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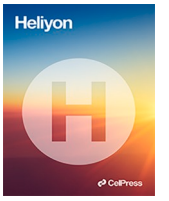

Research article

Humoral and cellular immune response after mRNA SARS-CoV-2 vaccine in children on treatment for cancer: A pilot observational study

Angela Mastronuzzi [a,*,1], Rita Carsetti [b,1], Maria Antonietta De Ioris [a], Chiara Agrati [a], Giada Del Baldo [a,c], Cristina Russo [d], Maria Giuseppina Cefalo [a], Pietro Merli [a], Carlo Federico Perno [d], Vito Andrea dell'Anna [a], Annalisa Serra [a], Veronica Bordoni [a], Eva Piano Mortari [b], Valentina Marcellini [e], Christian Albano [b], Giulia Linardos [d], Valentino Costabile [d], Matilde Sinibaldi [a], Marika Guercio [a], Stefano di Cecca [a], Concetta Quintarelli [a], Franco Locatelli [a,f]

[a] Department of Pediatric Haematology and Oncology, and Cell and Gene Therapy Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[b] B cell Unit, Immunology Research Area, IRCCS Bambino Gesù Children's Hospital, Rome, Italy
[c] Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy
[d] Multimodal Research Area, Unit of Microbiology and Diagnostics in Immunology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[e] Research Biobank, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy
[f] Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy

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ABSTRACT

Immunocompromised children are at risk of developing severe COVID-19 infection. We conducted a pilot prospective study to evaluate the impact of cancer treatment and stem cell transplantation on immunogenicity of two doses of BNT162b2 vaccine in pediatric patients.

Humoral, B- and T-cell responses to the BNT162b2 vaccine were assessed before, after the first and the second dose in patients aged 5–12 years (n = 35) and in a group of healthy donors (HD, n = 12). Patients were divided in three groups: solid tumors (ST, n = 11), hematological malignancies (HM, n = 14) and Hematopoietic Stem Cell Transplantation (HSCT) recipients (n = 10). After two vaccine doses, the seroconversion rate was 79.3 % (72.7 % in ST, 66.7 % in HM and 100 % in HSCT). The antibodies production was not associated to the presence of memory B and T-cells. Memory B-cells were measurable in 45.5 % ST, 66.6 % HSCT and in 22.0 % HM. The specific T-cell response was observed in most ST (81.8 %) and HSCT (85.7 %) patients and at lesser extent in those with HM (55.5 %). The combination of all immunological parameters (antibodies, memory B and T cells) showed that a significant fraction of HM (33.3 %) and ST (18.2 %) patients completely failed to respond to vaccination. Although able to produce antibodies, 11.1 % of HM and 27.3 % of ST had no B- and T-cell memory. HSCT subgroup showed the best immune function, with 80 % complete response and optimal T-cell function.

Combination of anti-RBD antibody, and specific memory B- and T-cell responses represents a reliable read-out of vaccine immune efficacy in frail patients.

* Corresponding author.
E-mail address: angela.mastronuzzi@opbg.net (A. Mastronuzzi).
[1] Angela Mastronuzzi and Rita Carsetti contributed equally to this work.

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1. Introduction

In cancer children, coronavirus disease 2019 (COVID-19) has been associated with an increased risk of hospitalization and death compared with the general population, as well as of discontinuation of treatment [1–4], [5], [6,7]. Vaccination has saved millions of lives in populations at risk of developing severe COVID-19 disease (i.e. elderly, immunocompromised, and cancer patients) [8], thus representing the best strategy to prevent disease severity. The BNT162b2 vaccine (Pfizer BioNTech) was authorized for use in children after 5 years of age [9,10], but patients diagnosed with solid tumor (ST) and hematological malignancies (HM) were not included in the registration trials. Several studies on frail adult patients reported highly variable immunogenicity, with the worst immune response observed in HM patients and those treated with B-cell targeting therapy [11–17].

