Corso di dottorato di ricerca in:

"Alimenti e Salute Umana"

34° Ciclo

Titolo della tesi

"Public Health and good practices in primary prevention: SARS-CoV-2 immune response monitoring in transplant recipients and waitlisted patients after a targeted vaccination campaign"

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ABSTRACT

Rationale & Objective: Sufficient data were not yet available to characterize SARS-CoV-2 vaccine immune response and the duration effectiveness in both transplant recipients and waitlisted patients. For this reason, a study was launched after the promotion of a targeted SARS-CoV-2 vaccination campaign in a University Hospital of Italy. We evaluated the humoral response after 2 doses of the BNT162b2 vaccine in waitlisted and transplanted patients in three different time points and the cellular immune response, once, in a subgroup of patients randomly selected.

Study Design: Longitudinal monocentric observational study.

Setting & Participants: Patients who received 2 doses of the BNT162b2 vaccine in a targeted vaccination campaign for transplanted (solid organ and hemopoietic stem cells) and waitlisted patients.

Findings: The percentage of the vaccine campaign adherence was 72,1% and 72.6% (n.440) of the vaccinated patients agreed to participate in the study. Patients (median age 63 years old, interquartile range (IQR) 55-69years old, 68,2% men) had a median SARS-CoV-2 antibody level of 8.3 (IQR 0.4-487) U/mL 30 days after the second dose, 45.4 (IQR 0. 4-387.5) U/mL after 90 days and 34.8 (IQR 0.6-288.5) U/mL after the 150 days. 50% of the subgroup of the patients (n°42) had a positive cellular immune response post vaccination. We found a statistically significant association between immunosuppressive regimen (mycophenolate mofetil, tacrolimus and corticosteroids) and humoral response in all three time points, excluding patients with an anamnestic recall for COVID19 and immunoglobulins value (IgG, IgM, IgA) not in range (p value: <0.001). In addition, the variation of the SARS-CoV-2 humoral response values among the three time points showed a statistically significant decrease up to 150 days after the second dose (p value: <0.001).

Limitations: Cellular immune response and pre-vaccination humoral response not assessed in the entire cohort. Observational study with no control group.

Conclusions: This study contributed to investigate the immune response after SARS-CoV-2 vaccination both in recipients and patients on waiting list, poorly represented in literature. Our findings on immunosuppressive regimen and post- vaccination humoral response were

consistent with recent literature though further studies among the population of waitlisted patients are needed.

Key words: vaccine, Public Health, SARS-CoV-2, transplantation, immune response

RIASSUNTO

Razionale e obiettivo: In un ospedale universitario italiano, è stata promossa una campagna di vaccinazione per SARS-CoV-2 rivolta esclusivamente a pazienti trapiantati ed in lista per trapianto. Non essendo ancora disponibili dati sufficienti per caratterizzare la risposta immunitaria a seguito di vaccinazione per SARS-CoV-2 e la durata della protezione immunitaria nel tempo per questi pazienti, è stato avviato uno studio. In particolare, abbiamo valutato la risposta umorale dopo 2 dosi di vaccino BNT162b2 in pazienti in lista d'attesa e trapiantati in tre diversi momenti e la risposta immunitaria cellulare, valutata una sola volta, in un sottogruppo di pazienti.

Disegno dello studio: Si tratta di uno studio osservazionale monocentrico longitudinale.

Partecipanti: Pazienti che hanno ricevuto 2 dosi del vaccino BNT162b2 in una campagna di vaccinazione promossa esclusivamente per pazienti trapiantati (organo solido e cellule staminali emopoietiche) e in lista d'attesa.

Risultati: La campagna vaccinale ha ottenuto una percentuale di adesione pari al 72.1% ed il 72.6% (n.440) dei pazienti vaccinati ha accettato di partecipare allo studio. Il valore della mediana riferito alla risposta umorale per SARS-CoV-2 dei pazienti (età mediana 63 anni, intervallo interquartile (IQR) 55-69 anni, 68.2% uomini) è stato rispettivamente di 8,3 (IQR 0.4-487) U/mL 30 giorni dopo la seconda dose, 45.4 (IQR 0.4-387.5) U/mL dopo 90 giorni e 34,8 (IQR 0.6-288.5) U/mL dopo 150 giorni. Il 50% del sottogruppo dei pazienti (n°42) ha avuto una risposta immunitaria cellulare positiva dopo la vaccinazione. Alcuni farmaci immunosoppressivi (micofenolato mofetile, tacrolimus e corticosteroidi) erano associati significativamente alla risposta umorale in tutti e tre i momenti di follow-up, escludendo i pazienti ex COVID19 e con valori anticorpali (IgG, IgM, IgA) non in range (p: <0,001). Inoltre, i valori della risposta umorale

SARS-CoV-2 nei tre momenti di osservazione, fino a 150 giorni dopo la seconda dose, hanno dimostrato una diminuzione statisticamente significativa nel tempo (p: <0,001).

Limiti: La risposta immunitaria cellulare e quella umorale prima della vaccinazione non sono state valutate per l'intera coorte e, inoltre, manca un gruppo di controllo.

Conclusioni: Questo studio ha contribuito a indagare la risposta immunitaria post vaccinazione sia nei pazienti trapiantati che in lista d'attesa, scarsamente rappresentati in letteratura. I nostri risultati relativi all'associazione tra immunosoppressione e risposta umorale post-vaccinazione sono coerenti con la letteratura più recente, sebbene siano necessari ulteriori studi che abbiano come target la popolazione dei pazienti in lista d'attesa per trapianto.

Parole chiave: vaccinazione, Sanità Pubblica, SARS-CoV-2, trapianti, risposta immunitaria

Aknowledgments

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To my family, friends, and colleagues: thank you so much for all the love and support!

1. Background

1.1 COVID-19

SARS-CoV-2 disease (COVID-19) is an infectious disease caused by a respiratory virus whom first cases were reported in Wuhan (China) in December 2019. (1,2) Firstly named "2019 novel coronavirus" (2019-nCoV) and later identified by WHO with the acronymous "SARS-CoV-2" (Severe acute respiratory syndrome coronavirus-2), on March 11th 2020, WHO Director-General declared the COVID-19 a pandemic: the first pandemic caused by a coronavirus.(3–5) During the first months of the pandemic, Italy was one of the worst hit countries in the world with more than 400,000 confirmed cases and thousands COVID-19 attributed deaths. (6–9)

In this scenario, studies have been conducted to evaluate the impact of SARS-CoV-2 among the population and especially frail and elderly people in order to identify risk factors associated with severe symptoms and probability of death. (10,11)

1.2 TRANSPLANT PATIENTS AND COVID-19

Considering categories at major risk for fatality outcome during pandemic, transplant recipients and patients on the waiting list for transplantation were identified to be at higher risk of severe disease. (12) Nacif et al. underlined, in their study, that solid organ transplantation patients (SOT) had higher mortality risk in comparison with non-transplanted populations with highest fatality rate in > 60 years old patients (SOT: 52.94% vs 4.54% , p=0.001). (13)

Besides SOT, Haematopoietic stem-cell transplantation (HSCT) recipients were also studied to evaluate the risk of mortality after COVID-19. Sharma et al., for example, described in their research that the development of COVID-19 within 12 months of transplantation were associated with a higher risk of mortality among allogeneic HSCT recipients. (14)

In general, many studies highlighted the association between severe COVID-19 and comorbidities especially showing that when these risk factors accumulate, mortality

increases.(15) Moreover, in their research, Hilbrands et al. found ≥75 years old dialysis patients with a frailty score of 5 or higher having the 28-day case-fatality rate at 44%. (15)

On the other hand, a Spanish study showed that chronically immunosuppressed liver transplant patients might have an increased risk of SARS-CoV-2 infection and lower risk of fatality outcome assuming chronic immunosuppression could represent a protective factor against COVID19.(16)

In a European multicenter prospective study of liver transplant recipients, the authors found that COVID-19 was associated with an overall and in-hospital case fatality rate of 12% (95% CI 5%to 24%) and 17% (95% CI 7% to 32%), respectively. (17)

Moreover, in Spain, a prospective observational cohort study pointed out that, especially in the early post-transplant phase, a shorter interval between transplantation and COVID-19 diagnosis had a negative impact on clinical prognosis.(18)

In addition, during the first wave of the pandemic, Trapani et al. showed that the cumulative incidence of SARS-Cov-2 infection in 8,500 patients awaiting solid organ transplantation reached 1.85% in Italy -about four times higher respect the general population in the same observation period- with a mortality rate close to 20%. The cumulative incidence of infection in the same period seemed to be reduced to 1.02% in the transplanted population, with a lethality rate of 27.3%. (19)

Another study conducted in Italy, among heart transplant centers, showed that, compared to the general population, prevalence (18 vs. 7 cases per 1,000) and related case fatality rate (29.7% vs. 15.4%) in heart transplant recipients were doubled. (20)

These data enlightened the frailty of transplant recipients because of the association with severe clinical outcomes and higher mortality and all available evidence, right from the beginning of the pandemic, suggested the need to protect this population to significantly avoid the risk of hospitalization and death caused by SARS-CoV-2.

