published paper showed that 10% to 20% of bilirubin does not come from senescent erythrocytes.[4] Red blood cell lifespan measured by CO breath test showed excellent agreement even in cases of severe anemia and hemolysis with RBC lifespan measured by labeling techniques.[5]

A prior study estimated the lifespan of RBCs in 16 healthy subjects and seven patients with aplastic anemia measured using the ^{51}Cr method.[6] 4 (57%) of the seven patients had a shortened RBC lifespan (14.8–18.5 days vs. 21–31 days in the normal value of RBC $T_{1/2}$).

Our previous study found that the serum levels of IL-2R and IL-6 were much lower in HR patients than NR patients (IL-2R: 4.3×10^5 U/L vs. 6.5×10^5 U/L, $p = 0.006$; IL-6: 2.6 [2.0–17.7] ng/L vs. 6.1 [2.0–14.4] ng/L, $p = 0.003$).[1]

In the present study, the CD3+CD8+T lymphocyte percentage correlated with RBC lifespan before IST ($p = 0.008$, $R^2 = 0.299$) after multivariate analysis. CD8+cytotoxic T cells with restricted T-cell receptor (TCR) diversity (oligoclonal T cells) are expanded in AA and secrete proinflammatory cytokines such as IFN-γ and TNF-α, which induce apoptosis of CD34+ cells in part through the Fas-dependent pathway. Systemic exposure to physiologically relevant levels of TNF-α is sufficient to cause acute cytopenias and hemophagocytosis. These could be the mechanism of the shortened lifespan in AA, but we did not observe in the present study a correlation between cytokines and RBC lifespan, even IFN-γ and TNF-α. Because of the small sample size, further studies with a large sample size should be carried out in the future.

In summary, we found the RBCs lifespan in untreated V/SAA patients is significantly shorter than normal and improves with response to therapy. Shortened lifespan of RBCs should therefore be considered in the pathogenesis of anemia in AA.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Lei Ye analyzed the data and wrote the paper; Liping Jing, Jie Guo, Xin Zhao, Guangxin Peng, Yuan Li, Jianping Li, Huihui Fan, Wenrui Yang performed and supervised the research; Fengkui Zhang and Li Zhang designed the study, reviewed the data, and revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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Antibody response to COVID-19 mRNA vaccine (Comirnaty) in myeloma patients treated with high-dose melphalan and/or immunotherapy

To The Editor:

COVID-19 infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) was first reported in December 2019 in Wuhan, China, and has since become a global pandemic. Symptoms range from none (subclinical infections) to acute respiratory failure, including acute respiratory distress syndrome (ARDS). Risk factors for a severe course of disease include old age, male gender and comorbidities such as cardiovascular disease, diabetes, chronic obstructive pulmonary disease (COPD) and cancer. Several studies have reported that the overall risk of cancer patients to become infected and developing critical symptoms requiring intensive care unit (ICU) admission and death is high. Notably, patients with hematological cancers seem to be at even higher risk of fatal outcome than those with solid tumors.[1] A high but variable mortality (27–57%) in COVID-19 hospitalized patients with a plasma cell disorder was reported by the International Myeloma Society.

Multiple myeloma (MM) accounts for 10% of all hematologic malignancies and it is associated with inherent cellular and humoral dysfunction, where patients are susceptible to respiratory tract infections in particular. The risk of infection is increased already at the stage of monoclonal gammopathy of undetermined significance (MGUS) and more so in active disease. Antitumoral treatments are known to further aggravate the immunosuppression impairing T-cell as well as antibody function and production,[2] and as a consequence, eliciting the patient's own immune response to common pathogens by means of scheduled vaccinations is usually highly recommended.

The COVID-19 mRNA vaccine BNT162b2 produced by Pfizer-BioNTech is a lipid nanoparticle-formulated, nucleoside-modified RNA vaccine, encoding the membrane-anchored SARS-CoV-2 full-length spike protein, stabilized in a prefusion conformation.[3] It was first authorized for active immunization in December 2020. However, the exclusion of cancer patients in the registry trials has raised questions about the efficacy and safety of mRNA vaccines in this often immunocompromised population. Several studies have reported good tolerance but reduced performance in cancer patients, including lower rates of seroconversion in patients with MM.[4]

Immunomodulatory drugs (IMiDs), proteasome inhibitors (PIs), and, later on, monoclonal antibodies have become standard treatments contributing to enhanced overall survival of patients with MM. In spite all these new agents, high-dose melphalan with autologous stem cell transplantation (ASCT) rescue has remained the standard of care for transplant-eligible patients. Due to its complex biology, MM will become refractory, rendering subsequent therapy less effective. The anti-CD38 human monoclonal antibody daratumumab alone gives an overall response rate of 30%–35%, in relapsed/refractory MM (RRMM), and it is used either as a single agent or in combination. We report here on the results of the two-dose BNT162b2 vaccine in MM patients after different treatment regimens, including ASCT and maintenance, at one institution.

