Immunogenicity and safety of SARS-CoV-2 mRNA vaccines in a cohort of patients with type 1 diabetes

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Abstract word count: 210
Text Word-count: 2039
Tables/Figures: 4 items
One sentence summary: cytotoxic immune response to SARS-CoV-2 mRNA vaccine is impaired in T1D patients.
Key-words: COVID-19, SARS-CoV-2 mRNA vaccine, Type 1 diabetes, Immunogenicity

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Abstract

Patients with type 1 diabetes (T1D) may develop severe outcomes during COVID-19 disease, but their ability to generate an immune response against the SARS-CoV-2 messenger RNA (mRNA) vaccines remains to be established. Here we evaluated the safety, immunogenicity and glycometabolic effects of the SARS-CoV-2 mRNA vaccines in patients with T1D. A total of 375 patients, 326 with T1D and 49 non-diabetics, who received two doses of the SARS-CoV-2 mRNA vaccines (mRNA-1273, BNT162b2) between March and April 2021 at the ASST FBF-Sacco Milan, Italy, were included in this monocentric observational study (NCT04905823). Local and systemic adverse events were reported in both groups after SARS-CoV-2 mRNA vaccination without statistical differences between them. While both T1D patients and non-diabetic subjects exhibited a parallel increase in anti-SARS-CoV-2S titers after vaccination, the vast majority of T1D patients (70% and 78% respectively) did not show any increase in the SARS-CoV-2-specific cytotoxic response as compared to the robust increase observed in all non-diabetic subjects. A reduced secretion of the T cell-related cytokines IL-2 and TNF-alpha in vaccinated patients with T1D was also observed. No glycometabolic alterations were evident in patients with T1D using continuous glucose monitoring during follow-up. Administration of the SARS-CoV-2 mRNA vaccine is associated with an impaired cellular SARS-CoV-2-specific cytotoxic immune response in T1D patients.

Introduction

Patients affected by both type 1 (T1D) and type 2 (T2D) diabetes may develop more severe outcomes during SARS-CoV-2 infection (1; 2) and the glycometabolic control is altered during COVID-19 (3; 4). The recent availability of novel vaccines against SARS-CoV-2 (5) suggests that primary prevention based on vaccination may be a key-strategy to dampen the risks associated with COVID-19 in patients with T1D. Because an impaired humoral/cellular immune response to vaccination was hypothesized in patients with diabetes (6-8), careful consideration of age and timing of disease onset has been proposed when considering vaccination for T1D. While benefits are known for common vaccinations, the use of novel vaccines, as it is the one for SARS-CoV-2, may be challenging (8-10). Given the more severe outcomes during SARS-CoV-2 infection (11), patients with T1D should represent a priority group to receive the COVID-19 vaccine (12), although studies in this population are lacking (13). Here we assessed the safety, immunogenicity and glycometabolic effects of the SARS-CoV-2 mRNA vaccines in patients with T1D, and we provide new evidence for an impaired SARS-CoV-2-specific cellular cytotoxic immune response in T1D patients.

Research Design and Methods

Study design and participants

This monocentric observational study was conducted at the Endocrinology and Diabetology Unit, ASST FBF-Sacco, Milan, Italy according to the principles of the Declaration of Helsinki and the protocol was approved by the Comitato Etico Milano Area 1 (CoVaxT1D, NCT04905823). 326 patients with T1D and 49 non-diabetic "frail" patients defined based on the guidelines issued by the Italian Ministry of Health and including also caregivers/family members of patients with T1D

(Table 1), received two doses of SARS-CoV-2 mRNA vaccines (Supplementary Table 1), between March 26, 2021, and April 18, 2021 and signed informed consent (Supplementary Fig. 1). Patients with active COVID-19 or diagnosis of COVID-19 within the previous 3 months were excluded. The primary outcomes of the present study were the safety of the mRNA vaccines in patients with T1D, as measured by the occurrence of local/systemic adverse events reported, humoral immunogenicity, as measured by anti-SARS-CoV-2 Spike antibody levels and cell immunogenicity, as measured by increase in SARS-CoV-2 cytotoxic cellular response *in vitro*. The secondary outcome was to evaluate the effect of the mRNA vaccines on glycemic control in patients with T1D, as measured by continuous glucose monitoring (CGM), (3). Samples and data were analyzed before vaccination, after dose 1 and 2.

Immunological studies

SARS-CoV-2 Spike antibody detection

The humoral response was analyzed using ROCHE Elecsys Anti-SARS-CoV-2 serology test (La Roche Ltd, Basel, Switzerland), which captured IgG, IgM and to a lesser extent IgA against SARS-CoV-2 Spike antigen (receptor-binding domain), with signal to cutoff (S/Co) values of ≥ 1.0 reported as positive (14). An in vitro neutralization assay (NTA), as already described (15), was used to validate anti-SARS-CoV-2 antibodies titers detected in seronegative vaccinated patients with T1D by performing a Spearman's correlation analysis between antibodies titers detected by NTA and the Elecsys assay.

In vitro immune cellular response

$1x10^6$ peripheral blood mononuclear cells (PBMCs) (16), obtained from frail subjects or T1D patients before and after vaccination, were cultured with/without SARS-CoV-2 spike and nucleocapsid recombinant proteins (PR-nCoV-3/PR-nCoV-1, Novatein Biosciences, Inc, Woburn,

MA), 500 ng/ml for 48 hours (17). Vaxigrip (quadrivalent split-virion influenza vaccine peptides, Sanofi-Pasteur, 0.25 ug/ml) and glutamate transporter-1 (GLT-1, Genscript, Piscataway, NJ, 50 ug/ml) were included as positive and negative controls. Supernatants were tested for perforin/granzyme A (HCD8MAG-15K, Merck-Millipore, Burlington, MA) and T cell-related cytokines (M500KCAF0Y, Bio-Rad Laboratories, Hercules, CA) by a Bio-Plex 200 System. An increase in cytotoxic response was assessed as a fold increase >0.1 for perforin and >0.01 for granzyme A respectively. Cut points of 0.1 and 0.01 were defined based on the difference detected for each analyte, measured at each timepoint and normalized to baseline value. To test the specific response to SARS-CoV-2 antigens, the number of IFN-γ-producing cells (BD Biosciences, USA) was assessed in an ELISpot assay as already described (17).