1.3 SARS-CoV-2 VACCINATION

On 22nd December 2020, Pfizer BNT162b2 (Comirnaty) mRNA vaccine was authorized by AIFA Italian Medicines Agency and on the 27th December the European Vaccination Day was organized. The initially recommended schedule of this vaccine required two doses, 21 days apart. (21)

Later, on 7th January 2021, another mRNA vaccine was approved to be used in Italy to prevent COVID-19: the Moderna mRNA-1273 vaccine, commonly known as "Moderna" vaccine. In this case, the second dose was scheduled after 28 days.

Due to initial organizational and supplying factors, a priority of vaccines' administration was defined at a national level, identifying healthcare professionals as a category to be given first, together with long term care facilities residents and elderlies (>80 years old). (22)
Secondly, frail people and 60-79 years old categories were identified to receive the SARS-CoV-2 available vaccines as soon as possible and transplant recipients and waitlisted patients were included. (22)

1.4 THE CONTEXT: TRANSPLANT PROGRAM IN OUR UNIVERSITY HOSPITAL

Udine-hospital is located in Northeast of Italy, in a region called "Friuli Venezia Giulia" (FVG). Three deceased donor transplant programs have been authorized, in particular:

- Heart Transplant Program – since year 1985-,
- Kidney Transplant Program -since 1993-.
- Liver and pancreas Transplant Program -since 1996-,

Moreover, the National Ministry of Health since 2010 authorized the Living-donor Kidney Transplant program. (23)
Besides solid organ transplantation, Hemopoietic Stem Cells Transplantation is also an operative program of our hospital.
Each transplant programs have a director (medical doctor) that oversees and evaluates all the different steps of that program, from adding the patients to the transplant waiting list to the

follow up, with the collaboration of the other wards and services of the hospital (such as the Department of Laboratory Medicine) and of the FVG region.

Following national and regional laws, the transplant programs are coordinated by the Regional Transplant Center (CRT – "Centro Regionale Trapianti") that has a key role in the transplant network organizational system, promoting quality and safety of all donation pathways. CRT is also an important link between each program center and the National Transplant center (CNT), that operates according to the program defined by the Ministry of Health and in agreement with the Italian Regions and Autonomous Provinces. (24)

2. SARS-CoV-2 vaccine campaign and immune response monitoring in transplant recipients and waitlisted patients

2.1 PLANNING SARS-CoV-2 VACCINATION CAMPAIGN FOR TRANSPLANT AND WAITLISTED PATIENTS

In Udine hospital, with the support of the Regional Transplant Center (CRT), the activation of vaccination agendas specifically dedicated to patients on the list for transplantation and transplant recipients was promoted, according to regional and national indications. (22)

The vaccination campaign in Udine hospital was organized into different phases, including:

1. planning,
2. patient enrollment phase,
3. organization of the sessions,
4. vaccine administration.

All the phases, activities and services involved in the process are detailed in Table1.

Besides CRT, Transplant Centers, Medical Direction and Healthcare professions Staff, other two services were involved in the vaccination campaign:

- The Hospital Pharmacy (in charge of dispensing and distribution of medications including vaccines throughout the hospital),
- the Department of Prevention (the operative branch of the local health authority for preventive medicine and public health, including pandemic response). (25)

Vaccination campaign's phases	Main Activities	Specialties and services involved
1.Planning	a) Identification and quantification of the population to be vaccinated (eligible population) b) Vaccine supply evaluation c) Estimate number of professionals and services to be involved d) Logistics	• CRT • Transplant Centers (Liver, Heart, Kidney and Hemopoietic Stem Cells) • Medical Direction and Healthcare professions Staff • Department of Prevention • Hospital Pharmacy
2.Patients' enrollment	a) Enrollment methods b) Patients' list	• CRT • Transplant Centers • Medical Direction and Healthcare professions Staff
3.Organization of the sessions	a) Creation of the informatic agenda to schedule vaccines' appointments b) Staff recruitment c) Materials	• CRT • Medical Direction and Healthcare professions Staff
4.Vaccine administration	a) Management of patient flows, avoiding crowding and delays b) Management of the different steps of vaccination (patients' identification, informed consent and anamnestic form, vaccine administration, post-vaccination observation, vaccine recording and certifications)	• CRT • Medical Direction and Healthcare professions Staff • Hospital Pharmacy

Table 1. SARS-CoV-2 Vaccination campaign for transplant and waitlisted patients: phases, activities and involved services in Udine Hospital

The very first step was to estimate the eligible population for vaccination (phase 1).

For this purpose, we considered the number of the patients both in follow up and in waiting list referred to the year 2021: on a total of about 1206 patients, 12.5% were waitlisted patients while were 87.5% recipients (Table 2).

We didn't include patients on dialysis because they could receive the vaccination directly into the dialysis centers. Considering also that only 153 (of 585) kidney transplanted patients were residents of Udine and that probably 85% of the kidney recipients living in other provinces of the region would have refused to join Udine vaccination campaign, with a rough approximation, we estimated the eligible population to be 840. Consequently, 1680 was the total number of the required vaccination appointments, counting the two doses of the vaccination schedule.

Transplant Centers	Transplant recipients (n)	Waitlisted patients (n)
Heart	120	5
Liver	260	6
Kidney	585	110
H. stem cells	90	30
Total	**1055**	**151**

Table 2. Transplant Centers' patients (year 2021 – the list could vary throughout the year).

As far as phase 1 is concerned, major critical issues were related to vaccine supply for which Hospital Pharmacy and the Department of Prevention were in charge and facilitated the procurement.

For the logistic issues (phase 1), since vaccination centers weren't yet available while planning the campaign, the blood sampling area for outpatients, located inside the hospital, was converted, in the afternoons, for vaccination sessions (see Figure 1).

A one-way path has been created, divided into several steps, allowing social distancing and non-intersection of patients' flows, following lean management principles.(26)

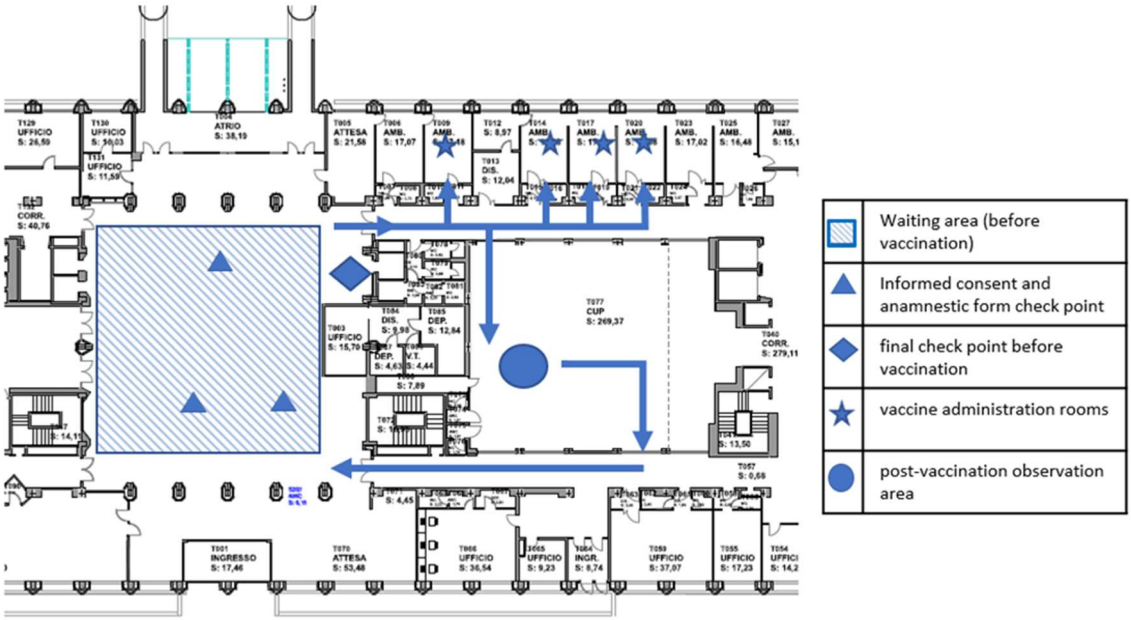

Figure 1. SARS-CoV-2 vaccination pathway, March-April 2021 [with the courtesy of Technical Services Group- Udine Hospital]

Regarding phase 2, patient enrollment procedures were discussed among the Transplant Centers, the Direction of the hospital and CRT to reach the better strategy in terms of effectiveness and feasibility.

