factor-alpha, TNF-α, upregulated p53 protein expression (Maacke et al., 1997). Since the spike protein also upregulates TNF-α (Khan et al., 2021), this could be the means by which p53 overexpression occurred in our patient.

Interestingly, it has become clear that p53 can misfold into amyloid fibrils and accumulate in the cytoplasm in an inactive form (Naeimzadeh et al., 2024). Excessive cytoplasmic levels of p53 facilitate such misfolding through crowding, but it can also be due to genetic mutations arising from DNA damage in cancer cells. It has been proposed that p53 amyloid formation could cause loss of function of p53 in association with cancer. Amyloid species p53 can also travel cell-to-cell via a prion-like mechanism (Ghosh et al., 2017). Experiments on yeast equipped with the human gene for p53 have clearly demonstrated that p53 can form self-replicating aggregates (prions) that can be spread to other yeast cells via cytoplasmic transfer (Park et al., 2020).

The activity of p53 is regulated through binding to other proteins, notably, murine double minute oncogene, MDM2, and the peptidyl-prolyl isomerase PIN1. MDM2 binding facilitates p53 clearance via proteasomal degradation through ubiquitination (Henningsen et al., 2021). PIN1 is essential to facilitate transport to the nucleus, where p53 activates gene expression for a large number of proteins involved in DNA repair and apoptosis (Zheng et al., 2002).

In an in-silico study, Singh and Bhara (2020) found that the S2 subunit of the spike protein strongly interacts with p53. This might lead to interference with p53 binding to MDM2 and/or PIN1. If that is so, p53 would be expected to accumulate in the cytoplasm if both degradation and migration to the nucleus are suppressed. This idea is supported by a bioRxiv preprint paper by Zhang & El-Deiry (2024), which showed that the spike protein interrupts p53-MDM2 interaction and also suppresses gene activation of p53 targets in the nucleus.

In their study of 25 people with long COVID, Appelman et al. (2024) showed that they suffered from severe exercise-induced myopathy and infiltration into the muscle tissues of amyloid-containing deposits, which could be misfolded p53 proteins. Furthermore, the participants in their study started producing lactate much sooner during exercise than healthier patients, suggesting that their mitochondria were operating at severely reduced capacity. These patients also showed more muscle atrophy and cell death, suggesting infiltration of immune cells causing an autoimmune response.

Are Nicotinic Aceylcholine Receptors Involved?

Nicotinic acetylcholine receptors (nAchRs) are present at the postsynaptic membrane in the neuromuscular junction, where they mediate synaptic transmission, by taking up acetylcholine released into the junction by the associated motor neuron. Denervation of skeletal muscle induces severe muscle atrophy and weakness, and it has been found that this effect is due to the loss of activation of the nAchRs, which induce muscle contraction (Cisterna et al., 2020).

By the early 1980s, the acetylcholine receptor was already identified as a locus where rabies virus could be taken up into muscle cells (Lentz et al., 1982). Molecular modelling demonstrated that a sequence in the spike protein just above the furin cleavage site forms stable complexes with three different acetylcholine receptor subtypes. A PRRA motif within this sequence, not found in the original SARS-CoV virus spike protein, is homologous to several neurotoxins known to target acetylcholine receptors (Oliveira et al., 2020).

The phenotypic changes associated with muscle atrophy can be reversed by supplying the muscle fibre with acetylcholine (Cisterna et al., 2020). A theoretical paper published in 2023 proposed that long COVID could be caused by spike protein binding to the nAchRs and blocking their access to acetylcholine. Nicotine is an agonist ligand to the nAchRs, showing up to 30-fold higher affinity to the receptors than acetylcholine. These authors proposed that a nicotine patch may be therapeutic in treating long COVID (Leitzke, 2023).

