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Waning of vaccine-induced immunity to measles in kidney transplanted children

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Abstract

Vaccine-preventable diseases are a significant cause of morbidity and mortality in solid organ transplant recipients who undergo immunosuppression after transplantation. Data on immune responses and long-term maintenance after vaccinations in such population are still limited.

We cross-sectionally evaluated the maintenance of immune response to measles vaccine in kidney transplanted children on immunosuppressive therapy. Measles-specific enzyme-linked immunosorbent assay and B-cell enzyme-linked immunosorbent spot were performed in 74 kidney transplant patients (Tps) and in 23 healthy controls (HCs) previously vaccinated and tested for humoral protection against measles. The quality of measles antibody response was measured by avidity test. B-cell phenotype, investigated via flow cytometry, was further correlated to the ability of Tps to maintain protective humoral responses to measles over time.

We observed the loss of vaccine-induced immunity against measles in 19% of Tps. Nonseroprotected children showed signs of impaired B-cell distribution as well as immune senescence and lower antibody avidity. We further reported as time elapsed between vaccination and transplantation, as well as the vaccine administration during dialysis are clinical factors affecting the maintenance of the immune memory response against measles.

Tps present both quantitative and qualitative alterations in the maintenance of protective immunity to measles vaccine. Prospective studies are needed to optimize the vaccination schedules in kidney transplant recipients in order to increase the immunization coverage over time in this population.

Abbreviations: DN = double negative, ELISA = enzyme-linked immunosorbent assay, ELIspot = enzyme-linked immunosorbent spot, HAA = high affinity antibodies, HCs = healthy controls, HD = hemodialysis, LAVV = live attenuated virus vaccines, MA = mature activated, MMR = measles, mumps, and rubella, MSMBC = measles-specific memory B-cells, NSP = nonseroprotected patients, OD = optical density, PBMCs = peripheral blood mononuclear cells, SOT = solid organ transplantation, SP = seroprotected patients, Tps = kidney transplant patients.

Keywords: kidney transplantation, long-term memory, measles, memory B-cell compartment, protective immunity, timing of vaccination

1. Introduction

Advances brought by modern medicine had led the solid organ transplantation (SOT) to be a routinely therapeutic option used to treat congenital or acquired irreversible kidney disorders.[1] Children who have undergone kidney transplantation are at an increased risk for infections due to the long-term use of immunosuppressants. Vaccine-preventable diseases are potentially life-threatening conditions that should be avoided in this population.[2] In this scenario, the implementation of active immunization pursued through vaccines is crucial.[3,4] The IDSA Clinical Practice Guidelines recommended vaccines administration prior to planned immunosuppression if feasible (strong, moderate).[5] For SOT on immunouppressive therapy, inactivated vaccines are generally considered safe and are usually administered.[6] Conversely, administration of live attenuated virus vaccines (LAVV), such as measles, mumps, and rubella (MMR) are currently contraindicated after SOT.[4] Published guidelines[5] agree that LAVV should be given before transplantation when the immune system can still trigger an effective immune response to vaccination and the safety profile is comparable to the 1 expected in healthy children.[3] However, less is known about the ability to maintain protective immunity developed prior to the SOT once the immunosuppressive therapy has been started. Few studies available have proven that

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protective antibody titers previously acquired following routine vaccinations wane in a considerable amount of pediatric SOT recipients.[7–9] Kano et al showed that the rate of seroprotection for measles 1 year after liver transplantation was 45% of totally immunized transplanted children suggesting that long-term maintenance of protective antibodies may be impaired by immunosuppressive therapy.[7] Loss of protective immunity acquired through immunization prior to transplantation is particularly alarming when considering live attenuated antigen vaccines. Indeed, clusters of MMR unvaccinated individuals across Europe reduce overall population coverage below the 95% which is the minimum threshold necessary for eradication. This phenomenon contributes to dissemination of the disease to high-risk population such as transplanted individuals.[10] Furthermore, informative correlates of protection other than standard serology test are needed in such population in order to design a personalized revaccination schedule.[11,12] In view of this background, we performed analysis of measles-specific avidity test to evaluate the quality of protective antibodies and the frequency of measles-specific B-cell memory responses through enzyme-linked immunosorbent spot (ELIspot). We further analyzed clinical factors related to the loss of vaccine-induced protective immunity in SOT population.