Considering what it was pointed out in literature, physicians, nurses and all allied health professionals play a central role in encouraging COVID-19 vaccination and influence patients' vaccination attitudes and beliefs. (27)

In the absence of in-depth studies on anti-SARS-CoV-2 vaccine hesitancy in stem cell transplant patients and solid organ recipients, researchers have pointed out that, for example, among liver transplant recipients, the acceptance rate for COVID-19 vaccination could be very high (reaching 96.6%) and that targeted communication strategies must be established, while promoting specific vaccination campaigns for transplant patients.(28,29)

While evaluating the enrollment methods, the call center system in use for clinical appointments did not seem to fit the purpose of a proactive and effective vaccine campaign strategy, leaving

the patients to decide alone without having medical or professional answer to specific questions that this population might have had.

For these reasons, there was an agreement between the Transplant Centers, the Medical Direction of the hospital and the CRT for actively calling the patient to offer vaccination and schedule the appointment. To promote COVID-19 vaccine campaign, in fact, as underlined by Schaffer et al., frontline health care workers should be taught how to make strong recommendations for COVID-19 vaccination, eventually sharing their personal experiences with COVID-19 and the vaccine. (27)

In our campaign, the calls were made by the personnel of the Transplant Centers (Liver, Heart, Kidney and Hemopoietic Stem Cells) and CRT in whom patients already trusted and that more likely had personal knowledge. Simultaneously, to promote a correct information about the vaccine campaign, a continue update both through official websites and through interviews to the healthcare leaders were provided.

For national and regional indications and vaccine supply reasons, Udine Hospital offered Pfizer mRNA BNT162b2 (Comirnaty) vaccination for transplant and waitlisted patients both solid organ and h. stem cells recipients.(22)

Anti-SARS-CoV-2 vaccination schedule began on the 27th March 2021 and consisted in a total of 6 vaccination sessions for the first and second dose, 3 weeks apart.

About 20 healthcare professionals (physicians, nurses) were engaged in each session for the different steps of the patients' pathway (from the patient identification to the post-vaccination observation).

Patients on the waiting list for kidney transplant (on dialysis) and patients living too far away from the hospital could decide to adhere to the vaccine campaign in other settings (vaccine hub or hospitals) since their vaccine priority was in any case guaranteed by law.(22)

For inpatients, vaccines administrations were organized directly by the ward, in relation with the clinical situation of the patients.

2.2 IMMUNE RESPONSE MONITORING

2.2.1 Background of the study

A two-dose regimen of Pfizer BNT162b2 mRNA vaccine demonstrated 95% efficacy for prevention of SARS-CoV-2 though initial studies did not include an assessment in immunocompromised individuals.(30)

Furthermore, as Aslam et al. underlined in his paper, solid-organ transplant recipients were excluded from previous SARS-CoV-2 vaccine trials, so neither efficacy nor the duration of protection of COVID-19 vaccines were known in this sub-population. (31)

Moreover, even if some literature studies estimated that the efficacy of mRNA vaccination could reach a percentage of about 94%, a research has shown that in solid organ transplant patients this percentage could drop to about 54%. (32,33)

However, sufficient data were not yet available to characterize SARS-CoV-2 vaccine immune response in patients undergoing through immunosuppressive therapies, such as those in use for both solid organ and hemopoietic stem cells transplant and waitlisted patients.

For this reason, according to the indications from the National Transplant Center and the Ministry of Health, a post-vaccine immune response monitoring, both humoral and cellular response, was highly recommended in transplant recipients. (34)

For all the considerations expressed above, running parallel with the organization of the of mRNA vaccines' agenda for to both transplanted/waitlisted patients for solid organ transplantation and hematopoietic stem cells, a study on the COVID19 vaccine response of this population was being launched.

This project was launched with the collaboration of the Regional Transplant Center, the Direction of the Hospital, as well as the Heart, Kidney, Liver, Hematopoietic Stem Cell Transplant Centers (Figure 2). Fundamental were also the multidisciplinary meetings with the expertise also

of the Clinical of Infectious Diseases, the Department of Laboratory Medicine, and the Department of Prevention.

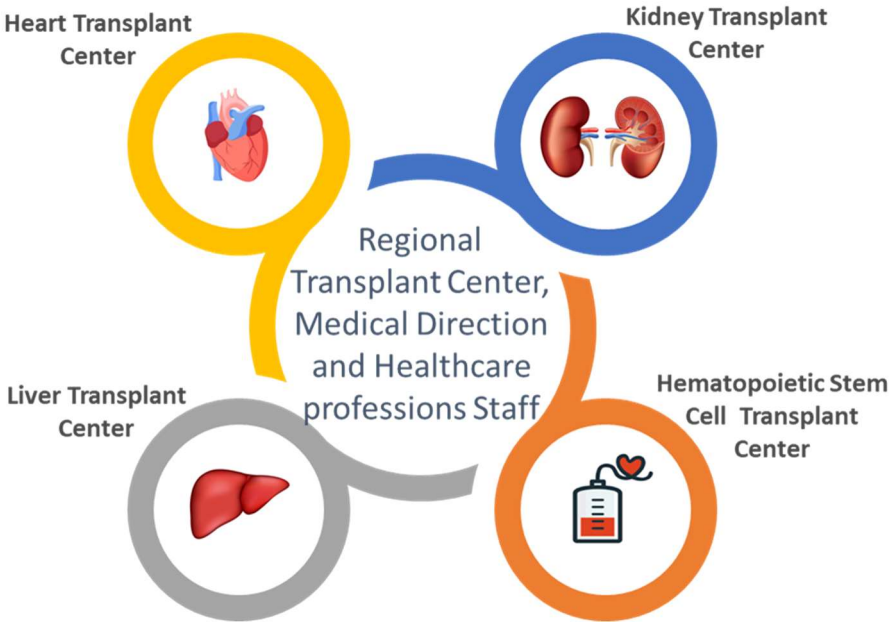

Figure 2. Visual representation of the Multi-professional Team: Transplant Centers coordinated by the Regional Transplant Center and the Medical Direction&Healthcare professions Staff

The multi-professional team (Fig.2) agreed to conduct an observational study after having reviewed literature, national and international guidelines, regulations and after having evaluated its feasibility.

2.2.2 Aim of the study

The objectives of the study, summarized in Table 3, are divided in: primary, secondary and exploratory endpoints.

Purpose of the research	
Primary endpoint	Detection and titration of antibodies to SARS-CoV-2 in transplanted / transplant patients 30 days after vaccination (2 doses)
Secondary endpoints	- detection and titration of SARS-CoV-2 antibodies in transplant recipients / waitlisted patients 90 and 180 days after vaccination (2 doses) - immunoglobulin evaluation (IgA, IgM and IgG) 30 days after vaccination - cell-mediated response collected in a randomly selected sub-sample of patients (about 10% of the total) on which the lymphocyte subset typing will also be tested - follow up of the patients and identification of any cases of positive covid test after vaccination, cell immunity + viral genetic typing from salivary / swab test.
Exploratory objectives	- detection and titration of SARS-CoV-2 pre-vaccination antibodies - detection of any adverse events / side effects after vaccination.

Table3. Brief description of the objectives of the study.

As far as the exploratory objectives concern, the vaccine related adverse events would have required further case studies for a correct assessment: our intent was only to report if any eventually occurred.

2.2.3 Methods

The study was designed as longitudinal monocentric observational study.

To evaluate the humoral response, as for the primary objective, the investigation was carried out through laboratory tests to detect of the presence and titration of antibodies directed towards the Spike protein of the virus. The chosen test was the test "ROCHE" - Elecsys Anti-SARS-CoV-2- that was also an internationally validated test for treatments with hyperimmune plasma (Convalescent plasma). (35)

The blood samples to study SARS-CoV-2 antibodies had to be drawn out and collected in three different times after the administration of the second dose of the mRNA vaccine, as described in Table 4. Serological samples taken within 2 months prior to the first dose (evaluation at “t0” time) would also be searched and analyzed, if still available in the laboratory or at the Transplant Centers, for evaluation and comparison with the antibody results after vaccination.

Together with the first sampling (t1) and just once, the IgA, IgG and IgM immunoglobulins would have been investigated.

To be able to fully understand the immune response, in accordance with literature, we also decided to test the cell-mediated response for SARS-CoV-2. Due to related costs and feasibility, we decided to randomly select a subgroup of patients (10% of the total sample) to whom perform the test. 50 people had to be selected at time t1 and to them we also measured lymphocytes (lymphocyte typing - a test that is common in clinical practice) to better interpret the results.

Highly specialized tests (salivary test or nasopharyngeal swab) to confirm positivity and determine the exact composition of the virus genome and cell-mediated response tests would have to be reserved only for vaccinated patients that had received two doses of SARS-CoC-2 vaccine and found to be positive for COVID19 both for screening and clinical needs, within 12 months after vaccination follow up, regardless of the severity symptoms.

Timeline	Days after 2-Dose SARS-CoV-2 mRNA Vaccine	Lab tests
t0	/	SARS-CoV-2 serological test
t1	30	- SARS-CoV-2 serological test + Antibody titration (IgA,IgG,IgM), -Cellular immune response + lymphocyte typing (10% patients)
t2	90	SARS-CoV-2 serological test
t3	180	SARS-CoV-2 serological test

Table 4. Reference times for serological samples

The test to evaluate humoral and cellular immune response were respectively: The Elecsys Anti-SARS-CoV-2 serological test and the IFNγ release assay (IGRA). For the interpretation of the results of the first test, according to the manufacturer's instructions, the test was considered negative if <0,8 U/mL.