Vaccination is generally not recommended during treatment for pediatric cancer and early after an autologous or allogeneic hematopoietic stem cell transplantation (HSCT). However, COVID-19 vaccination of children affected by HM, ST or recipients of HSCT is of fundamental importance in order to reduce the impact of the severity of SARS-CoV-2 infection [18].

Few data on the immunogenicity of BNT162b2 vaccine are available both in immunocompetent and immunocompromised children [19,20].

In particular, the degree and duration of vaccine-induced immunogenicity in immunocompromised children has been often limited to the analysis of the humoral immune response [21,22]. Few studies described both humoral and T cell response in frail children [23,24], but failed to give definitive results. Nowadays, data comparing the entire immune response to the SARS-CoV2 vaccine in different pediatric frailty contexts is not yet available.

The aim of this study was to compare the immunogenicity of two doses of the BNT162b2 vaccine in different groups of immunocompromised children (solid cancer, hematological malignancies, and HSCT recipients) through the evaluation of the three main players of the vaccine-induced immune response: the anti-RBD antibody titer, the frequency of vaccine-specific B and T cells. With this aim, we conducted a prospective pilot study at the Bambino Gesù Pediatric Hospital, Rome, Italy.

2. Materials and methods

2.1. Study design

Between January 2022 and March 2022, a prospective observational pilot study was planned to assess humoral and B- and T-cell response to SARS-CoV-2 vaccination in patients aged >5 and < 12 years with ST, HM and HSCT recipients. Healthy children (HD) matched for sex and age were used as control group. All patients received two doses of the BNT162b2 vaccine. Children within ST and HM groups were on treatment and had received chemotherapy in the last 30 days, whilst those in the HSCT group received transplantation at least 100 days before vaccination.

All patients and controls were sampled at three-time points: i) the day of the first dose administration (T0); the day of the second dose administration (21 ± 2 days from the first dose, T1), and iii) after 35 ± 2 days from the first dose (T2).

The endpoints of the study were: i) the assessment of the anti-receptor-binding domain (RBD) seroconversion rate at T1 and T2 in frail pediatric groups compared to HD; ii) evaluation of the impact of different frailties on the anti-RBD antibody levels and the specific response of the B and T memory cells after vaccination.

Inclusion criteria were: diagnosis as above, age >5 and < 12 years, SARS-CoV-2 mRNA full vaccination cycle, and life expectancy of at least 12 months at the time of vaccination. Exclusion criteria were: no chemotherapy course in the last 30 days, no transplantation in the last 100 days before vaccination, primary immunodeficiency.

The study was approved by the Internal Review Board (IRB) of the Bambino Gesù Children's Hospital. All investigations were conducted in accordance with the principles expressed in the Declaration of Helsinki.

2.2. Laboratory procedures

Anti-Spike SARS-CoV-2 antibodies and spike-specific B- and T-cell responses were monitored at T0, T1, and T2 in all enrolled patients and in HD. All the immunological analyses were performed during the planned evaluation for oncological treatment.

2.3. Humoral antibody response

The response to vaccination was assessed by quantifying the anti-RBD-IgG (Abbott Alinity anti Sars-Cov-2 IgG II Quantitative assay, Abbott Diagnostics, Chicago, IL). Anti-RBD-IgG were expressed as BAU/mL and values were considered positive when ≥ 50.

To define the seroconversion rate, patients, and HD with previous exposure to SARS-CoV-2 infection (identified by the positivity to a molecular/antigenic test or to anti-N antibodies) were excluded from this analysis to highlight the effect of vaccination in a *naïve* population. The median level of anti-RBD antibodies (Abs) was calculated using values from all patients (*naïve* and previously infected).

The anti-N quantification was performed to identify asymptomatic SARS-CoV-2 infections before or during the follow-up. Anti-N IgG were expressed as a ratio (S/CO) and values were considered positive when ≥ 1.1.