2. Materials and methods

2.1. Study subjects

Kidney transplant patients (Tps) followed-up in the Kidney Transplant Unit of the Bambino Gesù Children's Hospital (Rome, Italy) with a complete vaccine history available and evidence of pretransplantation seroprotection to measles were considered eligible for the study. In order to avoid potential discrepancy in terms of waning of seroprotection, only patients with a known level of measles-specific antibody titer at baseline were included in the study. Patients previously treated with anti-CD20 monoclonal antibodies were excluded. Age-matched HCs were enrolled at Bambino Gesù Children's Hospital (Rome, Italy). The study was approved by the local Ethical Committee and all subjects or parents/legal guardians provided informed consent before enrolment. Both HCs and Tps patients were previously immunized with live attenuated measles vaccine according to national vaccine schedule with a 1st dose at 13 months and 2nd booster dose at 6 years of age (http://www.salute.gov.it/).

2.2. Enzyme-linked immunosorbent assay

Serum antibody titers against measles were measured by Enzyme-Linked Immunosorbent Assay (ELISA) reader (Labsystem Multiscan RC photometer, Waltham, Massachussets, USA) using Enzygnost antimeasles Virus/IgG ELISA kit (Dade Behring, Deerfield, Illinois, USA) following manufacturer's instructions. The protective threshold was set to 170 mUI/mL.[13]

2.3. Peripheral blood mononuclear cell culture and B-cell ELIspot

Peripheral blood mononuclear cells (PBMCs) were polyclonally activated in vitro in complete RPMI medium (Invitrogen, Carlsbad, CA) supplemented with 2.5-μg/mL CpG type B (Hycult biotech, Uden, The Netherlands), 20-ng/mL interleukin-4 (Peprotech, Rock Hill, CT), and 20-ng/mL interleukin-21 (ProSpec, East Brunswick, NJ) and harvested after 5 days. The ELIspot 96-well filtration plates (Millipore, Billerica, MA) were coated with 5 μg/well Measles grade 2 antigen (Microbix Biosystem, Toronto, ON) with Na_2CO_3 coating buffer and subsequently loaded with 2×10^5 cells per well. The plates were then processed as previously described.[14] Spots were counted with the ELIspot Analysis Software version 5.1 (A.EL.VIS., Hannover, Germany).

2.4. IgG antibody avidity assay

The antibody avidity to the measles in sera from Tps and HCs was evaluated separately by Calbiotech Measles IgG ELISA kit. The assay was adapted from a previously described ELISA-based method with urea denaturation.[15] In summary, 10 μL of serum specimens each in 200 μL of a Tris-based diluent containing bovine serum albumin and skim milk powder were added to the appropriate wells of the ELISA plates and incubated for 20 min at 37°C. The plates were then washed twice for 5 min used wash solution and with or without urea according with plate's layout. After washing procedures, 100 μL of enzyme conjugated were dispensed in each well and incubated for 20 min at room temperature. The plates were then washed 3 times in wash buffer, and color development was carried out by the addition of 100 μL of the enzyme substrate tetramethylbenzidine per well. After a 10 min incubation in the dark at 37°C, the reaction was stopped by adding 100 μL of stop solution per well. Optical densities (ODs) were measured at 655 nm and the avidity index, expressed as a percentage, was calculated by (ΔOD with urea/ΔOD without urea) × 100.