Cellular immune response in vaccinated patients was evaluated and cut-offs were determined comparing results with a small control group of immunocompetent subjects.

The sample could be evaluated if:

1. negative control <8.6 pg / ml;
2. response to mitogen> 141 pg / ml.

The test was considered positive if the difference between the SARS-CoV-2 specific cell response and the negative control was > 12 pg / mL.

Summarizing, the results could be interpretated as: positive, undetermined and negative.

All laboratory tests were performed in clinical laboratories of the Department of Laboratory Medicine of Udine University Hospital.

Because of the nature of the study (observational), there were no associated side effects: any expected risks could be only related to the execution of venipuncture (such as haematoma, local complications, fainting).(36)

Population of the study

Sample

Transplant recipients and waitlisted patients afferent to the Heart, Kidney, Liver and Hematopoietic Stem Cells Transplant Centers of our University Hospital, for a total of about 550 patients to enroll.

Inclusion criteria

Adult patients (≥ 18 years old) able to provide valid consent and who have joined the vaccination campaign promoted by the CRT in our hospital.

Exclusion criteria

Patients unable to provide valid consent, <18 years old people and those who choose not to participate in the research project will be excluded.

Measured outcomes

Primary endpoint

Titration of antibodies to SARS-CoV-2 at 30 days after vaccination in transplanted / waitlisted patients.

Secondary endpoints

- titration of antibodies to SARS-CoV-2 at 90 and 180 days after vaccination in transplanted / waitlisted patients;
- antibody titration (IgA, IgM and IgG) 30 days after vaccination;
- cellular immune response and lymphocyte typing in the subgroup of 50 selected patients;
- n° patients tested positive for COVID-19 (post-vaccination), identification of their cell-mediated immune response and virus genome sequencing;

Exploratory objectives

- titration of anti-SARS-CoV-2 pre-vaccination antibodies;
-% of patients positive for COVID-19 (post vaccination) in association with SARS-CoV-2 antibody levels;
- detection of post-vaccination adverse events/side effects in the sample.

Methods of data collection

General aspects

All patients were required to sign the informed consent and privacy consent form, after the communication all the aspects of the conduction of the study, its purpose and duration.

Methods of conducting the study

The timeline of the project foresaw different steps: training the collaborators, withdrawals, data collection and analysis, preliminary and final report.

The very first stage of the study included meetings with the personnel to align behaviors and to ensure that the entire workforce would understand the project and each task.

Surveyors of the Transplant Centers, coordinated by the Principal Investigator and the CRT, were trained on the different aspects of the rational of the study, on the informed consent and recording the results. The working group also agreed on the method of enrollment, also sharing the contents of the phone call for patients who could express interest in participating in the study.

Another important part of the research was the collection of a brief self-administered anonymized questionnaire, made up of 3 pages, that regarded general personal aspects (age, occupation), vaccination and symptoms/adverse events, recall for eventual COVID positive test before vaccination, the therapy in use and the transplant (organ, date of transplant).

Figure 3: Timeline of the vaccination campaign and study monitoring

Sample size

It was estimated that 500 patients would have to be enrolled.

According to literature, we assumed that about 50% of patients had SARS-CoV-2 antibody response ≥0,8 U/mL 30 days after the second dose of COVID-19 vaccine, in order to get 95% confidence level, with approximately 4.5% of accuracy.(18)

Since for logistical and feasibility reason it was not possible to evaluate the cellular immune response for the entire cohort, it was decided to select about 10% of the total to test both the cell-mediated response and lymphocyte typing.

Ethical Committee

The study had to obtain the approval of the National Institute for Infectious Diseases "Lazzaro Spallanzani" in accordance with a national law approved in 2020. (37)

Statistical analysis

Descriptive statistics for categorical variables are presented as number (percent) and for continuous variables as mean ± standard deviation (SD) or median (interquartile range (IQR)). For categorical variables, comparisons between two groups were done using the Chi-squared test or Fisher Exact test, as appropriate. For continuous variables comparisons between two groups were done using the t-test or Mann–Whitney U test, according to the Shapiro-Wilk test establishing whether data were normally or non-normally distributed.

One-Way ANOVA with repeated Measures was used to assess the variation of values of SARS-CoV-2 antibody response among the three time points (t1, t2,t3).

All analyses were performed by the STATA 17 statistical software, and statistical significance was set at $p < 0.05$.

2.2.4 Results

The eligible population of the SARS-CoV-2 vaccination campaign was estimated to be 840 patients: 72,1% of them adhered to the vaccination sessions organized by Udine Hospital.
All the patients who have joined the vaccination campaign (606 people) were informed about the possibility of enrollment in the project.
Even if 450 transplant recipients/ waitlisted patients initially decided to join the study, 10 patients had to be excluded due to blood withdrawal refusal, informed consent, and privacy issues.
Therefore, a total of 440 patients, 72.6% of the patients who have joined the vaccination campaign for waitlisted patients and recipients, were included in the study. 93.9% of them were transplanted recipients while only 6.1% were transplant candidates (see Table 5).

We collected a total of 437 questionnaires and for the missing 3 we retrieved the information related to the transplantation and prescribed therapy with the help of the Transplant Centers.

As far as the timeline of the study is concerned, the third post-vaccination monitoring was anticipated and scheduled from 23rd September 2021 (new t3 at 150 days after 2nd dose). On the 14th of September 2021, in fact, the Ministry of Health gave preliminary indications on an additional vaccine dose for defined categories of patients, included transplanted recipients. (38) At this point we needed to guarantee the possibility of vaccination and at the same time to complete the evaluation of the SARS-CoV-2 antibody titration. The working group, in agreement with the hospital Direction, planned the collection of blood samples in the same location of the vaccine administration, to simplify the process. In this case, the third blood withdrawals were performed at the Vaccine Hub (outside the hospital) and then processed at the Laboratory Medicine Department.

During the first monitoring, a subgroup of 42 patients (of the 50 randomly selected) was tested for SARS-CoV-2 cellular immune response and lymphocyte typing (about 9.6% of the total).

At the first monitoring (t1 – about 30 days after vaccination) we collected a total of 437 samples with 3 missing due to organizational problems.

At t2 and t3, our laboratory tested a total of 412 and 392 blood samples respectively for the evaluation of the SARS-CoV-2 antibody titration. We were also able to analyze 162 blood samples collected before vaccination, at t0, to evaluate the SARS-CoV-2 humoral immune response before the vaccination (as described in detail below in Table 7).

Description of the sample

Most of the patients were Italian (96,8%), male (68,2%) and retired from work (58.3%).

Solid organ transplant recipients were more represented in comparison with hemopoietic stem cells recipients, with a total of 309 patients vs 104 patients enrolled. The description of the sample is summarized in Table 5.

	N=440
Age, years, median (IQR)	63 (55-69)
Female gender, n (%)	140 (31.8)
Nationality, n/N (%)	
Italian	420/440 (96.8)
Other	14/440 (3.2)
BMI, n/N (%)	
<18.5	15/435 (3.4)
18.5-25	193/435 (44.4)
25-30	149/435 (34.3)
≥ 30	78/435 (17.9)
Level of education, n/N(%)	
<High-school Diploma	230/435 (52.9)
≥ High-school Diploma	205/435 (47.1)
Occupation, n/N (%)	
Worker	131/437 (30.0)
Student	6/437 (1.4)
Retired	255/437 (58.3)
Unemployed	26/437 (5.9)
Others	19/437 (4.4)

Referred Transplant Centre, n (%)	
Kidney	119 (27.1)
Heart	44 (10.0)
Liver	170 (38.6)
H. Stem Cells	107 (24.3)
COVID-19 positivity, n/N (%)	19/439 (4.3)
Transplanted Recipients, n (%)	413 (93.9)
Transplanted Organ, n/N (%)	
Kidney	112/413 (27.1)
Heart	45/413 (10.9)
Liver	146/413 (35.3)
H.Stem cells	104/413 (25.2)
Combined	
Kidney and liver	3/413 (0.7)
Kidney and pancreas	2/413 (0.5)
Kidney and heart	1/413 (0.2)
Waitlisted patients, n (%)	27 (6.1)
Days after transplantation, median (IQR)	1975 (663-4639)

Table 5. Patients' characteristics: description of the sample.

Description of the subgroup

To 42 patients SARS-CoV-2 cellular immune response and lymphocyte typing were additionally analyzed.

In this subgroup of patients, 40 of them were recipients (n° 15 liver, n°10 kidney, n°10 h. stem cells, n° 5 heart recipients respectively) while 2 people were in transplant waiting list (n°1 haematological and n°1 hepatopathic patient).