Statistical analysis

The Fisher's exact test, one-way and two-way ANOVA, the Kruskal-Wallis tests, adjusted for multiple comparisons, and the Mann-Whitney tests, were used in the analyses of baseline characteristics, side effects, humoral and cellular response, CGM parameters. Multivariable logistic regression was used to model the relationships between co-factors and cellular response (Stata version 12; StataCorp). Statistical significance was determined at $\alpha < 0.05$. GraphPad Prism 7 (San Diego, CA) was used to generate graphs.

Data availability

The datasets and resources generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

Patients' characteristics

We included 375 patients consecutively administered a SARS-CoV-2 mRNA vaccine, of whom 326 patients had long-term T1D (22.9±14.2 years) and 49 subjects had no diabetes but were considered as medically frail/caregivers according to the Italian Ministry of Health guidelines (Supplementary Fig. 1, Table 1). The mean age of patients with T1D was 45.9 ± 9.0 years old, and 180 (55%) were male, while in nondiabetic medically frail patients/caregivers mean age was 61.0 ± 12.9 years old, and 21 (43%) were male (Table 1). No differences were observed with regard to type of vaccine administered (Supplementary Table 1), BMI, concomitant therapy (except for insulin treatment), and major clinical comorbidities, with a higher frequency of autoimmune thyroiditis observed in patients with T1D (Table 1).

Safety

No differences were observed between patients with T1D and non-diabetic subjects with regard to local and systemic side effects after vaccination (Supplementary Fig. 2A-B). Local pain was the most common local side effect, reported equally after dose 1 and 2 of the vaccine (72.6% vs 73.7%, p=0.78), (Supplementary Fig. 2A-B). Systemic adverse events, consisting mainly of weakness and fever, were more frequent after dose 2 (Supplementary Fig. 2A-B).

Immunogenicity

Humoral immunity

With regard to SARS-CoV-2 humoral response, patients with T1D who were seronegative for COVID-19 showed an increase in median IgG levels both after dose 1 and 2 (60.4 and 2058.0 U/ml respectively) (Fig. 1A-C). This observation was paralleled in subjects who had no diabetes at both timepoints (46.3 and 1965.0 U/ml respectively) (Fig. 1A-C), with a response rate comparable between the two groups. These data indicate that the administration of two doses of the SARS-CoV-2 mRNA vaccine elicited a humoral response in patients with T1D. In a subgroup

analysis performed on 15% (49/326) of patients with T1D and 14.3% (7/49, p=0.99) of non-diabetic subjects who underwent full vaccination but were seropositive for COVID-19 (i.e., experienced asymptomatic SARS-CoV-2 infection, baseline S/Co IgG value of ≥ 1.0), the analysis of the humoral response revealed no differences between the two groups (Supplementary Fig. 3). Finally, our assay captured neutralizing activity as shown by a positive correlation between anti SARS-CoV-2 titers determined by our assay with titers of neutralizing antibodies as measured by an *in vitro* neutralization assay (Fig. 1D).

Cellular immunity

To address whether an immune cytotoxic response against SARS-CoV-2 was elicited with vaccination, we re-challenged isolated PBMCs with SARS-CoV-2 peptides at baseline, after dose 1 and 2 in a Milliplex MAP Human assay. Patients with T1D showed no increase in the granzyme A and perforin SARS-CoV-2-specific T cell release in the supernatant after vaccination as compared to baseline, while a 2-3-fold increase in both granzyme A and perforin production was evident in non-diabetic subjects (Fig. 2A-B), which was also confirmed by testing negative (<1-fold increase) and positive controls (10- and 7-fold increase for granzyme A and perforin respectively). Interestingly, only 30% of T1D patients showed an increased SARS-CoV-2-specific T cell cytotoxic response after dose 1, as compared to 80% of non-diabetic subjects (Fig. 2C). This observation was confirmed after dose 2 in patients with T1D, with 22% of patients developing/maintaining an increased SARS-CoV-2-specific T cell cytotoxic response, although the rate of non-diabetic subjects developing a cytotoxic response was lower (57%, Fig. 2D). Furthermore, the reduced cytotoxic response detected in T1D was also confirmed after controlling for other factors potentially involved (age, sex, concomitant diseases, Supplementary Table 2). These findings suggest that SARS-CoV-2 mRNA vaccines fail to elicit a robust cytotoxic response

against SARS-CoV-2 in the majority of patients with T1D. Finally, a multiplex T cell-related cytokines analysis revealed lower levels of IL-2 and TNF-alpha measured in the supernatant of PBMCs cultured with SARS-CoV-2 peptides in patients with T1D as compared to non-diabetic subjects after vaccination (Fig. 3A), which was associated with a low response in an IFN-gamma ELISpot assay (Fig. 3B), regardless to the type of mRNA vaccination administered, thus confirming the existence of an altered immune cellular response to SARS-CoV-2 mRNA two doses-vaccination in T1D.

Glycemic control

Given that COVID-19 is associated with the development of dysglycemia and diabetes, we finally assessed whether the administration of SARS-CoV-2 mRNA vaccine may affect glycometabolic control in patients with T1D. Data collected by using continuous glucose monitoring showed no difference in all the parameters examined at each timepoint (Supplementary Table 3). Moreover, a multivariable analysis showed no association between estimated HbA1C levels and cytotoxic response to vaccination in patients with T1D, after adjusting for age, sex and concomitant therapies ($p=0.23$ and $p=0.65$ for response to dose 1 and 2 respectively). These findings suggest that the administration of the SARS-CoV-2 mRNA vaccine is not associated with altered glycometabolic control in patients with T1D.