The SARS-CoV-2 spike protein, when it is present internally in a cell, can suppress expression of acetylcholine receptors. The mechanism likely involves a hydrophobic helical motif in the S2 segment that is homologous to a similar motif in the intracellular domain of α7nAChR. It is plausible that the spike protein binds to the receptors inside the cell and interferes with their binding to chaperone proteins that would normally facilitate their transport to the membrane. This results in suppression of surface expression of α7nAChRs (Tillman et al., 2023). It is possible that, in the case of our patient, the spike protein is still being produced by skeletal muscle cells from Pfizer modified spike mRNA, causing interference with these receptors. Another possibility is impaired clearance of spike protein due to suppressed autophagy.

A Potential Role for the Prion Protein?

One possibility that should be considered for our patient is that the antibodies to the spike protein are attacking the human prion protein in the muscle tissues via molecular mimicry and disabling it. While the prion protein is known mostly for its causal association with Creutzfeldt Jakob disease (CJD) by misfolding into a toxic form of cellular prion protein, it actually serves important functions when it is properly folded. In fact, cellular prion protein activates the α7nAchRs (Jeong & Park, 2015). A sequence in the spike receptor binding domain, YQAGS, differs by only one amino acid from a sequence in the C-terminal domain of the prion protein, YQRGS. Antibodies to the C-terminal domain of the prion protein prevent it from performing

its functions (Seneff et al., 2023). Mice that are deficient in cellular prion protein have defective muscles, as shown in a paper by Smith et al., (2011). These authors wrote in the conclusion of their abstract that "prion protein content varies among murine skeletal muscles and is essential for maintaining normal redox homeostasis, muscle size, and contractile function in adult animals" (Smith et al., 2011).

Discussion

The patient we describe in this case is an individual with pure β^0-thalassemia (HBB:c.315+1G>A(IVSII-1 G>A) trait who has sustained an indisputable deterioration in his hemopoiesis after the mRNA COVID-19 injection, requiring blood transfusion due to the lowering of his blood hemoglobin levels. The patient was asymptomatic of any β-thalassemia pathological symptoms throughout his life, until he got vaccinated with the COVID-19 mRNA injectable. His fetal hemoglobin and hemoglobin A2 concentrations prior to the mRNA injection (see Table 1) were sustainable to keep him free from hemolytic anemia and iron accumulation defects since his birth (Tzetis et al., 1994). These values are consistent with the values of hematocrit, hemoglobin, red cell distribution width, mean corpuscular hemoglobin, and mean corpuscular volume prior to the mRNA injection. As a reminder, it must be kept in mind that the overall pathology of our patient began minutes or even seconds after his Pfizer mRNA injection.

After the mRNA injection, our patient encountered a significant increase in fetal hemoglobin concentrations in his blood compared with those that he had before the mRNA injection. Clearly, he suffered from hematologic stress after the mRNA injection. The reactivation of his γ-globin genes (fetal hemoglobin) due to inadequate amounts of normal hemoglobin β, is a survival mechanism that reduces β-thalassemia severity (Ruangrai et al., 2016).

An exceptional study by Cosenza et al. (2024) is relevant, we believe, to help understand what happened in the case of our patient. In their study, the researchers used erythroid precursor cell lines isolated from various patients with β-thalassemia traits (of different genotypes) and exposed them to either the SARS-CoV-2 spike protein or to the mRNA of the BNT162b2 (Pfizer-BioNTech) COVID-19 injectable. Although it may sound contradictory to our case that the Corteza results show that the SARS-CoV-2 spike protein inhibits fetal hemoglobin expression, in the same study, and also by an in-silico investigation, the researchers showed that the spike protein has an overwhelming binding affinity to fetal hemoglobin, similar to its affinity to angiotensin-2. It therefore may be plausible that, in our patient's case, the impairment of the oxygen-carrying capability of fetal hemoglobin by the SARS-CoV-2 spike protein binding has led to hematologic stress, and this increased the expression of fetal hemoglobin systemically. Several genetic and molecular factors influence the production of fetal hemoglobin that are distant from the already known β-thalassemia mutations, such as the micro-RNAs, that may have become operational in our patient's case to withstand hematological stress after the mRNA injection This may have resulted in an increase in fetal hemoglobin circulating in the blood (Carrocini et al., 2011).