2.4. Cell isolation and cryopreservation

Whole blood was collected at all time points (T0, T1, and T2). Peripheral blood mononuclear cells (PBMCs) were isolated by standard density gradient centrifugation (Ficoll Paque™ Plus 206, Amersham PharmaciaBiotech) and immediately stored in liquid nitrogen until use.

2.5. Detection of antigen-specific B cells

For the detection of SARS-CoV-2 specific memory B cells, biotinylated protein antigens were individually multimerized with fluorescently labeled streptavidin as previously described [25]. Separate aliquots of recombinant biotinylated spike were mixed with streptavidin BUV39 or streptavidin PE at 25:1 ratio 20:1 ratio respectively. Streptavidin PE-Cy7 (BD Bioscience) was used as a decoy probe to gate out SARS-CoV-2 antigen non-specific streptavidin-binding B cells. The antigen probes individually prepared as above were then mixed in Brilliant Buffer (BD Bioscience). 4×10^6 previously frozen PBMC samples were prepared and stained with the antigen probe cocktail containing 100 ng of Spike per probe (total 200 ng), and 2 ng of streptavidin PE-Cy7. After one step of washing, surface staining with antibodies was performed in Brilliant Buffer at 4C for 30 min.

B-cell subsets were identified based on the expression of CD19, CD27, CD24, and CD38 markers by flow-cytometry. Memory B cells (MBCs) were defined as $CD19^+CD24^+CD27^+$. Spike-specific MBCs were defined as $CD19^+CD24^+CD27$ + PE + BUV395+ (Spike double positive, S++). Stained PBMC samples were acquired on FACs LSRFortessa (BD Bioscience). The gating strategy is described in Supplementary Figure 1.

At least 2×10^6 cells were acquired and analyzed using Flow-Jo10.7.1 (BD Bioscience). Phenotype analysis of antigen-specific memory B cells was only performed in subjects with at least 10 cells detected in the respective antigen-specific gate. Blank was determined in unexposed donors, before vaccination. LOB (limit of blank) was set as the mean of the blank + 1.645xSD. LOD (limit of detection) as the mean of the blank + 3xSD or the LOB + 1.645xSD. LOS (limit of sensitivity) was set as the median + 2xSD of the results in unexposed donors before vaccination. The intra-subject biological coefficient of variation (CV) and the inter-subject biological coefficient of variation values were calculated on 10 replicates each. The percent of CV for intra-assay was 3.7 % and for inter-assay was 5.2 %.

2.6. Detection of antigen-specific T cells

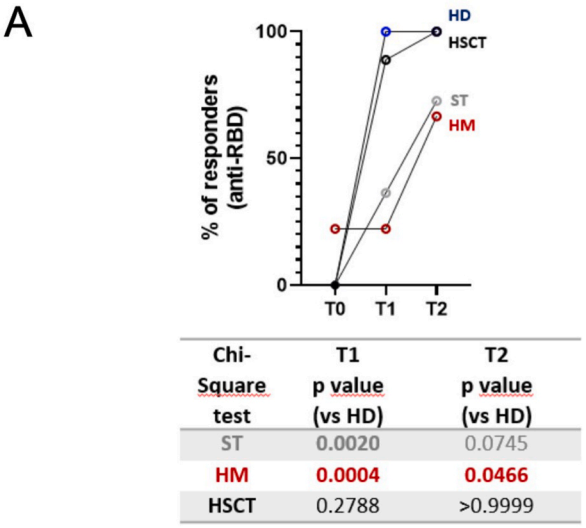
Peripheral Blood Mononuclear Cells (BMCs) from patients and controls were thawed and seeded in 96-well cell plates at 1×10^6 cells/well in RPMI 1640 culture medium containing 10 % AB human serum. PBMCs were stimulated for 5 h at 37 °C, 5 % CO2, with a pool of SARS-CoV-2 viral peptides (PepTivator® SARS-CoV-2 Prot_S, Miltenyi Biotech, Bergisch Gladbach, Germany) at the final concentration of 1 μg/mL in the presence of anti-CD49d/anti-CD28 (2 μg/mL) (Becton Dickinson). As positive control, PBMC were stimulated with CytoStim® (Miltenyi Biotech, Bergisch Gladbach, Germany) and, as negative control, PBMC were maintained in the culture medium supplemented with anti-CD49d/anti-CD28 co-stimulation. After 1-h stimulation, brefeldin A (Miltenyi, Biotech, Bergisch Gladbach, Germany) was added to the cells for 4 h to inhibit the transport of proteins to the cellular membrane. At the end of the stimulation, cells were fixed, permeabilized, and stained with anti-CD3, IFN-γ, and CD154 (CD40L). Stained PBMC samples were acquired on FACS Fortessa (BD Biosciences). The frequency of Spike-specific T cells was quantified as IFN-γ+ among CD3 T cells. Moreover, we also analyzed IFN-γ+CD40L + specific T cells. The gating strategy is described in Supplementary Figure 1.