2.5. Flow cytometry

Purified PBMCs were thawed and stained with the following conjugated monoclonal antibodies: CD19- and CXCR5-Alexa 488, CD27-PerCP-Cy5.5, CD21- and CD45RO-APC, IgD, and CD4-APC-H7 (all from BD Biosciences, Buccinasco, Italy) and CD3- and CD10-Pe/Cy7 (BD Biosciences and Biolegend, respectively). Multicolor flow cytometry was performed on a FACSCanto, interfaced to a FacsDiva software (BD Biosciences) and analyzed through Flow-Jo software version 8.8.3 (Three Star Inc., Ashland, Oregon, USA). The different CD19+ B-cell subpopulations were defined as follows: resting memory (CD27+CD21+), switched memory (CD27+IgD−), unswitched-memory (CD27+IgD+), naïve (CD27−IgD+), double negative (DN) (CD27−IgD−), and mature activated (MA) (CD10−CD21−).

2.6. Statistical analysis

Continuous data have been assessed for Gaussian distribution by Kolmogorov–Smirnov test and differences between Tps and HCs were analyzed using the independent sample T test or the nonparametric Mann–Whitney test for the normally and non-normally distributed variables, respectively. Similarly, the correlation among parameters was evaluated by Pearson and Spearman, respectively. One-way ANOVA with Bonferroni post hoc test was used for comparison of 3 groups. Significance was assigned for $P < 0.05$. The GraphPad Prism software for Windows was used to perform the analyses. To identify the influences of immunosuppressive drugs multiple regression analyses were performed. In particular (I) impaired B subpopulations, measles-specific antibody titers, avidity of specific measles antibodies, frequencies of measles-specific memory-B cells (MSMBC) were considered as dependent variable while (II)

Table 1

Characteristics of subjects.

Characteristics	Tps (n = 74)	HCs (n = 23)
Female, n (%)	30 (40)	10 (43)
Age, y*	16.8 ± 6.4	13.42 ± 10
Years from vaccine*	10.52 ± 6.2	11.42 ± 8
Age at transplantation*	11.7 ± 5.2	NA
Years from transplant*	4.9 ± 3.8	NA
Years between vaccine-transplant*	5.5 ± 5.6	NA
Pretransplant measles serology, mIU/mL*	1078.6 ± 738.4	NA
Creatinine, mg/dL*	0.95 ± 0.36	0.51 ± 0.23
GFR, mL/min per 1.73 m^{2*}	84 ± 23	133 ± 28
Immunosuppressive regimens, n (%)		
Prednisone	74 (100)	NA
Ciclosporine vs tacrolimus	43 (58)/31 (42)	NA
Mycophenolate mofetil vs azathioprine vs everolimus	42 (57)/20 (27)/4 (5)	NA
Primary diseases, n (%)		
Uropathy	42 (56)	NA
Hypodysplasia	10 (14)	NA
Nephrotic syndrome/glomerulonephritis	7 (9)	NA
Hereditary	6 (8)	NA
Cystinosis	5 (7)	NA
HUS	2 (3)	NA
Others	2 (3)	NA

GFR = glomerular filtration rate according to Schwartz formula, HCs = healthy controls, HUS = hemolytic uremic syndrome, NA = not applicable, Tps = transplant patient.
* Values are given as mean ± standard deviation.

age, time since transplantation and immunosuppressive drugs as predictor variables.

3. Results

3.1. Study subjects

A total of 74 Tps on different immunosuppressive regimen and 23 HCs were enrolled at the Bambino Gesù Children's Hospital (Rome, Italy). All Tps had received induction immunosuppression with basiliximab at day 0 and day 4 post-transplant, and were treated with a triple immunosuppressive regimen: a calcineurin inhibitor (cyclosporine or tacrolimus), an antiproliferative agent (mycophenolate mofetil or azathioprine), and steroids which represented the common backbone therapy in all patients. All Tps were clinically stable at the time of examination. Moreover, our multiple regression analysis did not reveal significant influence of immunosuppressive drugs on seroprotection rata, B subpopulations, avidity of measles antibodies, and frequency of MSMBC. Characteristics of all individuals at the time of enrollment are reported in Table 1.