The overwhelmingly high IgG antibodies against SARS-CoV-2 spike protein that our patient still produces almost three years after the mRNA single injection to the best of our knowledge have never been described in the literature (Swadźba et al., 2024). These sky-rocketing SARS-CoV-2 spike protein neutralizing antibody levels, according to the study of Yang & Du (2021), indicate that there should be a substantial presence of SARS-CoV-2 spike protein in our patient's tissues and circulation. These findings can have a tremendous impact on the health of patients with βthalassemic traits, since the ability to reactivate the γ-globin gene in these adults to express more fetal hemoglobin is the best and most natural way to improve their clinical symptoms. Nowadays, energisation of fetal hemoglobin expression has become probably the best therapeutic option to treat β-thalassemia and sickle cell diseases (Ju & Zhao, 2018).

According to the case study by Gamberini et al. (2023), the production of fetal hemoglobin in a βthalassemia trait patient who was being affected hematologically by sirolimus treatment to produce more fetal hemoglobin requires further investigation in association with SARS-CoV-2 mRNA injection. Sirolimus, which is an immunosuppressant and an mTOR inhibitor, suppresses the humoral response and therefore the production of SARS-CoV-2 neutralizing antibodies upon mRNA injection in organ transplant patients (Mazzola et al., 2022). However, this was not the case with the β-thalassemia trait individual presented by Gamberini et al. (2023), who, although he was receiving sirolimus treatment, and, due to that, had enhanced fetal hemoglobin production, unexpectedly, sirolimus did not inhibit the production of SARS-CoV-2 neutralizing antibodies after the mRNA injection. In our case, the β^0-thalassemia trait patient showed a marked elevation of fetal hemoglobin production and humoral response (neutralizing SARS-CoV-2 spike protein antibodies), after his single mRNA injection, despite not having taken any sirolimus treatment to enhance his fetal hemoglobin expression, as, prior to injection, he was in no need for any treatment.

Moreover, our patient, before the mRNA injectable, had a mean corpuscular hemoglobin at 18.6 pg, a mean corpuscular volume at 61.3 fL, a mean corpuscular hemoglobin concentration at 30.4 g/dL and a red cell distribution width at 16.3 % (see Table 1). These hematological data are compatible with those found in individuals with pure β^0-thalassemia traits and, in such asymptomatic individuals, the hemoglobin A2 concentration is usually around 5.7+/- 0.6 % for pure β^0, and around 6.9 +/- 0.8 % for pure $\beta^{3.4}$-thalassemia traits (Soontornpanawet et al., 2023). Furthermore, the patient's hemoglobin A2 concentration before the mRNA injectable has been measured at 5.1%, a concentration which is compatible with his other hematological readings and compatible with the ranges of hemoglobin A2 concentrations usually encountered in wide numbers of asymptomatic individuals with pure β^0-thalassemic traits (Soontornpanawet et al., 2023).

Alarmingly, the drop of hemoglobin A2 concentration in our patient's case is found in the most severe anemias. Especially in β-thalassemia heterozygotes, folate deficiency, as in our patient's case (see Table 1), suppresses hemoglobin A2 levels in the blood (Alperin et al., 1977). Due to a lack of hemoglobin B in patients with β-thalassemia, hemoglobin A2 is elevated and forms a tetramer with α- and δ-globin chains. Although the physiological role of hemoglobin A2 has not yet been fully established, it is important to mention that it prevents polymerization of the deoxy-sickle hemoglobin, and in sickle cell anemia it is considered a therapeutic target to raise the oxygen transport levels sufficiently, since it can have similar beneficial effects as fetal hemoglobin on oxygen carrying deficiency (Steinberg & Rodgers, 2015). Therefore, a concurrent significant drop of hemoglobin A2 and a rise in fetal hemoglobin in our patient contributes to the prediction of oxygen transport insufficiency throughout his body and hematologic stress that emerged after the mRNA injection. This is also reflected by his drop of hematocrit concentration and the changes in mean corpuscular volume, mean corpuscular hemoglobin concentration, iron, ferritin, folate, and red cell distribution width (see Table 1) after the mRNA injection requiring blood transfusion. Our patient's complaints of intense arthralgias and myalgias are also clinical characteristics of β-thalassemia major, leading through deterioration to rheumatic disease (Noureldine et al., 2018).