Discussion

Our study evaluated the safety, immunogenicity and glycometabolic effects of two doses of SARS-CoV-2 mRNA vaccines in patients with T1D. No differences were observed with regard to the humoral response or the incidence of side effects in patients with T1D as compared to non-diabetic subjects, and no effects on glycometabolic control were observed using continuous glucose

monitoring analysis during follow-up. Interestingly, both dose 1 and 2 of SARS-CoV-2 mRNA vaccines were not associated with an increase in cytotoxic factors granzyme A and perforin in the majority of patients with T1D nor with an increase in the production of the T-cell related cytokines IL-2 and TNF-alpha, as compared to non-diabetic subjects, who showed an increased rate of both cytotoxic factors and cytokines after vaccination. The SARS-CoV-2 mRNA vaccines have been shown to elicit a humoral and T cell-specific immune response in healthy subjects (18; 19), but little is known on their effects in patients with T1D. An impairment in the immune response following vaccination in patients with T1D has been previously hypothesized for other immunization strategies such as influenza, rotavirus, and Hemophilus B (6; 8) and attributed to an impaired cellular immune response. Therefore, boosting immunization strategies with multiple doses has been recommended (8; 20). Indeed, immune dysregulation of both T and B cell compartments are a key feature of patients with T1D from the onset of the disease and may indicate abnormalities in the immune response (21; 22), including a higher risk of developing infectious disease (23; 24). This was particularly evident during the COVID-19 pandemic, with T1D patients experiencing severe COVID-19 disease, with worsened outcomes as compared to the non-diabetic general population (1; 2; 11). In our study, patients with T1D did not show an increase in T cell-specific SARS-CoV-2 cytotoxic factors release (granzyme A and perforin), which may indicate a defective ability in inactivating the virus. Indeed, perforin and granzyme A levels, as observed for other vaccines and immunization strategies (25; 26), detected after vaccination, increased in non-diabetic subjects in response to SARS-CoV-2 *in vitro* challenge, while no effect was observed in the majority of T1D patients. This suggests a reduced cytotoxic effector function in T1D and a less immunogenic/efficient vaccination in these patients. Despite this, no significant differences were observed in SARS-CoV-2 Spike-specific neutralizing antibody titers between patients with T1D

and non-diabetic subjects. To the best of our knowledge, this is the first study designed to demonstrate the safety and immunogenicity of SARS-CoV-2 mRNA vaccines in a large cohort of patients with T1D, without any significant perturbation in continuous glucose monitoring parameters, thus supporting the complete safety of vaccine for patients with T1D in our large population (13; 27). We acknowledge that this study has some limitations, which include the study design, that may have led to potential selection bias primarily due to the pandemic situation, such as the smaller and not fully age-matched "frail" control population, the limited number of samples available for some analyses (e.g., Elispot), and the assay employed that may capture one aspect of the vaccine-specific cellular response. In summary, an impaired cytotoxic SARS-CoV-2-specific cellular immune response has been observed in the majority of patients with T1D following two doses of SARS-CoV-2 mRNA vaccination, with the humoral response and glycemic control unaffected.

Author contributions

F.D. and GM.S. designed the study, analyzed data, and wrote the paper; E.A., V.U., A.A., A.M., AJ.S, M.BN., C.L., D.M., and G.R. performed experiments and analyzed data; I.P., L.M., P.M., L.P., A.R., E.C., AM.B., E.L., C.M. and M.M. collected human data and samples; S.A., S.R., M.G., F.F., C.B., M.G., MR.G. and GV.Z. coordinated and designed research and edited the paper; P.F. conceived the idea, designed the study, and wrote and edited the paper.

Acknowledgments

F.D. is supported by an SID Lombardia Grant and by an EFSD/JDRF/Lilly Programme on Type 1 Diabetes Research 2019. P.F. is supported by the Italian Ministry of Health grant RF-2016-02362512 and by the Linea-2 2019 funding from Università di Milano. We thank the "Fondazione Romeo e Enrica Invernizzi" for extraordinary support.

Prof. Paolo Fiorina is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure

The authors have nothing to disclose.

TABLES

Table 1. **Baseline characteristics of patients included in the study**.

	T1D (n =326)	**CTRL° (n =49)**	***p value***
Age – yr (mean ± SD)	45.9 ± 9.0	61.0 ± 12.9	<0.0001
Male – n (%)	180 (54.9)	21 (42.9)	0.12
Diabetes duration – yr (mean ± SD)	22.9 ± 14.2	-	
Body mass index – kg/m^2 (mean ± SD)	25.1 ± 4.1	26.4 ± 4.9	0.16
Comorbidities – n (%)			
Hypertension	85 (25.9)	18 (36.7)	0.13
Cardiovascular disease	18 (5.5)	1 (2.0)	0.48
Cerebrovascular disease	12 (3.7)	2 (4.1)	0.70
Chronic kidney disease†	14 (4.3)	1 (2.0)	0.70
Diabetic neuropathy	32 (9.8)	-	
Diabetic retinopathy	86 (28.3)	-	
Malignancy	9 (2.9)	0	0.61
Autoimmune diseases – n (%)			
Autoimmune thyroiditis	72 (23.5)	6 (12.2)	<0.0001
Other autoimmune disease	24 (7.8)	5 (10.2)	0.57
Diabetes treatment – n (%)			
Insulin	326 (100)	-	
Other treatments			
ACE-I	39 (11.9)	10 (20.4)	0.11
ARB	31 (9.5)	5 (10.2)	0.80
B-blockers	32 (9.8)	4 (8.2)	0.99
Aspirin	45 (13.8)	8 (16.3)	0.66
Statins	27 (12)	4 (14)	0.75

†Chronic kidney disease defined as eGFR ≤ 60 ml/min/m^2
°Non-diabetic "frail" patients defined based on the guidelines issued by the Italian Ministry of Health (i.e., elderly subjects > 75 years old, or patients of any age with respiratory illness, severe cardiovascular disease, neurological disabilities, multiple sclerosis, cystic fibrosis, kidney failure, autoimmune diseases, liver disease, strokes and cerebrovascular disease, cancer, Down's syndrome, organ or bone marrow transplants and severe obesity) and caregivers/family members of patients with T1D. Data are reported as mean ± standard deviation (SD)

Abbreviations: ACE-I, Angiotensin-converting-enzyme inhibitors; ARB, Angiotensin II receptor blocker; T1D, type 1 diabetes; B-blockers, beta blockers; SD, standard deviation.