3.2. Evaluation of measles vaccine-induced immunity in Tps and HCs groups

The specific antibody titer against measles was measured in HCs and in Tps. Among Tps, 14 out of the 74 transplant patients (19%) showed level of measles-specific antibodies below the

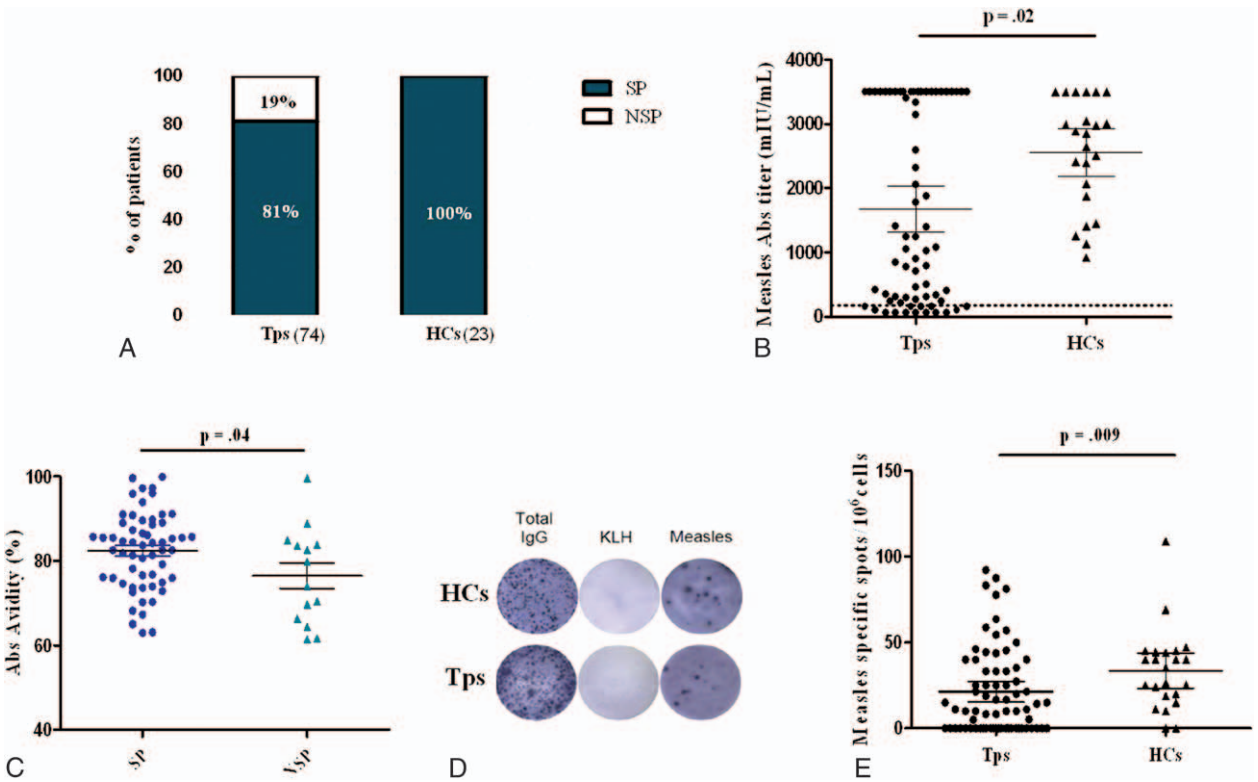

Figure 1. Humoral and cellular measles immunity in Tps and HCs. (A) Comparison between the percentages of Tps and HCs showing protective measles-specific antibody titers. (B) Scatter plot analyses on the measles-specific antibody titers measured in Tps and HCs. The dashed line represents the threshold of seroprotection. (C) Scatter plot analyses on the levels of measles-specific antibodies avidity index, expressed as a percentage, among SP and NSP. (D) Representative examples for the different measles-specific spots in HCs and Tps. Total IgG and keyhole limpet hemocyanin were used a positive and negative controls, respectively. (E) Scatter plot analyses on the frequencies of spots/million cells specific for measles among Tps and HCs. HCs = healthy controls, NSP = nonseroprotected patients, SP = seroprotected patients, Tps = kidney transplant patients.