Table 1
Characteristics of frail patients. Solid Tumor, ST; Central Nervous System, CNS; Hematologi-cal Malignancies, HM; Acute Lymphoblastic Leukemia, ALL; Acute Myeloid Leukemia, AML; Non Hodgkin Lymphoma, NHL; Hematopoietic Stem Cell Transplantation (HSCT) recipients; Matched Unrelated Donor, MUD; Matched Familial Donor, MFD.

		N°	Previous COVID-19	Age	Gender (M/F)
HD		12	2	8.7 ± 2.2	5/7
Frail patients		36	6	8.1 ± 2.0	20/16
ST		11	0	9.8 ± 1.9	3/8
	CNS tumor	7			
	Sarcoma	5			
HM		14	5	7.6 ± 1.8	9/5
	ALL	12			
	AML	1			
	LNH	1			
HSCT		10	1	8.1 ± 2.0	8/2
	MUD	4 with ATLG (+2 with abatacept)			
	MFD	3			
	Haploidentical	3 ex-vivo T cell-depletion (TCRαβ/CD19)			

2.7. Statistical methods

Quantitative variables were summarized using median and Interquartile Range (IQR), while categorical variables were reported using absolute counts and percentage. Differences in seroconversion rate across subgroups were tested using the chi-square test. Differences between immunological quantitative variables (anti-RBD Abs, B- and T–cell response) before and after vaccination within the same group was evaluated by Wilcoxon paired test. Differences across groups were evaluated by Mann–Whitney *t*-test. Statistical



Chi-Square test	T1 p value (vs HD)	T2 p value (vs HD)
ST	0.0020	0.0745
HM	0.0004	0.0466
HSCT	0.2788	>0.9999