Regarding the complement-reactive-protein measurements of our patient, listed in Table 1, it is clear that, immediately before the mRNA injection, our patient had an elevated, but within normal limits, concentration of the inflammatory interacting protein which rises during increased pathology of various organs in patients with β-thalassemia major — commonly, if diagnosed soon enough and treated appropriately — it will be kept within normal limits in patients with β-thalassemia traits (Ehteram et al., 2014; Moftah et al., 2020). Also, our patient's hematocrit, ferritin and blood iron were within normal margins for a pure β-thalassemia trait, predicting that the patient we describe did not have a severe anemia condition prior to the mRNA injection. All the hematological data, combined with the previous measurement of hemoglobin A2 at 5.1%, suggest that our patient, prior to the mRNA injection, exhibited a normal hemoglobin A2 β^0-

thalassemia trait, as the hemoglobin A2 concentration can be as high as 10% in individuals with β^0 traits originating from Greece (Colaco et al., 2022).

Moreover, only the double β-thalassemia mutation heterozygotes with high hemoglobin A2 concentrations are characterized with relatively low levels of fetal hemoglobin (less than 20%) (Colaco et al., 2022), and, when this happens, it is alarming because of their life-threatening anemia conditions (Musallam et al., 2022). Furthermore, the high levels of ferritin our patient scored (see Table 1) closely match the high ferritin levels of β-thalassemic major children patients described by Moftah et al. (2020). If this is also the case, however, with our patient, then the mRNA injection has converted his anemia condition from that of a pure β^0-thalassemia trait from normal hemoglobin A2 levels to those encountered in patients with intermedia and major β-thalassemia conditions.

It has been shown that chronically elevated levels of complement-reactive-protein promote tumor suppressor p53-dependent Gap 2, or growth phase 2, halting of the mitosis cycle. If the mature cell's DNA is too damaged for cell division to proceed — the mitosis cycle is arrested and mitochondrial-dependent apoptosis of circulating monocytes takes place (Kim et al., 2014). Given the observed symptoms and elevated complement-reactive-protein in our patient's vascular system, it seems likely that the tumor-suppressor p53 is blocking normal cell divisions that are essential for repairing cell damage from toxicity. The latter we believe is reasonably attributed to the Pfizer injection the patient received. According to the patient's medical history report, he has sustained multiple viral infections, apart from SARS-CoV-2 and including SARS-CoV-2, throughout the 2.5 years after the mRNA injection, indicating an impairment of his immune defenses. The examinations of complement-reactive-protein included in this study were collected from periods when the patient was free from any viral infection for at least a month. The near upper borderline elevated levels of anti-p53 autoantibodies with the concurrent elevated levels of complement-reactive-protein suggest widespread systemic inflammation and impairment of immunity in our patient's body (Suppiah & Greenman, 2013).