protective threshold (nonseroprotected patients, NSP). Conversely, all 23 HCs (100%) resulted seroprotected (Fig. 1A). Measles-specific antibody titers resulted significantly lower in Tps compared with HCs group ($P=0.02$) (Fig. 1B). To further investigate whether the quality of antibodies could differ among the Tps group, we evaluated the avidity of measles-specific antibodies. A significant difference was found between seroprotected patients (SP) and NSP, with NSP having antigen-specific IgGs characterized by lower avidity ($P=0.04$) (Fig. 1C).

Finally, we performed ELIspot assay in order to evaluate the frequency of MSMBCs (Fig. 1D). We observed a significant difference between Tps and HCs ($P=0.009$), indicating lower frequency of MSMBC in Tps group (Fig. 1E).

3.3. Differences in B-cell phenotype among Tps maintaining measles immunity over time

Phenotypic characterization of B-cell compartment was performed in order to identify differences in B-cell subsets among the groups (Fig. 2A). A significant lower percentage of total B cells (CD19+) was present in SP ($P=0.001$) and NSP ($P=0.003$) compared with HCs (Fig. 2B) whereas the frequencies of naïve B cells (CD27−IgD+) were lower in NSP ($P=0.03$) compared with HCs (Fig. 2B). No statistical difference was found between study groups in both switched and unswitchted memory B cells.

Remarkably, the frequency of abnormally expanded B cell subset such as DN and MA, already described in other models of immune senescence,[16–18] was higher in Tps when compared with HCs. Specifically, higher frequencies of DN (IgD−CD27−) and MA (CD21−CD10−) B cells were reported in NSP compared with SP ($P=0.02$ and 0.03, respectively) and HCs (both DN and DN, $P<0.001$) (Fig. 2B). These differences were confirmed also in SP as compared with HCs ($P=0.04$ for DN and $P=0.01$ for MA).

3.4. Clinical conditions related to lower persistence of measles-specific immunity

Timing of vaccination and dialysis were taken into account in the analysis in order to identify potential additional factors influencing the maintenance of specific measles immunity over time in our cohort.

Spearman test revealed a positive correlation between serum measles antibody titer and time elapsed between vaccination and