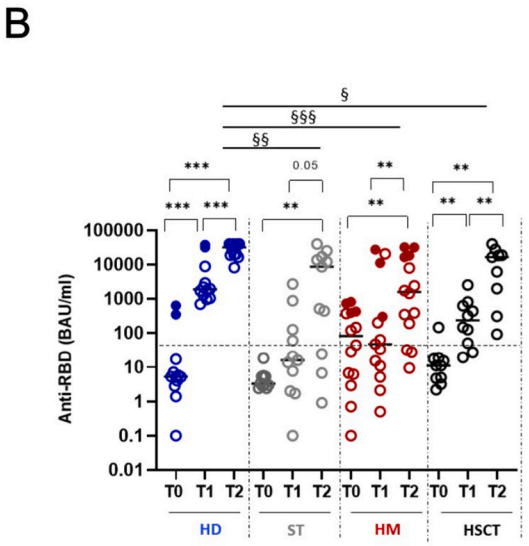
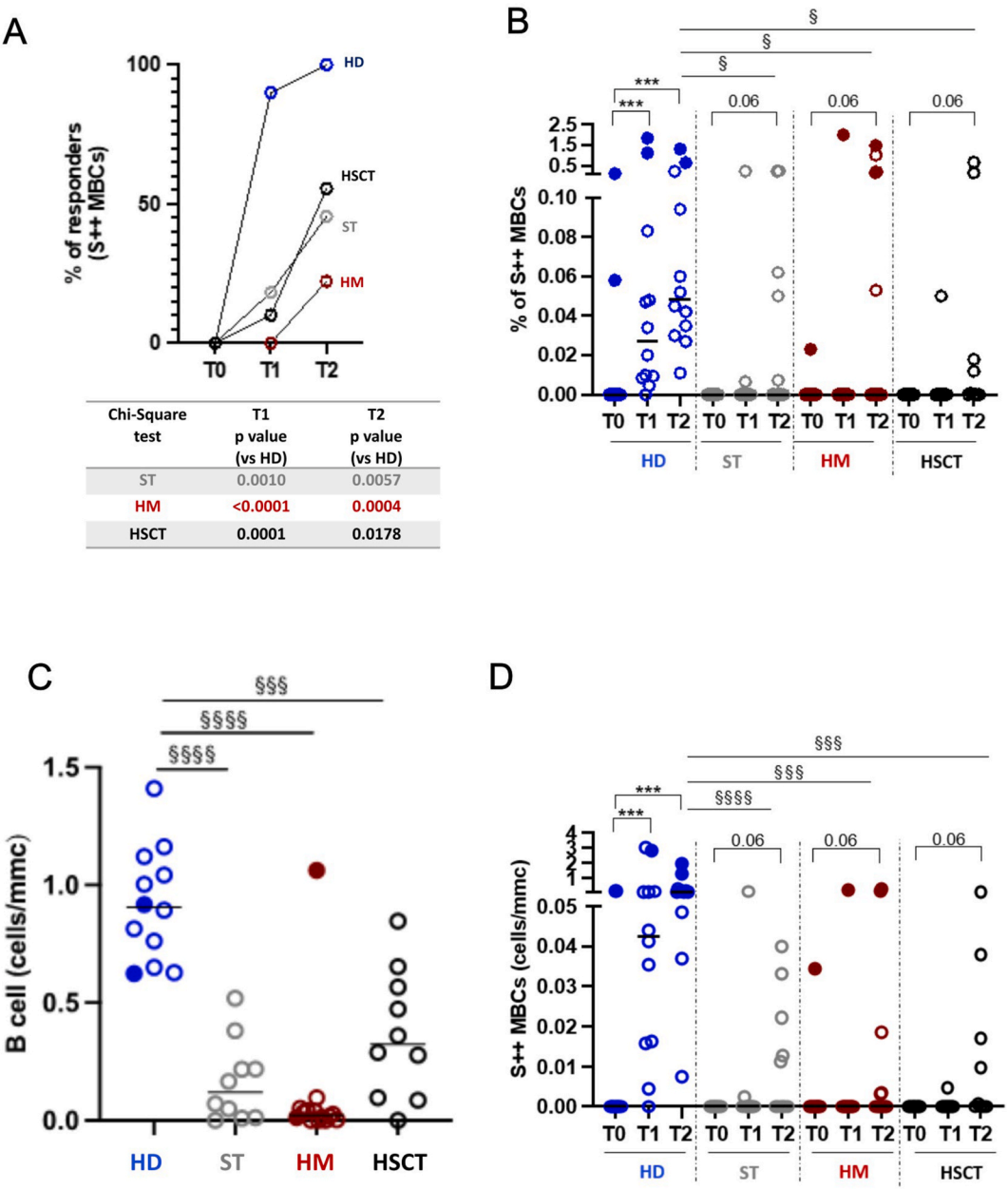


Fig. 1. Kinetic of anti-RBD response in different patient groups

A, The percentage of patients [HD, blue dots, (n = 10); ST, grey dots (n = 11); HM, red dot (n = 9); HSCT, black dots (n = 10)] presenting a positive anti-RBD response (50 BAU/mL) at each time point (T0, T1, and T2) is shown. The table below the graph reports statistical data (chi-square test) about the comparison of the seroconversion rate between each frail group and HD.