The selected transplanted patients were similarly representative of the different Transplant Centers of the whole champion (37.5% liver, 25% kidney 25% h. stem cells 12.5% heart VS 35.3%, 27.1%, 25.2%, 10.9%), excluding combined organs.

We also recorded a drop out of the subgroup for the SARS-CoV-2 humoral response (42 patients at t1, 38 at t2 and 40 at t3).

Results of SARS-CoV-2 cellular immune response and lymphocyte typing in the subgroup of 42 patients are shown in Table 6.

		N=42
Cellular immune response	**Blank** pg/ml, median (IQR)	2.58 (1.11-5.95)
	Mitogen pg/ml, median (IQR)	374.5 (193-1236)
	Spike pg/ml, median (IQR)	15.8 (5.51-110)
	Interpretation of the results, n (%) Positive Negative Undetermined	 21 (50.0) 14 (33.3) 7 (16.7)
Lymphocyte typing	White blood cells/µL, median (IQR)	5611 (4744-8133)
	Total lymphocytes/µL, median (IQR)	1045.5 (818-1560)
	CD3+ %, median (IQR)	78.5 (63-85)
	CD3+, median (IQR)	796 (534-1291)
	CD4+ %, median (IQR)	33.5 (25-42)
	CD4+, median (IQR)	390 (224-560)
	CD8+ %, median (IQR)	38 (29-47)
	CD8+, median (IQR)	426.5 (236-651)
	CD4+/CD8+, median (IQR)	1 (0.6-1.5)
	NK %, median (IQR)	15.2 (9-21)
	NK, median (IQR)	161 (115-281)
	CD19+ %, median (IQR)	6.5 (3-13)
	CD19+, median (IQR)	73.5 (29-134)

Table 6. SARS-CoV-2 cellular immune response and lymphocyte typing in the subgroup of 42 patients.

For 422 of 440 patients we were able to analyze also the antibody titration (IgA,IgG,IgM). Most of the sample had a positive test result for SARS-CoV-2 antibodies in all post-vaccination monitoring (30, 90 and 150 days after the 2nd dose of mRNA SARS-CoV-2 vaccine), as depicted in Table 7.

	N=422
IgA (mg/dL), **median (IQR)**	184.5 (115-284)
IgG (mg/dL),**, median (IQR)**	970 (743-1250)
IgM (mg/dL),**, median (IQR)**	74.5 (42-126)
	N=162
Abs t 0 (U/mL), median (IQR)	0.4 (0.4-0.4)
Abs t 0 (U/mL), ≥ 0.8, n (%)	12 (7.4)
	N=437
Abs t 1 (U/mL), median (IQR)	8.3 (0.4-487)
Abs t 1(U/mL), ≥ 0.8, n (%)	264 (60.4)
	N=412
Abs t 2 (U/mL), median (IQR)	45.4 (0.4-387.5)
Abs t 2 (U/mL), ≥ 0.8, n (%)	300 (72.8)
	N=392
Abs t 3 (U/mL), median (IQR)	34.8 (0.6-288.5)
Abs t 3 (U/mL), ≥ 0.8, n (%)	293 (74.7)

Table 7. Antibody titration (IgA,IgG,IgM) and SARS-CoV-2 serological test at t0, t1, t2 and t3.

As shown in Figure 4, after the vaccination, the humoral response of the patients gradually decreased from 30 days after vaccination (t1) to the last monitoring (t3).

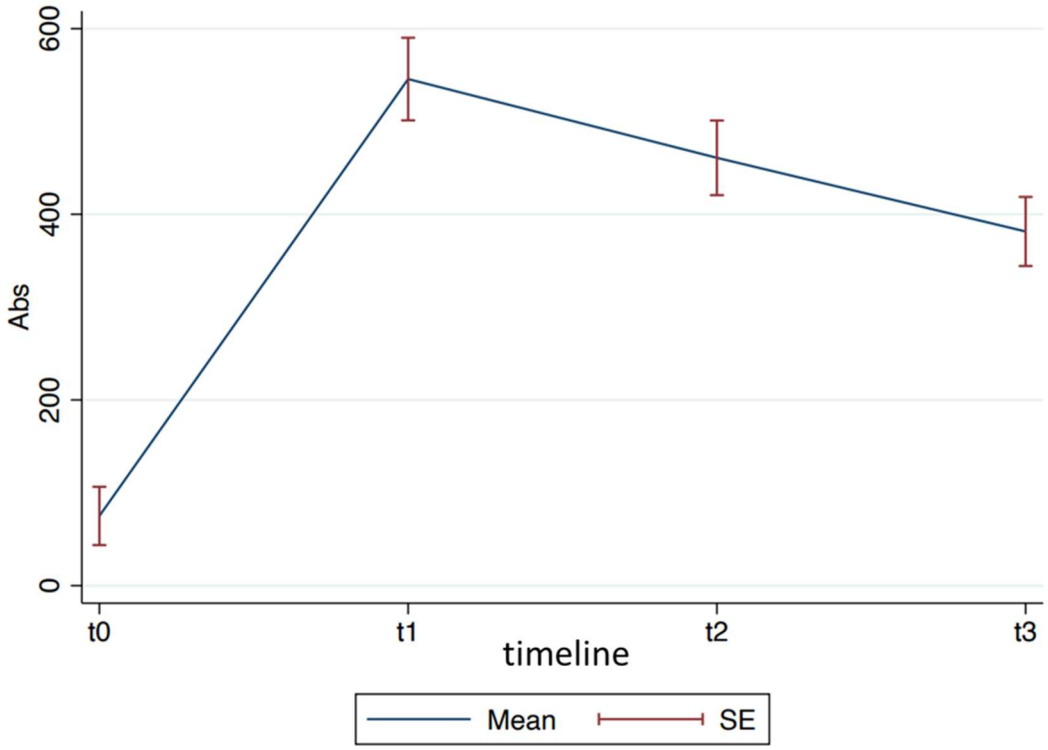

Figure 4. The visual representation of SARS-CoV-2 humoral response in all of the time points of the study.

Questionnaire information

Through the administration of the questionnaire, we collected various general information of the patients (see Table 5) and we were also able to detect SARS-CoV-2 positivity before vaccination (mean 169 days prior to the first monitoring).

Local and systemic adverse effects after the first and the second dose of vaccination were reported on the anamnestic form and recorded. A total of 155 and 179 patients have experienced the presence of adverse effects symptoms after the 1st and 2nd dose respectively (see Table 8). Moreover, no post-vaccination major immediate adverse events occurred during the vaccination sessions.

We also collected information about the immunosuppressive regimen of the patients, revealing that tacrolimus was the most common immunosuppressor in use (45.7%), registered in 201 patients, followed by mycophenolate mofetil (in 158 patients – 35.9%) and corticosteroids (139 – 31.6%). Most of the patients under immunosuppression regimen had a double-therapy immunosuppression regimen (123 patients), 37 of whom received mycophenolate mofetil/mycophenolic acid in combination with tacrolimus. Other registered therapies included: azathioprine, cyclosporine, sirolimus, everolimus, lenalidomid and nivolumab (only 1 patient).

To analyze the titration of antibodies directed towards the Spike protein of the virus, the sample was divided into two groups in relation to the antibodies' value: the one with the test considered negative (<0,8 U/mL) and the one with a positive response (≥0,8 U/mL).

	Abs <0.8 (N=173)	Abs ≥ 0.8 (N=264)	p-value
Age ≥ 65, n/N (%)	79/172 (45.9)	108/262 (41.2)	0.333
Female gender, n (%)	57 (32.9)	82 (31.1)	0.679
Transplant, n (%)	169 (97.7)	241 (91.3)	**0.007**
Transplanted Organ, n/N (%)			**<0.001**
Kidney	77/169 (45.6)	35/241 (14.5)	
Heart	14/169 (8.3)	31/241 (12.9)	
Liver	41/169 (24.3)	102/241 (42.3)	
H. Stem cells	34/169 (20.1)	70/241 (29.0)	
Combined			
Kidney and liver	2/169 (1.2)	1/241 (0.4)	
Kidney and pancreas	1/169 (0.6)	1/241 (0.4)	
Kidney and heart	0/169 (0.0)	1/241 (0.4)	
Days since transplant ≤365, n/N (%)	144/169 (85.2)	214/240 (89.2)	0.233
Presence of adverse effects after 1st dose, n/N (%)	57/171 (33.3)	98/262 (37.4)	0.388
Presence of adverse effects after 2nd dose, n/N (%)	70/171 (40.9)	109/260 (41.9)	0.839
COVID positivity, n/N (%)	3/172 (1.7)	16/264 (6.1)	**0.031**
Immunosuppressive regimen, n (%)			
Mycophenolate mofetil	103 (59.5)	55 (20.8)	**<0.001**
Tacrolimus	99 (57.2)	100 (37.9)	**<0.001**
Cyclosporine	25 (14.4)	58 (22.0)	0.050
Corticosteroids	90 (52.0)	49 (18.6)	**<0.001**
Everolimus	7 (4.0)	24 (9.1)	**0.045**
Sirolimus	2 (1.2)	4 (1.5)	1.000
Azathioprine	2 (1.2)	4 (1.5)	1.000
Lenalidomid	2 (1.2)	4 (1.5)	1.000
Nivolumab	0 (0.0)	1 (0.4)	1.000

Table 8. SARS-CoV-2 antibodies at t1 (30 days after 2nd dose) in relation with the major characteristics.