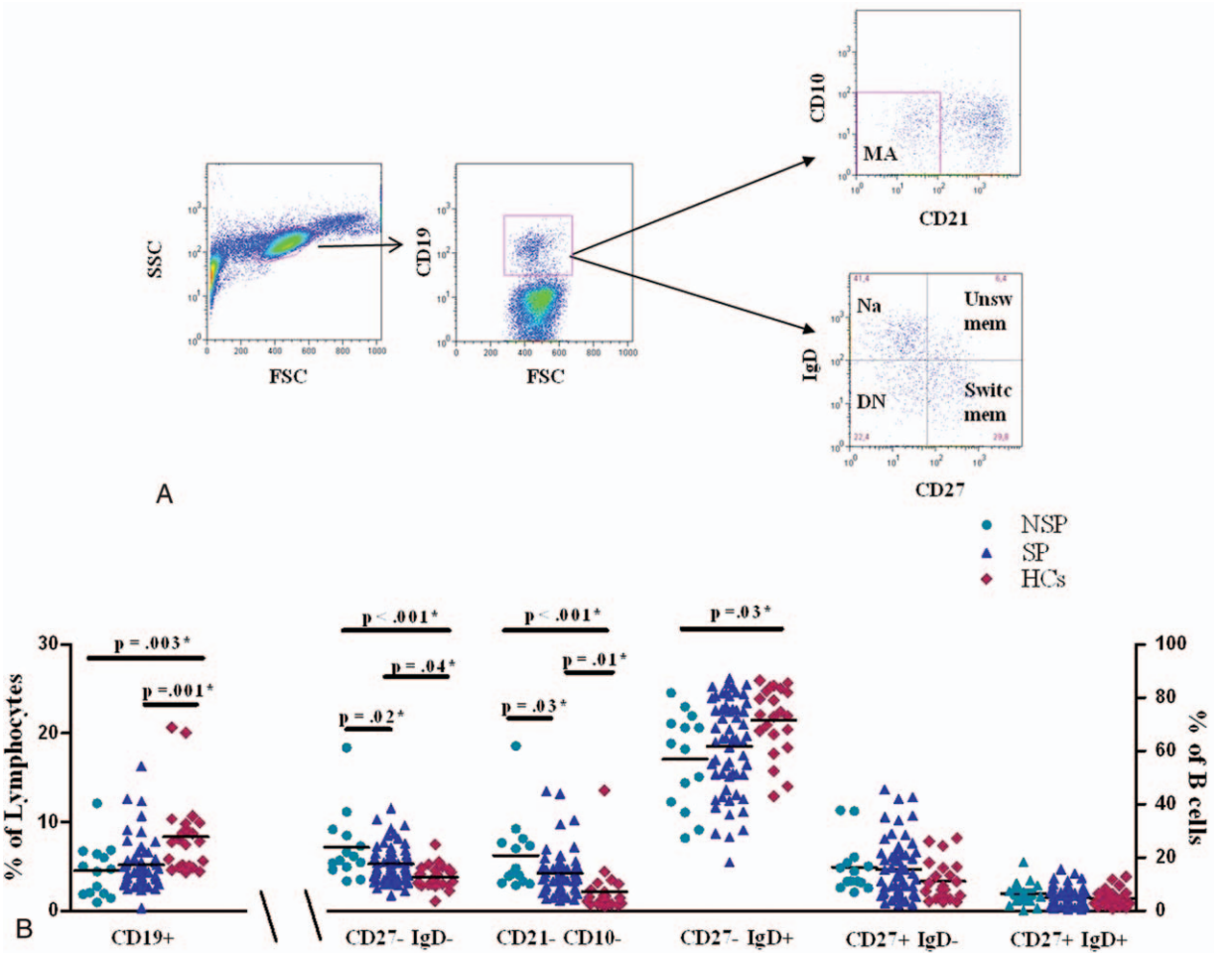

Figure 2. B-cell phenotype of HCs, SP, and NSP patients. (A) Gating strategy for the identification of the different B-cell subpopulations. (B) Scatter plot analyses on the differences of B-cell subpopulations among groups are shown. Only significant *P* values ($P<0.05$) have been reported in the figure. CD19+ cells established the B-cell population and expression of CD27, CD21, IgD, and CD10 was used to define total naïve (CD27− IgD+), switched memory (CD27+ IgD−), double negative (CD27− IgD−), unswitched memory (CD27+ IgD+), and mature activated (CD21− CD10−). FSC = forward scatter, HCs = healthy controls, NSP = nonseroprotected patients, SP = seroprotected patients, SSC = side scatter.