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FIGURE LEGENDS

Figure 1. **Anti–SARS-CoV-2 antibody response in patients with T1D and in non-diabetic subjects**.

(A, **B**, **C)**. Boxplots representing Anti–SARS-CoV-2 S antibody levels measured in patients with T1D and in non-diabetic subjects at baseline (A), after the first dose (B) and after the second dose (C) of the SARS-CoV-2 mRNA vaccines. Anti-SARS-CoV-2 antibody titers are reported as median with interquartile range. **(D)**. Line graph showing the correlation between SARS-CoV-2 antibodies titers detected by an *in vitro* neutralization assay and through the Elecsys assay (n=30). Measurements for 'after dose 1' were performed 3-4 weeks after the first dose, and measurements 'after dose 2' were performed 4 weeks after the second dose.

Abbreviations: AU, Arbitrary Units; NTA, neutralization assay.

Figure 2. Cytotoxic response to the SARS-CoV-2 peptides after prime and boost doses of SARS-Cov-2 mRNA vaccine administered to T1D patients and to non-diabetic subjects.

(A, **B)**. Line graphs representing normalized secretion of SARS-CoV-2 -specific T cell cytotoxic factors perforin (A) and granzyme A (B), measured in the supernatant of PBMCs obtained from patients with T1D or from non-diabetic subjects at each timepoint (baseline, after dose 1 and after dose 2) and cultured in the presence/absence of SARS-CoV-2 peptides for 48 hours. Data expressed are mean ± standard error of the mean (SEM). Secretion data have been normalized to baseline levels for each cytokine analyzed. Cut points of 0.1 and 0.01 were assessed based on the difference observed in the levels of each analyte, perforin and granzyme A respectively, measured at each timepoint as compared to baseline and normalized to baseline value. Analysis was performed for 'after dose 1' at 3-4 weeks after the first dose, and for 'after dose 2' at 4 weeks after the second dose. **(C)**. Bar graph representing proportions of patients with T1D as compared to non-diabetic subjects showing increased or no change in the SARS-CoV-2-specific T cell cytotoxic response before receiving the second dose of SARS-Cov-2 mRNA vaccine. **(D)**. Bar graph representing proportions of patients with T1D as compared to non-diabetic subjects showing increased or no change in the SARS-CoV-2-specific T cell cytotoxic response at 4 weeks of follow-up after administration of the second dose of the SARS-Cov-2 mRNA vaccine. The SARS-CoV-2-specific T cell cytotoxic response was analyzed as an increase/lack of increase observed in both perforin and granzyme A levels at each timepoint.

Abbreviations: PBMCs, peripheral blood mononuclear cells; A.U., arbitrary units; T1D, type 1 diabetes.

Figure 3. T cell-related cytokines and immune response to SARS-CoV-2 peptides after administration of SARS-Cov-2 mRNA vaccines in T1D patients and in non-diabetic subjects.

(A). Dot plots representing secretion of SARS-CoV-2 -specific T cell-related cytokines, measured in the supernatant of PBMCs obtained from patients with T1D or from non-diabetic subjects at baseline and after dose 2 and cultured in the presence/absence of SARS-CoV-2 peptides for 48 hours. Data are expressed as mean ± standard error of the mean (SEM) of secretion levels corrected to baseline levels for each cytokine analyzed. **(B)**. Bar graphs representing the number of IFN-γ spots detected by ELISpot analysis using PBMCs isolated from patients with T1D (n=10) and from non-diabetic subjects (n=10), after the administration of the SARS-CoV-2 mRNA vaccines, following challenge with spike and nucleocapsid SARS-CoV-2 peptides. Data are expressed as mean ± SEM.

Abbreviations: PBMCs, peripheral blood mononuclear cells; T1D, type 1 diabetes.