As far as the first monitoring is concerned, we found a statistically significant association between the 2 groups and the transplanted organ, COVID-19 positivity, and mycophenolate mofetil, tacrolimus and corticosteroids.

We decided also to test these characteristics at t2 and t3, as reported in Table 9 and Table 10.

	Abs <0.8 (N=112)	**Abs ≥0.8 (N=300)**	**p-value**
Age ≥ 65, n/N (%)	53/112 (47.3)	126/298 (42.3)	0.359
Female gender, n (%)	38 (33.9)	92 (30.7)	0.526
Transplant, n (%)	112 (100)	274 (91.3)	**<0.001**
Transplanted Organ, n/N (%)			**<0.001**
Kidney	58/112 (51.8)	51/274 (18.6)	
Heart	9/112 (8.0)	33/274 (12.0)	
Liver	29/112 (25.9)	112/274 (40.9)	
H. Stem cells	14/112 (12.5)	74/274 (27.0)	
Combined			
Kidney and liver	1/112 (0.9)	2/274 (0.7)	
Kidney and pancreas	1/112 (0.9)	1/274 (0.4)	
Kidney and heart	0/112 (0.0)	1/274 (0.4)	
Days since transplant ≤365, n/N (%)	91 (81.2)	244 (90.4)	**0.013**
Presence of adverse effects after 1st dose, n/N (%)	38/111 (34.2)	108/298 (36.2)	0.706
Presence of adverse effects after 2nd dose, n/N (%)	49/110 (44.5)	119/297 (40.1)	0.415
COVID positivity, n/N (%)	1/111 (0.9)	17/300 (5.7)	0.053
Drugs /Immunosuppressive regimen, n (%)			
Mycophenolate mofetil	77 (68.7)	78 (26.0)	**<0.001**
Tacrolimus	74 (66.1)	118 (39.3)	**<0.001**
Cyclosporine	17 (15.2)	64 (21.3)	0.162
Corticosteroids	68 (60.7)	67 (22.3)	**<0.001**
Everolimus	5 (4.5)	23 (7.7)	0.251
Sirolimus	2 (1.8)	4 (1.3)	0.733
Azathioprine	1 (0.9)	5 (1.7)	1.000
Lenalidomid	0 (0.0)	6 (2.0)	0.196
Nivolumab	0 (0.0)	0 (0.0)	-

Table 9. SARS-CoV-2 antibodies at t2 (90 days after 2nd dose) in relation with the major characteristics.

	Abs < 0.8 (N=99)	Abs ≥ 0.8 (N=293)	p-value
Age ≥ 65, n/N (%)	46 (46.5)	126 (43.3)	0.584
Female gender, n (%)	34 (34.3)	86 (29.3)	0.351
Trasplant, n (%)	99 (100)	271 (92.5)	**0.002**
Transplanted Organ, n/N (%)			**<0.001**
Kidney	57/99 (57.6)	51/271 (18.8)	
Heart	8/99 (8.1)	32/271 (11.8)	
Liver	25/99 (25.2)	110/271 (40.6)	
H. Stem cells	7/99 (7.1)	74/271 (27.3)	
Combined			
Kidney and liver	1/99 (1.0)	2/271 (0.7)	
Kidney and pancreas	1/99 (1.0)	1/271 (0.4)	
Kidney and heart	0/99 (0.0)	1/271 (0.4)	
Days since transplant ≤365, n/N (%)	82/99 (82.8)	247/268 (92.2)	**0.009**
Presence of adverse effects after 1st dose, n/N (%)	33/98 (33.7)	106/291 (36.4)	0.623
Presence of adverse effects after 2nd dose, n/N (%)	43/98 (43.9)	115/290 (40.0)	0.462
COVID positivity, n/N (%)	0/98 (0.0)	15/293 (5.1)	**0.016**
Drugs /Immunosuppressive regimen, n (%)			
Mycophenolate mofetil	73 (73.7)	79 (27.0)	**<0.001**
Tacrolimus	72 (72.7)	118 (40.3)	**<0.001**
Cyclosporine	14 (14.1)	60 (20.5)	0.164
Corticosteroids	62 (62.6)	66 (22.5)	**<0.001**
Everolimus	5 (5.0)	22 (7.5)	0.404
Sirolimus	1 (1.0)	4 (1.4)	1.000
Azathioprine	1 (1.0)	5 (1.7)	1.000
Lenalidomid	0 (0.0)	4 (1.4)	0.576
Nivolumab	0 (0.0)	0 (0.0)	-

Table 10. SARS-CoV-2 antibodies at t3 (150 days after 2nd dose) in relation with the major characteristics.

Statistically significant association with mycophenolate mofetil, tacrolimus and corticosteroids were confirmed in all 3 post-vaccination monitoring (p value: <0.001). To avoid bias we decided to repeat the analysis excluding the patients that showed antibody titration not in range (IgG,

IgM, IgA) and those who were previously tested positive for COVID19, finding no difference in the statistical significance.

Results are presented in Table 11, 12 and 13.

	Abs < 0.8 (N=152)	**Abs ≥ 0.8 (N=180)**	**p-value**
Age ≥ 65, n/N (%)	68/151 (45.0)	68/178 (38.2)	0.210
Female gender, n (%)	47 (30.9)	58 (32.2)	0.799
Trasplant, n (%)	148 (97.4)	172 (95.6)	0.378
Transplanted Organ, n/N (%)			**<0.001**
Kidney	70/148 (47.3)	29/172 (16.9)	
Heart	12/148 (8.1)	19/172 (11.0)	
Liver	37/148 (25.0)	61/172 (35.5)	
H. Stem cells	26/148 (17.6)	61/172 (35.5)	
Combined			
Kidney and liver	2/148 (1.3)	0/172 (0.0)	
Kidney and pancreas	1/148 (0.7)	1/172 (0.6)	
Kidney and heart	0/148 (0.0)	1/172 (0.6)	
Days from transplantation ≤365, n/N (%)	128/148 (86.5)	149/171 (87.1)	0.864
Presence of adverse effects after 1st dose, n/N (%)	52/150 (34.7)	69/178 (38.8)	0.444
Presence of adverse effects after 2nd dose, n/N (%)	60/150 (40.0)	76/177 (42.9)	0.591
Drugs /Immunosuppressive regimen, n (%)			
Mycophenolate mofetil	95 (62.5)	35 (19.4)	**<0.001**
Tacrolimus	91 (59.9)	68 (37.8)	**<0.001**
Cyclosporine	21 (13.8)	35 (19.4)	0.172
Corticosteroids	79 (52.0)	42 (23.3)	**<0.001**
Everolimus	7 (4.6)	16 (8.9)	0.126
Sirolimus	2 (1.3)	3 (1.7)	1.000
Azathioprine	1 (0.7)	2 (1.1)	1.000
Lenalidomid	2 (1.3)	2 (1.1)	1.000
Nivolumab	0 (0.0)	0 (0.0)	-

Table 11. SARS-CoV-2 antibodies at t1 (30 days after 2nd dose) in relation with the major characteristics, excluding patients with IgM, IgG and IgA not in range and ex COVID19 patients

	Abs < 0.8 (N=103)	Abs ≥ 0.8 (N=208)	p-value
Age ≥ 65, n/N (%)	48/103 (46.6)	83/206 (40.3)	0.290
Female gender, n (%)	33 (32.0)	64 (30.8)	0.820
Transplant, n (%)	103 (100)	197 (94.7)	**0.018**
Transplanted Organ, n/N (%)			**<0.001**
Kidney			
Heart	56/103 (54.4)	40/197 (20.3)	
Liver	7/103 (6.8)	21/197 (10.7)	
H. Stem cells	29/103 (28.2)	69/197 (35.0)	
Combined	9/103 (8.7)	64/197 (32.5)	
Kidney and liver			
Kidney and pancreas	1/103 (1.0)	1/197 (0.5)	
Kidney and heart	1/103 (1.0)	1/197 (0.5)	
	0/103 (0.0)	1/197 (0.5)	
Days from transplantation ≤365, n/N (%)	85/103 (82.5)	173/193 (89.6)	0.081
Presence of adverse effects after 1st dose, n/N (%)	36/102 (35.3)	78/206 (37.9)	0.660
Presence of adverse effects after 2nd dose,, n/N (%)	44/101 (43.6)	82/206 (39.8)	0.529
Drugs /Immunosuppressive regimen, n (%)			
Mycophenolate mofetil	74 (71.8)	53 (25.5)	**<0.001**
Tacrolimus	71 (68.9)	83 (39.9)	**<0.001**
Cyclosporine	15 (14.6)	39 (18.7)	0.359
Corticosteroids	62 (60.2)	55 (26.4)	**<0.001**
Everolimus	5 (4.8)	15 (7.2)	0.425
Sirolimus	2 (1.9)	3 (1.4)	0.668
Azathioprine	1 (1.0)	2 (1.0)	1.000
Lenalidomid	0 (0.0)	4 (1.9)	0.306
Nivolumab	0 (0.0)	0 (0.0)	-

Table 12. SARS-CoV-2 antibodies at t2 (90 days after 2nd dose) in relation with the major characteristics, excluding patients with IgM, IgG and IgA not in range and ex COVID19 patients.