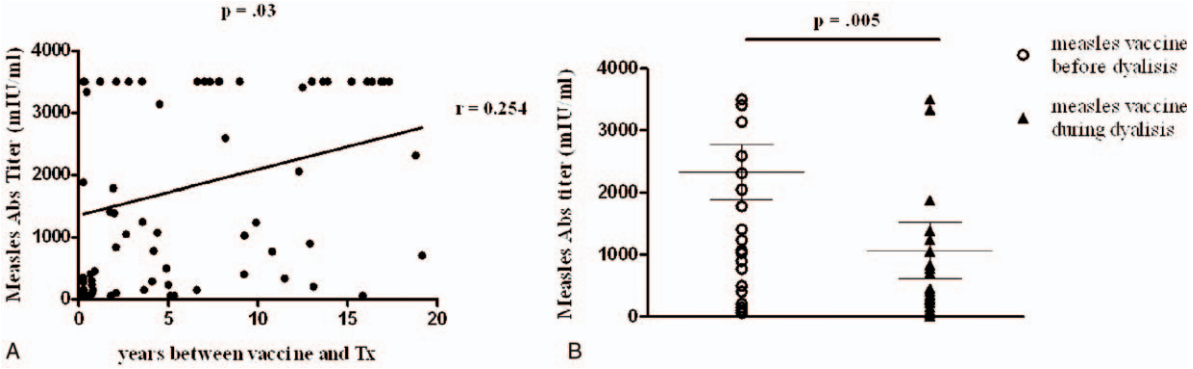

Figure 3. Clinical conditions influencing measles vaccine-induced immunity. (A) Correlation analyses (Spearman test) between measles antibody titers and years elapsed between vaccine and transplant. (B) Aligned dot plot between measles antibody titers in Tps vaccinated during dialysis and in Tps vaccinated before dialysis. Tps = kidney transplant patients.

transplant ($P=0.03$), indicating that patients transplanted close to vaccination had lower measles antibody titer compared with patients vaccinated earlier before transplantation (Fig. 3A).

Finally, we found that patients vaccinated during dialysis show a lower level of measles antibody titer after transplantation than patients vaccinated before dialysis ($P=0.005$) (Fig. 3B).

4. Discussion

Vaccination is certainly among the most effective clinical interventions to prevent infectious diseases. However, the immune responses obtained following vaccination in immunocompromised children are likely inadequate. The ability of this population to respond to vaccinations depends on immune-genetic factors, but also on the degree of immunologic impairment at the time of immunization.[19] With steadily improving survival rates due to the availability of novel therapeutic tools, the population composed of immunocompromised children with special vaccination needs is burgeoning.[20] In parallel, many uncertainties remain about the optimal strategies for identifying susceptible individuals, and to offer them sustained protection through an appropriate immunization schedule, both in terms of timing and number of vaccine doses. Thus, a deeper knowledge of the immunological basis for revaccination is necessary in such susceptible patients. Understanding the factors that regulate the development of protective immune responses is essential for rationally devising novel vaccination strategies against old and emerging infections, particularly in a large group of immunocompromised patients such SOT patients, in whom maintenance of protective immunity remains a major clinical challenge.

Life-long-lasting serological response following immunization with the measles live attenuated vaccine has been clearly reported in healthy population,[21] whereas few data are available in immunocompromised patients.

Warmington et al demonstrated that after 3 to 6 months following a kidney, heart, or liver transplantation, loss of antibodies occurred in 4 of 18 children (22.2%) who were seropositive for measles prior to transplantation.[22]

In this study, 19% of Tps lost protective antibody titers previously acquired following measles vaccinations, whereas all HCs retained antibodies against measles. This high percentage of NSP should alert clinicians and caregivers. Indeed, the reduced overall population measles coverage in Europe below the 95% minimum, necessary for eradication, allows the dissemination of the disease to high-risk population such transplanted individuals.[23] Particularly, in Italy, MMR vaccination coverage with 2 doses dropped from 90.6% to 88.3% between 2010 and 2013. Noteworthy, between January and December 2014, Italy experienced the highest incidence of measles in Europe, with a total of 1676 cases (28.1 cases per million).[24]

Measles infection may be complicated by pneumonia or encephalitis and can became disseminated with significant mortality in immunocompromised transplanted patients.[25,26] Severe forms of measles despite vaccination have been associated with suboptimal immunity to vaccination and previous history of immunosuppressive therapy or underlying immune deficient condition.[27] In addition, current serological markers predictive of vaccine-induced protection are only partially informative in immunocompromised population[13] and achievement of a protective titer does not always correlate with true protection from infection.[28] In this context, the investigation of additional parameters should be carefully considered in order to define more comprehensive immune correlates in immunocompromised populations. In line with this, the analysis of the antibody avidity and frequencies of measles-specific B-cell memory responses trough ELIspot could represent informative additional assays in patients being treated with immunouppressive therapies.