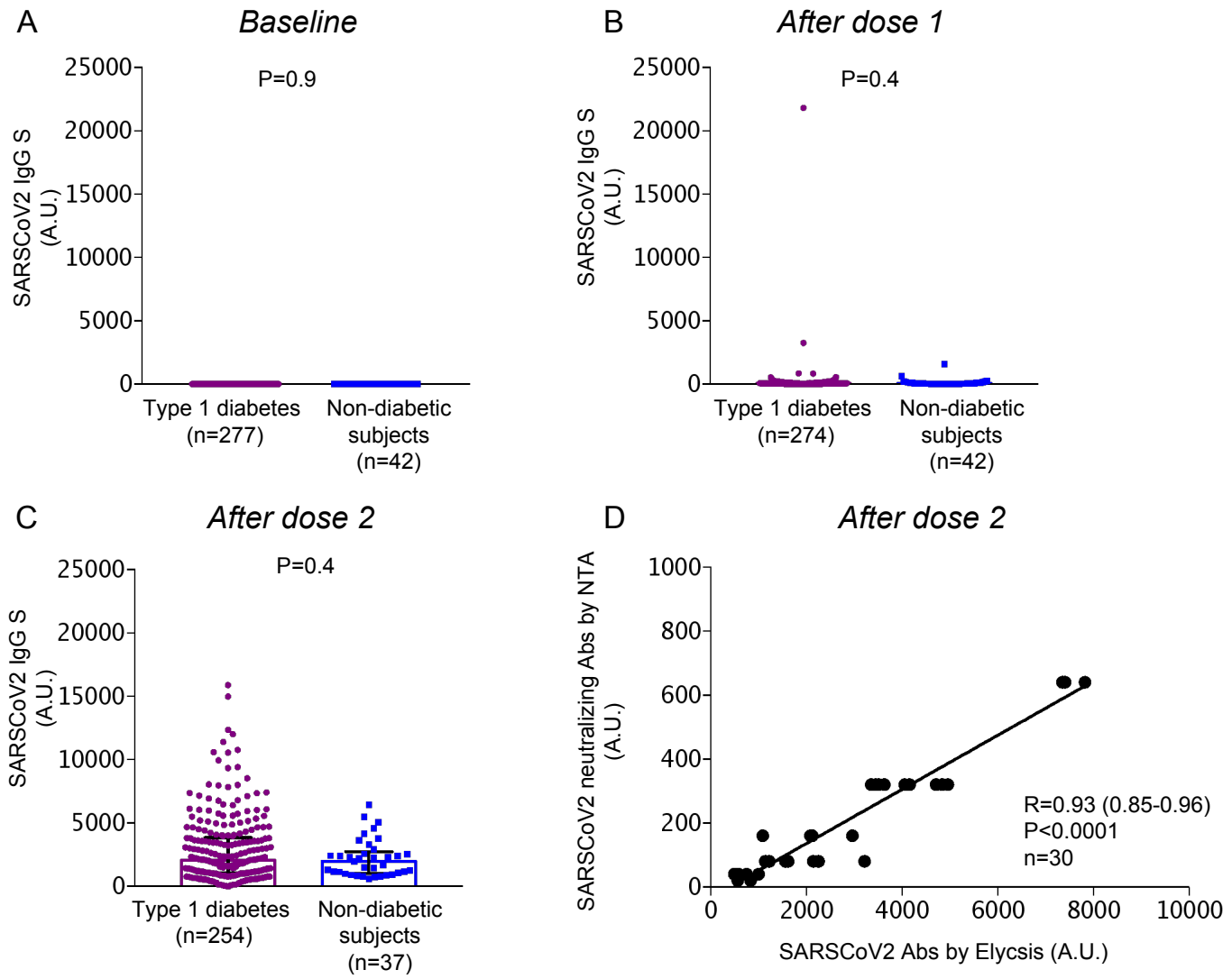

Figure 1. **Anti–SARS-CoV-2 antibody response in patients with T1D and in non-diabetic subjects**.
(A, **B**, **C)**. Boxplots representing Anti–SARS-CoV-2 S antibody levels measured in patients with T1D and in non-diabetic subjects at baseline (A), after the first dose (B) and after the second dose (C) of the SARS-CoV-2 mRNA vaccines. Anti-SARS-CoV-2 antibody titers are reported as median with interquartile range. **(D)**. Line graph showing the correlation between SARS-CoV-2 antibodies titers detected by an *in vitro* neutralization assay and through the Elecsys assay (n=30). Measurements for 'after dose 1' were performed 3-4 weeks after the first dose, and measurements 'after dose 2' were performed 4 weeks after the second dose. AU, Arbitrary Units; NTA, neutralization assay.