	Abs < 0.8 (N=95)	Abs ≥ 0.8 (N=204)	p-value
Age ≥ 65, n/N (%)	44/95 (46.3)	80/202 (39.6)	0.274
Female gender, n (%)	31 (32.6)	60 (29.4)	0.573
Transplant, n (%)	95 (100)	196 (96.1)	0.059
Transplanted Organ, n/N (%)			**<0.001**
Kidney			
Heart	57/95 (60.0)	39/196 (19.9)	
Liver	7/95 (7.4)	21/196 (10.7)	
H. Stem cells	24/95 (25.3)	68/196 (34.7)	
Combined	5/95 (5.3)	65/196 (33.2)	
Kidney and liver			
Kidney and pancreas	1/95 (1.0)	1/196 (0.5)	
Kidney and heart	1/95 (1.0)	1/196 (0.5)	
	0/95 (0.0)	1/196 (0.5)	
Days from transplantation ≤365, n/N (%)	80/95 (84.2)	177/193 (91.7)	0.054
Presence of adverse effects after 1st dose, n/N (%)	33/94 (35.1)	77/202 (38.1)	0.618
Presence of adverse effects after 2nd dose, n/N (%)	41/94 (43.6)	81/202 (40.1)	0.567
Drugs /Immunosuppressive regimen, n (%)			
Mycophenolate mofetil	71 (74.7)	55 (27.0)	**<0.001**
Tacrolimus	70 (73.7)	81 (39.7)	**<0.001**
Cyclosporine	14 (14.7)	38 (18.6)	0.409
Corticosteroids	60 (63.2)	53 (26.0)	**<0.001**
Everolimus	5 (5.3)	15 (7.3)	0.501
Sirolimus	1 (1.0)	3 (1.5)	1.000
Azathioprine	1 (1.0)	2 (1.0)	1.000
Lenalidomid	0 (0.0)	3 (1.5)	0.554
Nivolumab	0 (0.0)	0 (0.0)	-

Table 13. SARS-CoV-2 antibodies at t3 (150 days after 2nd dose) in relation with the major characteristics, excluding patients with IgM, IgG and IgA not in range and ex COVID19 patients.

Considering the characteristic of the subgroup of patients selected for the cellular immune response test (n.42 patients) and comparing them with the interpretation of

the test, a statistically significant association was found for SARS-CoV-2 antibody response ≥ 0.80 U/mL (data are presented in Table 14).

	Negative (N=14)	Undetermined (N=7)	Positive (N=21)	p-value
Age ≥ 65, n/N (%)	4/14 (28.6)	2/7 (28.6)	6/20 (30.0)	1.000
Female gender, n (%)	1 (7.1)	2 (28.6)	7 (33.3)	0.181
Transplant, n (%)	14 (100)	7 (100)	19 (90.5)	0.659
Transplanted Organ, n/N (%)				0.085
Kidney	2/14 (14.3)	5/7 (71.4)	3/19 (15.8)	
Heart	2/14 (14.3)	0/7 (0.0)	3/19 (15.8)	
Liver	7/14 (50.0)	0/7 (0.0)	8/19 (42.1)	
H. Stem cells	3/14 (21.4)	2/7 (28.6)	5/19 (26.3)	
Days since transplantation≤365, n/N (%)	12/14 (85.7)	4/7 (57.1)	17/19 (89.5)	0.195
Presence of adverse effects after 1st dose, n/N (%)	6/14 (42.9)	1/7 (14.3)	10/20 (50.0)	0.254
Abs ≥ 0.8 at t1, n (%)*	9 (64.3)	3 (42.9)	19 (90.5)	**0.021**
Abs ≥ 0.8 at t2, n/N (%)*	8/11 (72.7)	3/7 (42.9)	19/20 (95.0)	**0.008**
Abs ≥ 0.8 at t3, n/N (%)*	10/13 (76.9)	2/7 (28.6)	19/20 (95.0)	**0.002**
COVID positivity, n/N (%)	0 (0.0)	0 (0.0)	1 (4.8)	1.000
Drugs /Immunosuppressive regimen, n (%)				
mycophenolate mofetil/ mycophenolic acid	4 (28.6)	3 (42.9)	4 (19.0)	0.407
Tacrolimus	8 (57.1)	3 (42.9)	8 (38.1)	0.497
Cyclosporine	2 (14.3)	1 (14.3)	6 (28.6)	0.600
Corticosteroids	2 (14.3)	4 (57.1)	4 (19.0)	0.107
Everolimus	1 (7.1)	1 (14.3)	2 (9.5)	1.000
Sirolimus	0 (0.0)	0 (0.0)	0 (0.0)	-
Azathioprine	0 (0.0)	0 (0.0)	1 (4.8)	1.000
Lenalidomid	1 (7.1)	0 (0.0)	0 (0.0)	0.500
Nivolumab	0 (0.0)	0 (0.0)	0 (0.0)	-

Table 14. Cellular immune response Interpretation of the results (Positive, Negative, Undetermined) with the main characteristics of the subgroup.
**data referred to a total of 42 patients at t1, 38 at t2 and 40 at t3.*

To investigate if the numbers and relative percentages of lymphocytes could interfere with the SARS-CoV-2 cellular immune response, we tested the interpretations of the

cellular immune response (negative, undetermined and positive) with all of lymphocyte typing results (see Table13). We found a statistically significant association with the white blood cells numbers count per µL (p-value: 0.032), as well as other elements of lymphocyte typing, as shown in detail in Table15.

	Negative (N=14)	**Undetermined (N=7)**	**Positive (N=21)**	**p-value**
White blood cells/µL, n (%)				**0.032**
<4800	6 (42.9)	1 (14.3)	4 (19.0)	
4800-10800	8 (57.1)	3 (42.9)	16 (76.2)	
>10800	0 (0)	3 (42.9)	1 (4.8)	
Total lymphocytes/µL, n (%)				0.097
<1500	12 (85.7)	7 (100)	12 (57.1)	
1500-4000	2 (14.3)	0 (0)	8 (38.1)	
>4000	0 (0)	0 (0)	1 (4.8)	
lymphocytes T CD3+ %, n (%)				0.520
<67%	5 (35.7)	3 (42.9)	4 (19.0)	
67-80%	3 (21.4)	1 (14.3)	9 (19.0)	
>80%	6 (42.9)	3 (42.9)	8 (38.1)	
lymphocytes T CD3+/ µL, n (%)				**0.020**
<1000	12 (85.7)	6 (85.7)	9 (42.9)	
1000-3200	2 (14.3)	1 (14.3)	12 (57.1)	
>3200	0 (0)	0 (0)	0 (0)	
lymphocytes T helper CD4+/CD3+ %, n (%)				0.345
<35%	7 (50)	5 (71.4)	10 (47.6)	
35-55%	7 (50)	1 (14.3)	10 (47.6)	
>55%	0 (0)	1 (14.3)	1 (4.8)	
lymphocytes T helper CD4+/CD3+ /µL, n (%)				0.089
<500	11 (78.6)	7 (100)	12 (57.1)	
500-2200	3 (21.4)	0 (0)	9 (42.9)	
>2200	0 (0)	0 (0)	0 (0)	
lymphocytes T CD8+/CD3+%, n (%)				0.212
<20%	0 (0)	0 (0)	3 (14.3)	
20-38%	9 (64.3)	4 (57.1)	6 (28.6)	
>38%	5 (35.7)	3 (42.9)	12 (57.1)	

Lymphocyte T CD8+/CD3+ /µL, n (%)				**0.025**
<300	7 (50)	4 (57.1)	3 (14.3)	
300-1520	7 (50)	3 (42.9)	18 (85.7)	
>1520	0 (0)	0 (0)	0 (0)	
CD4+ / CD8+, n (%)				0.536
<1.2	6 (42.9)	4 (57.1)	14 (66.7)	
1.2-2.2	7 (50)	2 (28.6)	5 (23.8)	
>2.2	1 (7.1)	1 (14.3)	2 (9.5)	
Natural Killer CD56 + CD16 + /CD3- %, n (%)				**0.031**
<5%	0 (0)	1 (14.3)	2 (9.5)	
5-25%	11 (78.6)	4 (57.1)	19 (90.5)	
>25%	3 (21.4)	2 (28.6)	0 (0)	
Natural Killer CD56 + CD16 + /CD3- /µL				0.600
<90	2 (14.3)	1 (14.3)	6 (28.6)	
90-540	12 (85.7)	6 (85.7)	15 (71.4)	
>540	0 (0)	0 (0)	0 (0)	
lymphocytes B CD19+ %				0.511
<7%	6 (42.9)	5 (71.4)	10 (47.6)	
7-14%	5 (35.7)	0 (0)	6 (28.6)	
>14%	3 (21.4)	2 (28.6)	5 (23.8)	
lymphocytes B CD19+ /µL				0.574
<105	10 (71.4)	5 (71.4)	10 (47.6)	
105-560	4 (28.6)	2 (28.6)	10 (47.6)	
>560	0 (0)	0 (0)	1 (4.8)	

Table 15. Cellular immune response (negative, undetermined and positive) in comparison with lymphocyte typing results.