Avidity can be defined as the strength of the complex between an antigen and an antibody[29] and it rises after a natural infection or a vaccination because of the progressive increase in the amount of more specific antibodies (high affinity antibodies [HAA]). HAA are indeed associated with a better and stronger protection against the reinfection as they are more effective than Low Affinity Abs and they are also correlated with the development of a strong adaptive response.[30] We found that measles-specific IgGs from NSP patients present a significant lower avidity compared with those isolated from SP children. In contrast with recent data produced in children affected by rheumatic diseases under immunosuppressive therapy,[31] avidity data from this study suggest that Tps may present an overall impairment of the antibody functionality.

In addition, a significant percentage of Tps presents a lower frequency of antibody-secreting cells to measles compared with HCs group. Similarly, recent evidences reported reduced frequencies of MSMBC in children under treatment with immunomodulating agents such as methotrexate or anti-TNFα.[31] The lower frequency of MSMBC can be also partially

explained by the underlying alterations reported into the B-cell compartment of these patients. Indeed, when being challenged by the chronic presence of a not-self antigen (the transplanted organ), the immune system is forced to be over-activated. This condition can cause the premature aging of the system itself and several abnormalities in the distribution of the B cell subpopulations which have been further related to an altered ability to maintain of protective vaccine-induced immunity after vaccinations.[12,17,18,32] In agreement with these data, in the present study, the waning of protective vaccine-induced immunity, previously acquired through immunization, was directly related to the degree of immune senescence.

Taken together, these data indicate that a significant portion of patients receiving an SOT loses vaccine-induced serological and cellular-specific memory response to measles.

Additional analyses were further performed in this cohort to identify whether timing of immunization and dialysis could influence the maintenance of specific measles immunity over time. We found a positive correlation between the antibody titer and the time elapsed between vaccination and transplant. Indeed, patients transplanted close to vaccination had lower titers of specific antibodies compared with patients vaccinated earlier before transplantation. In particular, 7 out of 14 NSP were transplanted within the 1st year after vaccination. This finding suggests a higher negative impact of the immunosuppressive therapy when started before the MSMBC pool is fully established.[31] In addition, other immunological factors belonging to the T cell compartment directly affecting the establishment of long-term memory responses should be considered. In line with other authors, we previously showed in this cohort a progressive impairment of T-follicular Helper cells.[17] This subset was found to be crucial for the T–B interaction within the germinal center and to develop and maintain a memory response.[33,34] Taking into account hemodialysis (HD), we observed that patients being vaccinated while undergoing HD tended to wane protective antibody titers over time. According with several other authors showing a deep immune dysregulation in patients under dialysis we hypothesize a direct role of such treatment with long-term maintenance of memory responses.[35–38] Indeed, as reported by Griveas et al, HD as well as continuous ambulatory peritoneal dialysis leads to a significant reduction in the frequencies of T and B cell subsets.[38] In addition, qualitative dysfunctions both in terms of altered activation and proliferation of T-lymphocytes and of antigen-presenting cells have been reported in these patients.[35] Accordingly, several studies frequently reported an impaired responsiveness to standard vaccinations in patients undergoing HD.[39] Schulman et al reported that only 70% of dialysis patients developed protective titers to measles.[40]

4.1. Study limitations

To the best of our knowledge, the present study represents the largest evaluation of measles' vaccine-induced immunity maintenance in kidney transplanted patients. Nevertheless, some limitations should be reported. First, this is a cross-sectional observational study presenting all the general limitations for this type of study design.[41] Secondly, the sample size does not allow to draw definitive conclusions. Consequently, these results await confirmation from further larger studies. Thirdly, the high rate of unprotected individuals in the Tps group has not been evaluated for the effective risk of developing measles disease in a long-term follow-up.

5. Conclusions

In this study, we report as Tps present a significant waning of measles vaccine-induced immunity. Furthermore, these data suggest as the degree of immunologic impairment at the time of immunization should be taken into account in the future effort to define a personalized vaccination schedule for solid organ transplant recipients. In this context, either time elapsed between vaccination and transplant than dialysis on immune memory responses appear to be crucial.

Further studies are needed in order to link these evidences with clinical outcomes and risk of developing diseases in this population.

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