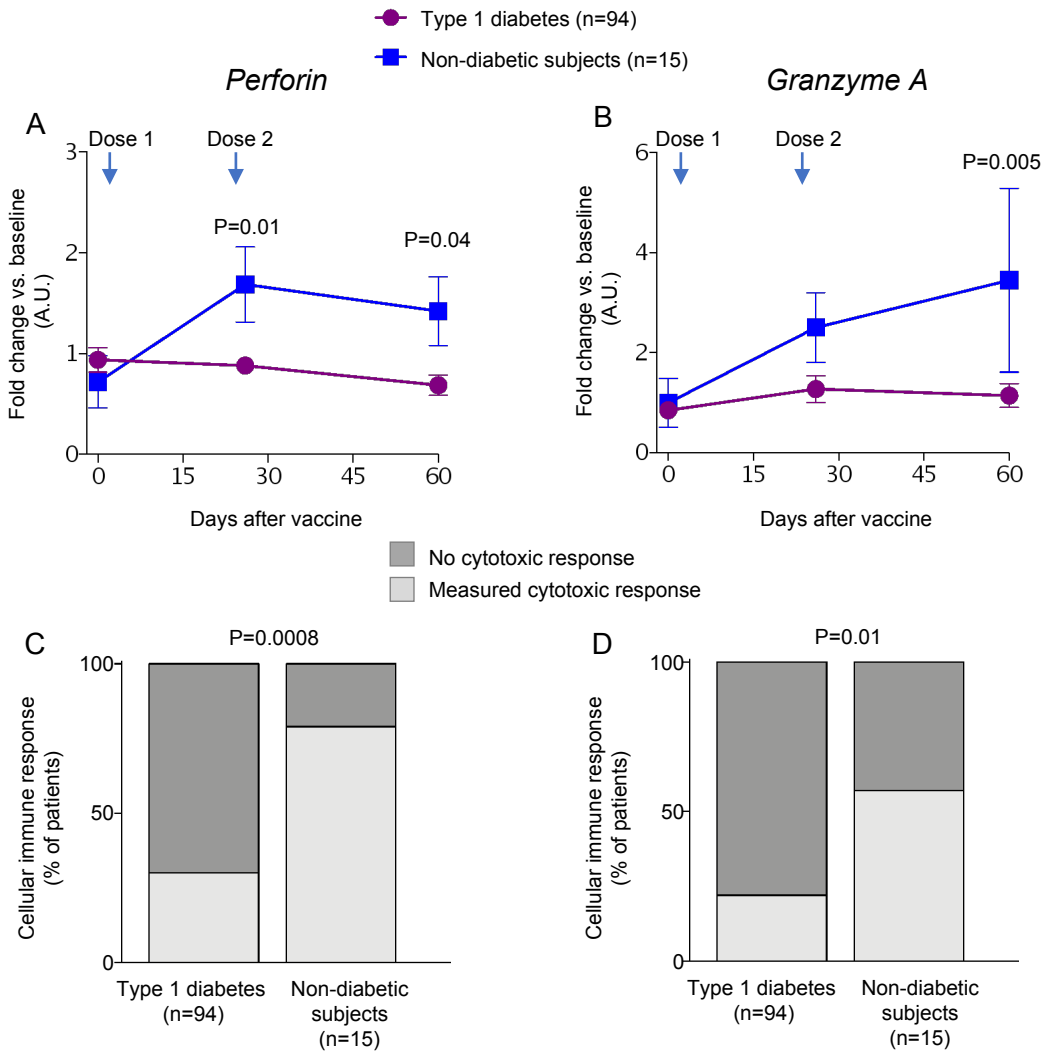

Figure 2. Cytotoxic response to the SARS-CoV-2 peptides after prime and boost doses of SARS-Cov-2 mRNA vaccine administered to T1D patients and to non-diabetic subjects. (A, **B)**. Line graphs representing normalized secretion of SARS-CoV-2 -specific T cell cytotoxic factors perforin (A) and granzyme A (B), measured in the supernatant of PBMCs obtained from patients with T1D or from non-diabetic subjects at each timepoint (baseline, after dose 1 and after dose 2) and cultured in the presence/absence of SARS-CoV-2 peptides for 48 hours. Data are expressed are mean ± standard error of the mean (SEM). Secretion data have been normalized to baseline levels for each cytokine analyzed. Cut points of 0.1 and 0.01 were assessed based on the difference observed in the levels of each analyte, perforin and granzyme A respectively, measured at each timepoint as compared to baseline and normalized to baseline value. Analysis was performed for 'after dose 1' at 3-4 weeks after the first dose, and for 'after dose 2' at 4 weeks after the second dose. **(C)**. Bar graph representing proportions of patients with T1D as compared to non-diabetic subjects showing increased or no change in the SARS-CoV-2-specific T cell cytotoxic response before receiving the second dose of SARS-Cov-2 mRNA vaccine. **(D)**. Bar graph representing proportions of patients with T1D as compared to non-diabetic subjects showing increased or no change in the SARS-CoV-2-specific T cell cytotoxic response at 4 weeks of follow-up after administration of the second dose of the SARS-Cov-2 mRNA vaccine. The SARS-CoV-2-specific T cell cytotoxic response was analyzed as an increase/lack of increase observed in both perforin and granzyme A levels at each timepoint. PBMCs, peripheral blood mononuclear cells; A.U., arbitrary units; T1D, type 1 diabetes.

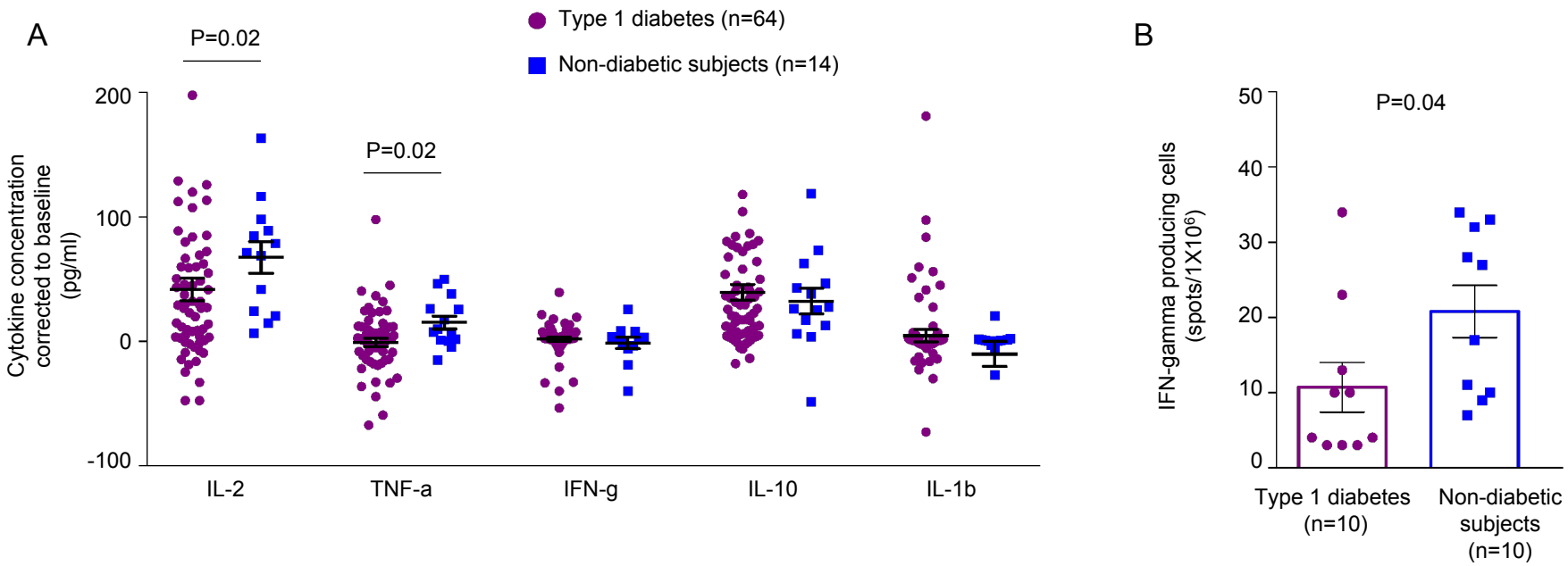

Figure 3. T cell-related cytokines and immune response to SARS-CoV-2 peptides after administration of SARS-Cov-2 mRNA vaccines in T1D patients and in non-diabetic subjects.
(A). Dot plots representing secretion of SARS-CoV-2 -specific T cell-related cytokines, measured in the supernatant of PBMCs obtained from patients with T1D or from non-diabetic subjects at baseline and after dose 2 and cultured in the presence/absence of SARS-CoV-2 peptides for 48 hours. Data are expressed as mean ± standard error of the mean (SEM) of secretion levels corrected to baseline levels for each cytokine analyzed. **(B)**. Bar graphs representing the number of IFN-γ spots detected by ELISpot analysis using PBMCs isolated from patients with T1D (n=10) and from non-diabetic subjects (n=10), after the administration of the SARS-CoV-2 mRNA vaccines, following challenge with spike and nucleocapsid SARS-CoV-2 peptides. Data are expressed as mean ± SEM. PBMCs, peripheral blood mononuclear cells; T1D, type 1 diabetes.