The variation of the SARS-CoV-2 humoral response values among the three time points showed a statistically significant decrease, as depicted in Figure 5.

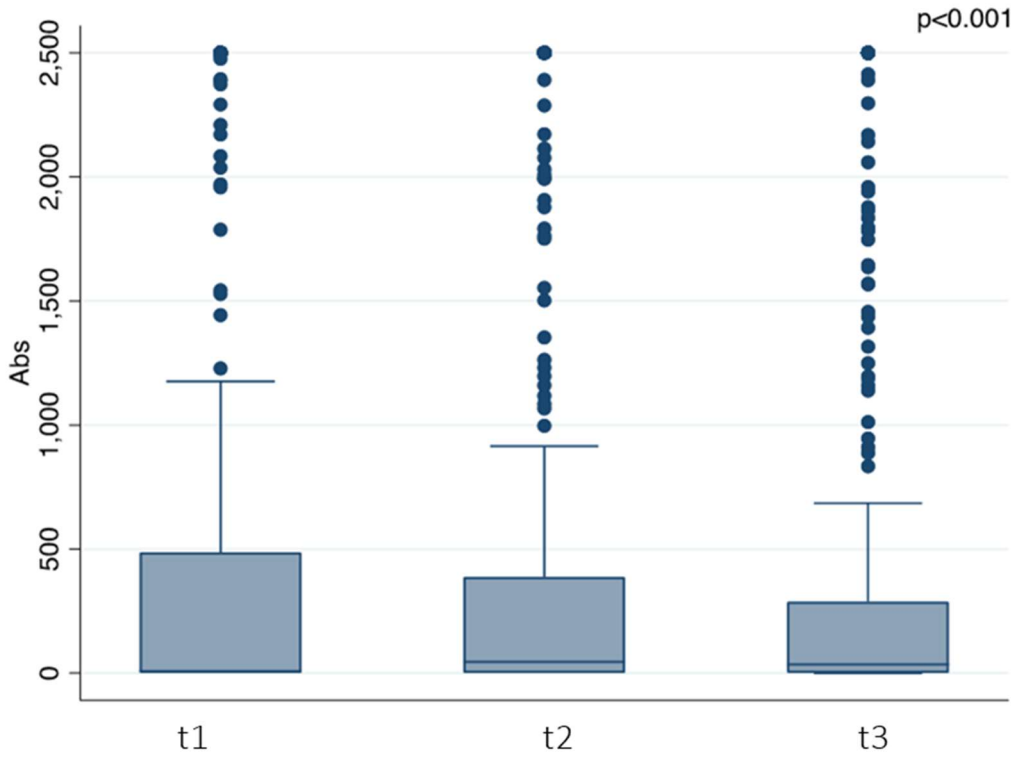

Figure 5. Box plot of the SARS-CoV-2 antibody titration 30, 90 and 150 days after SARS-CoV-2 vaccination (*One-Way ANOVA).*

As far as the other endpoints of the study are concerned, patients' follow-up is still ongoing and we didn't record yet any data about COVID-19 positivity after immunization.

2.2.5 Discussion

The vaccination campaign for transplant recipients and waitlisted patients had high adherence, considering that 72.1% of the eligible population joined the vaccination sessions organized by Udine Hospital.
Comparing our adherence to national data, we were in line with the mean percentage (72.9%) of transplant recipients that had received at least one dose of mRNA SARS-CoV-2 vaccine in the same period in Italy (National Transplant Center's data).

As far as the population included in the study is concerned, 93.9% of them were transplanted recipients while only 6.1% were waitlisted patients, showing low adherence of this subpopulation, if we consider that 12.5% was the percentage of waitlisted patients in 2021.
Unfortunately, we couldn't collect any additional data about the vaccination adherence and hesitancy of this subpopulation. Considering the importance of identifying the reasons influencing COVID-19 vaccination hesitancy, as underlined in literature, this phenomenon should be further analyzed. (29,39)

As far as the primary endpoint is concerned, we found out that most of the patients had a positive humoral response (60.4%) with a median of 8.3 (IQR: 0.4-487) after 1 month from the second dose of SARS-CoV-2 vaccine.
Our findings were higher considering the SOTr research of Boyarsky et al. where, at a median (IQR) of 29 (28-31) days after dose 2, antibody was detectable in 357 participants (54%) (95% CI, 50%-58%).(33)
This gap could be related to the high prevalence of h. stem cell transplant patients in our population and to the immunosuppressor regimen. Many studies, in fact, have underlined the association between immunosuppressors and SARS-CoV-2 immune response in transplant recipients. (33,40)
Similar results were also found in our research: the use of mycophenolate mofetil, tacrolimus and corticosteroids were associated with a significantly diminished humoral response in all three

monitoring points (p value: <0.001). Further investigations on the dosage couldn't be performed because, for organizational reasons, the immunosuppression regimen dosage hasn't been recorded in the database.

Most of the sample had a positive test result for SARS-CoV-2 antibodies in all post-vaccination monitoring, decreasing from the second to the third monitoring, 45.4 (0.4-387.5) to 34.8 (0.6-288.5). Although there is still no consensus on the cut-off that can be considered protective, SARS-CoV-2 antibody test results were below what it was observed in immunocompetent adults.(41)

Cell-mediated vaccine immunogenicity determined in the subgroup of patients showed a statistically significant association with the humoral response (p-value 0.021) and even if difficult to compare with other studies, due to the variability of the test, it remains a useful tool to fully investigate the immune response, as suggested in literature. (33)

Presence of side effects and adverse reactions after the vaccine administration were recorded and no statistically significant association with antibody response was found. Furthermore, no immediate systemic adverse event was registered after immunization.

2.2.6 Limits of the study

In Udine COVID-19 vaccine campaign, all transplant waitlisted patients and recipients received two doses of "Pfizer" BNT162b2 (Comirnaty) mRNA vaccine, therefore immunization after the administration of other vaccines authorized for these categories of patients, such as "Moderna" mRNA vaccine, could not have been compared and would need further explorations.(42) Moreover, we didn't include a control group, since post-vaccination immune responses have been extensively studied in immunocompetent people. (41)

Even if we investigated SARS-CoV-2 mRNA vaccine immune response, we didn't evaluate vaccine effectiveness in terms of COVID-19 illness or infection prevention and neither we tested the effectiveness for variants of concerns of SARS-CoV-2 virus, as it was not the purpose of our study. Based on the real-world studies, in fact, SARS-CoV-2 vaccines have already demonstrated to be capable of effectively reduce severe outcomes. (43)

The major limit of the study is linked to the laboratory tests used. Moreover, we did not assess cellular immune responses in the entire cohort, for feasibility reasons.
In addition, we didn't have the possibility to retrieve and process blood pre-vaccination samples for all the patients included in the study to test antibody response, though we evaluated previous SARS-CoV-2 infection also through the information reported in the questionnaires.
Referring to the questionnaire, it must be specified that even if it indeed helped collecting many data, this method could be partially affected by recall bias.

Finally, ours was a heterogeneous sample of transplanted and waitlisted patients of both solid organ and hematopoietic stem cells. For this reason, detailed considerations would have been required for the h. stem cell transplant population as the association between vaccine immune response and the type of transplantation, if autologous, allogeneic or chimeric antigen receptor (CAR) T-cell therapy, as suggested by other authors in literature. (44)

2.2.7 Conclusions

The main strengths of this study are the assessment of humoral and cellular immune response after SARS-CoV-2 mRNA vaccination, even if at a local level, in a population that was only partially considered in previous studies.

We also confirmed the association between immunosuppression regimen and post vaccination humoral response in transplanted and waitlisted patients, as presented in recent literature, though further studies among the population of waitlisted patients should be needed.

Moreover, significantly decreased of the SARS-CoV-2 antibody titration 90 and 150 days after SARS-CoV-2 vaccination, reinforced the need for tailored evaluations for this category of patients, as booster vaccination.

It should also be noted that research on this topic must be constantly updated, in consideration of national and international indications on SARS-CoV-2 vaccination and continuous emerging evidences.

Recently, in fact, the 4th dose -better defined as booster dose- has been recommended for transplanted and immunosuppressed patients, for which new SARS-CoV-2 vaccination agendas have been opened and to which a part of this population has already adhered. (45)

2.2.8 Conflict of interest

None.

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