In this prospective study a total of 93 MM patients, all aged >18 years, were included. Consecutive patients were offered vaccine against SARS CoV-2 according to guidelines of the Swedish authorities. After consenting to the BNT162b2 mRNA regimen, patients in active surveillance were asked for blood samples for serological tests at 4 weeks following the second dose. The general advice for patients with ongoing antitumoral treatment was to schedule, whenever feasible, both vaccine doses at least 3–4 days apart from administration of the disease-modifying drug and at least 3 months following ASCT. The primary end-point was spike protein (SP) detection after the 4 weeks from the second dose. Blood samples were drawn starting early in the year 2021 on February 1 until July 7. Antibodies against the SP receptor-binding domain (RBD) were measured as units/ml (U/ml) (normal reference value is >250 U/ml).

Clinical data focusing on, age, cytogenetics, disease status and ongoing disease-related treatment were extracted from the patient medical records. No adverse events were measured if not reported by the patients.

The study was approved by the Ethical Committee in Sweden.

For statistical analyses, continuous variables were described as the mean and range while the categorical data were represented by the frequency. Chi-squared and fisher's exact tests were used to test the association between predictors and SP levels. The SP levels were grouped into ≥250 U/ml versus <250 U/ml and ≥100 U/ml versus <100 U/ml, where levels of <100 U/ml were defined as no response and levels of ≥100 U/ml were considered as response. In the text, we discussed the latter group only, while results for both are presented in Table 1. Statistical analyses were performed using R and machine-learning was performed by random-forest using Python.

Patient characteristics are presented in Table S1. The mean age of 93 patients was 62.4 years (range 37–86). Among them, 61 patients were in a RRMM phase and 62 had ongoing treatment. A majority of patients ($n = 58$) received monotherapy while only four patients were treated with three or more drugs. A total of 45 patients showed very good or better partial response/complete response (VGPR/CR) while only 48 patients exhibited a less than VGPR/CR response to treatment. Fourteen patients were infected with COVID-19 before the vaccination and 73 patients were undergoing high-dose treatment.

We categorized the patients, on the basis of their current treatment status, into non-ongoing ($n = 31$), and ongoing ($n = 62$) treatment groups. Patients with non-ongoing treatment showed significantly higher SP levels ($p = 0.002$) than patients with ongoing treatment (see Table 1). Importantly, the association diminished by removing patients either treated with anti-B cell maturation antigen (anti-BCMA) and daratumumab (more than once per month) or anti-BCMA, daratumumab (more than once per month) and dexamethasone from analyses ($p = 0.18$ and $p = 0.46$, respectively). This suggests that in general, disease-related, ongoing treatment besides treatment with anti-BCMA, daratumumab (more than once per month) and dexamethasone did not have an impact on SP levels.

None of the patients on anti-BCMA therapy ($n = 10$) responded to BNT162b2 vaccination suggesting a strong association between anti-BCMA and lower levels of SP ($p < 0.0001$). Treatment with daratumumab ($n = 27$) resulted into nonsignificant changes in SP. Interestingly, when daratumumab patients were subgrouped into those receiving more than one dose per month ($n = 5$) and those receiving one dose per month ($n = 22$) the analysis resulted in a strong association of the former group with lower levels of SP ($p < 0.0001$). The dexamethasone treatment group

TABLE 1 Effect of treatments and clinical parameters on the levels of spike proteins in the NT162b2 vaccinated patients ($n = 93$)