SUPPLEMENTARY INFORMATION

Immunogenicity and safety of SARS-CoV-2 mRNA vaccines in a cohort of patients with type 1 diabetes

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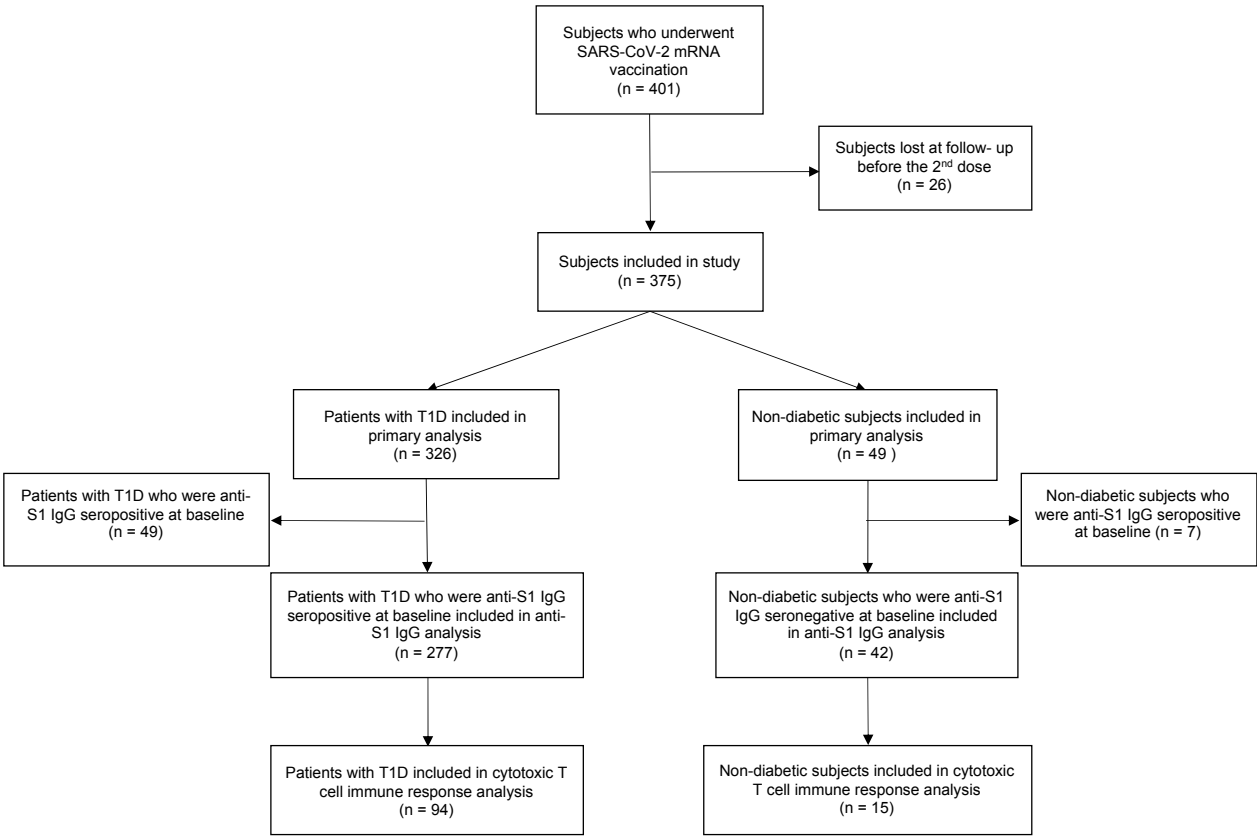
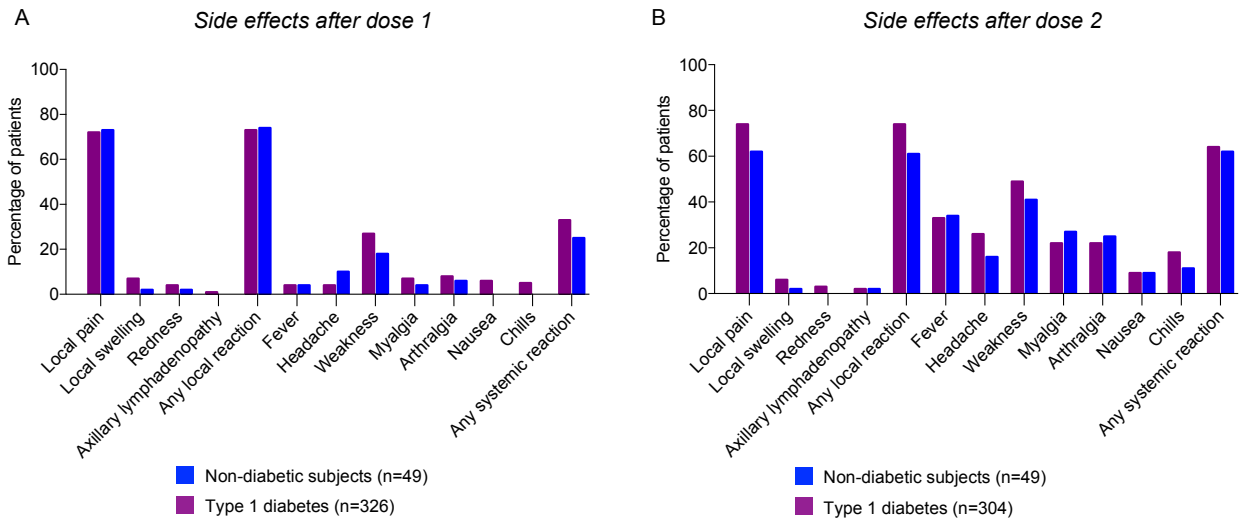
Supplementary Figure 1. Study flow-chart.