To summarize, we are hypothesizing, and this requires further investigation, that our patient's symptoms of muscle atrophy may be a consequence of a complex interplay among the spike protein, spike protein antibodies, α7nAChRs, MDM2, the p53 protein and the prion protein. Spike protein binding to p53 causes its accumulation in the cytoplasm, which triggers misfolding into amyloidogenic fibrils. Failure to activate DNA repair enzymes leads to increased risk of mutations in p53, another way in which it could be inactivated. Low p53 expression leads to increased serum lactate, which was a positive metric for our patient. Meanwhile, spike antibodies bind to cellular prion protein, disabling it, which prevents both α7nAChRs mediated muscle contraction and autophagy clearance of the misfolded p53 proteins. Cellular prion protein facilitates recovery from muscle injury, so its suppression would aggravate the muscle damage due to the misfolded p53 and the toxic spike protein. Cellular prion protein is expressed in several other cell types besides neurons, including skeletal muscle cells, endothelial cells, erythrocytes and platelets. Cellular prion protein was shown to play a neuroprotective effect in a mouse model of ALS (Steinacker et al., 2010). Cellular prion protein plays a role in erythropoiesis, and its deficiency could help explain the hematological defects including anemia in our patient that developed after-injection. An experiment on mice involved chemically inducing acute hemolytic anemia in cellular prion protein-/- mice and wild type controls. cellular prion protein-/- mice produced less erythropoietin during recovery, and their erythroid precursor cells experienced a higher rate of apoptosis (Zivny et al., 2008).

Conclusion

In this paper, we have described a β^0thalassemia trait patient who was asymptomatic prior to the mRNA injection. However, after the mRNA injection, the lowering of his hemoglobin concentration from 13 g/L to 11.2 g/L, along with splenomegaly, suggested that the patient sustained a significant deterioration of his condition. The patient sustained a medically important

rise of fetal hemoglobin and drop of hemoglobin A2 concentrations after the mRNA injection, which were not due to additional mutations in the α and δ globin genes. This leads us to conclude that the rise in fetal hemoglobin was due to hematologic stress.

The lowering of his mean corpuscular volume and the rise of his red cell distribution width values also incline to the conclusion that the patient has suffered from an anemia condition consequential to the lowering of his hemoglobin concentration. The hematologic deterioration can explain most of the cardiological, neurological, respiratory and muscle weakness symptoms that the patient experiences until now. The continuously increased complement-reactive-protein values after the mRNA injection and the upper borderline p53 autoantibodies and high ferritin levels, apart from pointing to chronic inflammation also point to immune dysregulation. Moreover, the patient's chronic mild leucocytosis and elevated platelet count after the mRNA injection point to an autoimmune hematological disorder.

One puzzling aspect of this case is that the patient still has an abnormally high level of SARS-CoV-2 spike protein neutralizing antibodies after so many days from his single mRNA COVID-19 injection, and, to the best of our knowledge, similarly high levels have not been recorded elsewhere in the research literature so far. We hypothesize that the mRNA injectable caused an upregulation of mTOR which interfered with autophagy, and this prevented clearance of the spike protein. Autophagy suppression also leads to upregulated expression of prion protein, and this may have contributed to the development of hypothesized autoantibodies to the C-terminal region of prion protein, via molecular mimicry with antibodies to the RBD domain of the spike protein. This hypothesis could explain the observed issues with muscle weakness and muscle atrophy. Our hypothesis is strengthened by yet another recently published case of a β-thalassemia trait carrier who showed abnormal mTOR upregulation after the mRNA COVID-19 injection. According to the extensive literature review conducted for this unique case, the excessive activation of innate immunity and thus an mTOR hyperactivation, produces a downregulated autophagic process that leads to autoimmunity. The change in β^0-thalassemia phenotype in our patient's case, after mRNA injection, may be an alarming concern for all β-thalassemia minor trait patients receiving this kind of genetic prophylactic against COVID-19.

Conflict of Interest Statement

The authors deny any conflict of interest. Seneff is on the Editorial Board for the *IJVTPR* but did not participate in the decision to publish this paper and receives no remuneration for her work as an editor of the journal, a non-profit, non-advertising, independent academic entity.

Consent

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

Data Availability

Supplementary material of clinical reports is available upon request.

Author Contributions

Anthony Kyriakopoulos: Conceptualization. Investigation. Methodology. Writing of the original draft. Providing all the figures and tables. Participating in multiple revisions.

Stephanie Seneff: Writing of the original draft. Participating in multiple revisions.

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