Variable	Category	Spike proteins [mean (*n*)]	*p*-value (≥ 100 U/ml vs. <100 U/ml)	*p*-value (≥ 250 U/ml vs. <250 U/ml)
Treatment	No treatment	227 (31)	0.002	0.012
	Any ongoing treatment	160 (62)		
Treatment[a]	No treatment	227 (31)	0.18	0.31
	Any ongoing treatment	198 (49)		
Treatment[b]	No treatment	227 (31)	0.46	0.86
	Any ongoing treatment	211 (44)		
Anti-BCMA	Treated	3 (10)	2.4E−7	7.9E−6
	Untreated	204 (83)		
Daratumumab	Treated > once per month	31 (5)	1.2E−5	7.4E−5
	Treated < once per month	247 (22)		
Dexamethasone	Treated	58 (7)	0.011	3.5E−4
	Untreated	192 (86)		
IMiDs	Treated	177 (25)	0.98	1
	Untreated	184 (68)		
HDT	Treated	190 (73)	0.44	0.39
	Untreated	154 (20)		
6 months	Within 6 months	154 (12)	1	0.06
	> 6 months	197 (61)		
12 months	Within 12 months	187 (22)	1	0.75
	>12 months	191 (51)		
Age (years)	<65	191 (60)	0.63	0.33
	≥65	165 (33)		
IgG	<4	152 (35)	0.09	0.045
	≥4	200 (58)		
RRMM	Yes	164 (61)	0.01	0.039
	No	217 (32)		
COVID-19	Yes	244 (14)	0.02	0.029
	No	171 (79)		
CR	Yes	191 (45)	0.60	0.39
	No	173 (48)		

Abbreviations: anti-BCMA, anti-B cell maturation antigen; HDT, high-dose treatment; IgG, Immunoglobulin G; IMiDs, immunomodulatory drugs; RRMM, relapsed/refractory multiple myeloma.

[a]Treatment group without anti-BCMA and daratumumab.

[b]Treatment group without ant-BCMA, daratumumab and dexamethasone.

($n = 7$) also had a significant reduction in SP ($p = 0.01$) (see Table 1). Machine learning using an ensemble method random-forest ranked daratumumab (more than once per month), anti-BCMA and dexamethasone as top three important variables in predicting the categories of SP response.

Patients undergoing HDT followed by ASCT ($n = 73$) did not show an association between changes in SP levels and prior HDT ($n = 20$) patients ($p = 0.44$), as shown in Table 1. A full antibody response was seen in patients infected and presenting with symptoms of COVID-19 ($p = 0.02$).

In the cohort of 93 patients, 32 had newly diagnosed MM (NDMM) and 61 had RRMM. We found that vaccinated RRMM patients had lower SP levels than NDMM patients ($p = 0.01$), suggesting involvement of disease progression to lower the effect of vaccination, but when excluding patients with ongoing treatment with antibodies (BCMA/CD38) and dexamethasone, no difference was observed.

This study illustrates the safety and tolerability of BNT162b2 vaccine. It highlights the benefit in patients with MM previously treated with ASCT regardless when the vaccine was given from the ASCT date, but also patients on antibody treatment whose response was inadequate. Our findings suggest that this type of vaccine can induce an antibody response while maintaining a tolerable safety profile, which is of great importance in this population.

In an earlier study by Avivi et al.,[5] the BNT162b2 vaccine was found to be safe and to provide a high seropositivity rate after two

doses in MM patients overall. Elderly, hypogammaglobulinemic and heavily pretreated patients had lower response rates. The reasonable serological response in hematological patients after ASCT was also described by Maneikis et al.,[6] perhaps reflecting a younger population with an effectively treated underlying disease.

In this study, we found that anti-BCMA, daratumumab (more than once per month) and dexamethasone treatments are strongly associated with lower SP levels, results that are well in line with those reported by Oekelen et al.[7] It had already been shown that[6] daratumumab treatment tended towards reduced response, but the association was not significant for any agent included in a combination regimen.

In this study we did not monitor the T-cell immunity as a response to the vaccination and thus the results need to be interpreted with caution. None of the patients reported a serious unexpected adverse event.

In conclusion, the BNT162b2 mRNA vaccine against SARS CoV-2 virus was found to be safe and often efficient (73% responded) in inducing an antibody response in patients with MM. Response to vaccination seems to be affected more by the type of ongoing treatment than by the current presentation of disease. In our study anti-BCMA therapy and frequent administration of daratumumab (more than once per month) were the strongest predictors of a reduced response. Future randomized trials will be needed to validate if withholding of the ongoing antibody/dexamethasone treatment promotes better responses to COVID-19 vaccination.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Katarina Uttervall, Johan Lund and Hareth Nahi were involved in study design. Carina Svärd, Katarina Malmsten, Evellyn Fletcher-Torres, Johan Lund and Hareth Nahi contributed to data collection. Sandra Lockmer, Muhammad Kashif, Evren Alici and Hareth Nahi contributed to data analysis and interpretation. Sandra Lockmer, Katarina Uttervall, Muhammad Kashif, Johan Lund and Hareth Nahi wrote the manuscript. All authors reviewed the draft and approved the final version to be submitted.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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