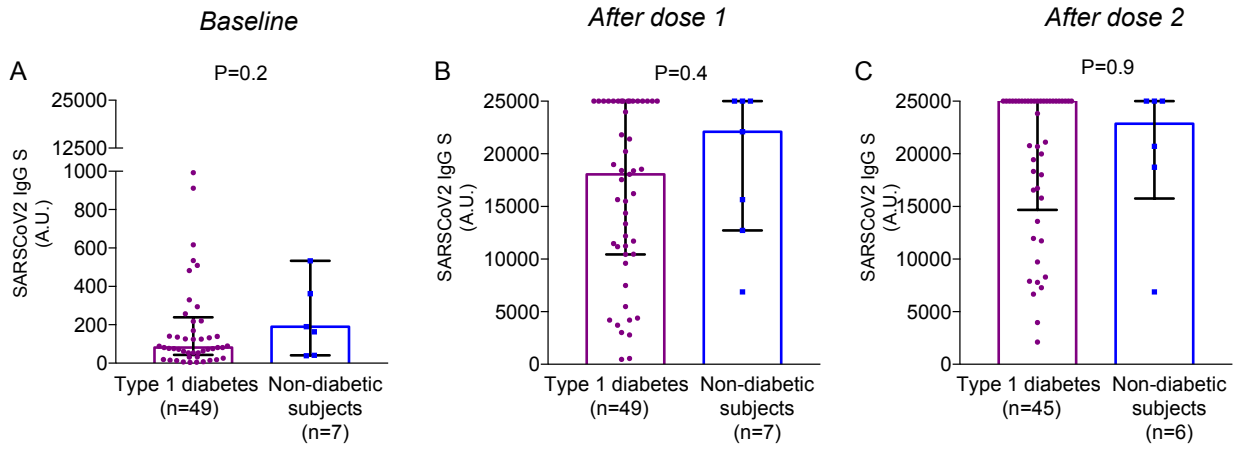
Supplementary Figure 2. Local and systemic adverse effects in T1D patients and non-diabetic subjects who received two doses of SARS-CoV-2 mRNA vaccine.



(A, **B)**. Bar graphs representing percentage of T1D patients and non-diabetic subjects reporting local and systemic reactions after dose 1 (A) and after dose 2 (B) of the SARS-CoV-2 mRNA vaccines. Assessment 'after dose 1' was performed 3-4 weeks after the first dose, and assessment 'after dose 2' was performed 4 weeks after the second dose.

Abbreviations: T1D, type 1 diabetes.

Supplementary Figure 3. Anti–SARS-CoV-2 antibody response in patients with T1D and non-diabetic subjects who were seropositive before vaccination.



(A, **B**, **C)**. Anti–SARS-CoV-2 antibody levels in SARS-CoV-2-seropositive patients with T1D and non-diabetic subjects at baseline (A), after dose 1 (B), and after dose 2 (C). Anti-SARS-CoV-2 antibody titers are reported as median with interquartile range. 'After dose 1' was 3-4 weeks after the first dose, and 'after dose 2' was 4 weeks after the second dose.

SUPPLEMENTARY TABLES

Supplementary Table 1. Distribution of mRNA SARS-CoV-2 vaccines in the study population.

	BNT162b2	**mRNA-1273**
T1D – n of patients (%)	168 (51.5)	158 (49.5)
Controls – n of patients (%)	29 (59.1)	20 (40.9)

Abbreviations: n, number; T1D, type 1 diabetes; BNT162b2 Pfizer mRNA SARS-CoV-2 vaccine administered at day 0 and 21; mRNA-1273, Moderna mRNA SARS-CoV-2 vaccine administered at day 0 and 28.

Supplementary Table 2. Multivariable analysis of factors associated with the development of cytotoxic response in the whole study population after having received the first and the second dose of the SARSCoV2 mRNA vaccine.

Cytotoxic response after dose 1		
Variables	**Regression coefficient (95% CI)**	**P value**
Type 1 diabetes	-3.68 (-5.49 to -1.88)	0.0001
Gender (Male)	-0.01 (-0.97 to 0.93)	0.96
Age (years)	-0.04 (-0.08 to -0.01)	0.01
Cardiovascular disease	1.92 (-0.57 to 4.47)	0.12
Hypertension	-0.31 (-1.99 to 1.36)	0.71
Autoimmune thyroid disease	-0.25 (-1.45 to 0.95)	0.68
Cytotoxic response after dose 2		
Variables	**Regression coefficient (95% CI)**	**P value**
Type 1 diabetes	-1.76 (-3.22 to -0.30)	0.01
Gender (Male)	0.42 (-0.61 to 1.46)	0.42
Age (years)	-0.02 (-0.05 to 0.01)	0.21
Hypertension	0.43 (-1.01 to 1.88)	0.55
Autoimmune thyroid disease	-0.78 (-2.43 to 0.86)	0.35

Abbreviations: CI, confidence interval; after dose 1, the first dose; after dose 2, after the second dose of the SARS-CoV-2 mRNA vaccines.

Supplementary Table 3. **Continuous glucose monitoring in patients with T1D receiving the SARS-CoV-2 mRNA vaccine at three timepoints**.

	Baseline (n=150)	**After the 1st dose (n=150)**	**After the 2nd dose (n=150)**	**p value**
Time in range %	64.1 ± 18.8	65.0 ± 18.2	62.6 ± 18.6	0.91^; 0.76$^{\lrcorner}$; 0.51$^{\perp}$
Time above range %	32.9 ± 19.8	32.2 ± 18.7	34.3 ± 19.1	0.95^; 0.79$^{\lrcorner}$; 0.60$^{\perp}$
Time below range %	3.0 ± 2.6	2.9 ± 3.2	2.9 ± 3.3	0.98^; 0.99$^{\lrcorner}$; 0.99$^{\perp}$
Use of CGM %	91.7 ± 12.2	91.0 ± 13.0	89.2 ± 16.7	0.89^; 0.30$^{\lrcorner}$; 0.56$^{\perp}$
estimated HbA1c § *%*	7.2 ± 0.8	7.2 ± 0.8	7.3 ± 0.8	0.99^; 0.93$^{\lrcorner}$; 0.93$^{\perp}$
Coefficient of variation† *%*	33.2 ± 6.7	33.2 ± 6.4	34.5 ± 6.8	0.99^; 0.33$^{\lrcorner}$; 0.34$^{\perp}$
Glucose level (mg/dl)	161.1 ± 30.0	160.6 ± 30.0	163.6 ± 31.0	0.99^; 0.76$^{\lrcorner}$; 0.69$^{\perp}$

Plus–minus values are means ± SD.
^Baseline vs "After the 1st dose"; $^{\lrcorner}$ Baseline vs. "After the 2nd dose"; $^{\perp}$ "After the 1st dose" vs. "After the 2nd dose"; § Complete data from 122 patients; † Complete data from 118 patients.

Abbreviations: CGM, continuous glucose monitoring; HbA1c, glycated hemoglobin.