B, Kinetics of humoral immune response before and after vaccination in HD (blue dots, n = 12), ST (grey dots, n = 11), HM (red dot, n = 14), and HSCT (black dots, n = 10). Anti-RBD Abs are expressed as BAU/mL and values higher than 50 BAU/mL are considered positive. Differences were evaluated by Wilcoxon paired test. ***p < 0.001. The level of anti-RBD Abs at T2 was compared among groups (Mann-Whitney *t*-test §p < 0.05, §§p < 0.01, §§§p < 0.001) and was expressed as BAU/mL. Full dots depict subjects with a previously reported SARS-CoV-2 infection.

Abbreviations: Abs, antibodies; HD, healthy donors; ST, solid tumors; HM, hematological malignancies; HSCT, Hematopoietic stem cell transplantation; IQR, interquartile range; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome 2 virus; T0, before vaccination; T1, after 3–4 weeks from T0; T2, 5–8 weeks from T0. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



Chi-Square test	T1 p value (vs HD)	T2 p value (vs HD)
ST	0.0010	0.0057
HM	<0.0001	0.0004
HSCT	0.0001	0.0178

Fig. 2. Kinetic of Spike specific B cells response in different group

A, The percentage of *naïve* HD (blue dots, n = 10), ST (grey dots, n = 11), HM (red dots, n = 9) and HSCT (black dots, n = 10)] presenting a positive S++ MBC response at each time point (T0, T1, and T2) is shown. The table below the graph reports statistical data (chi-square test) about the comparison of the percentage of positive S++ MBC response between each frail group and HD.

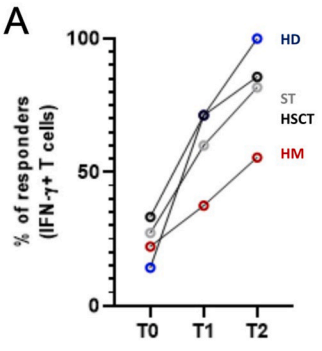
B, Kinetics of S++ MBC response before and after vaccination in HD (blue dots) ST (grey dots), HM (red dot), HSCT (black dots). Differences were evaluated by Wilcoxon paired test. *$p < 0.05$, ***$p < 0.001$. The frequency of S++ MBC response at T2 was compared among groups (Mann-Whitney *t*-test $^{§}p < 0.05$) and was expressed as a percentage of MBCs.

C, The absolute number of $CD19^{+}$ B cells in HD (blue dots) ST (grey dots), HM (red dot), and HSCT (black dots) is shown.

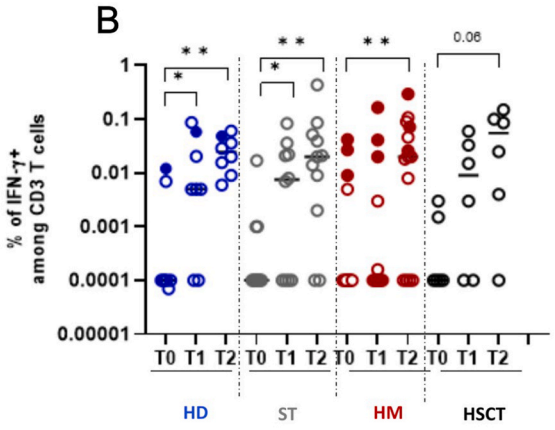
D, The absolute number of S++ MBC cells in HD (blue dots, n = 12); ST (grey dots, n = 11); HM (red dot, n = 14); HSCT (black dots, n = 10) at each time point (T0, T1, and T2) is shown. The absolute number of S++ MBC responses at T2 was compared among groups (Mann-Whitney test $^{§}p < 0.05$, $^{§§§}p < 0.001$, $^{§§§§}p < 0.0001$) and was expressed as S++ MBCs cells/mmc.

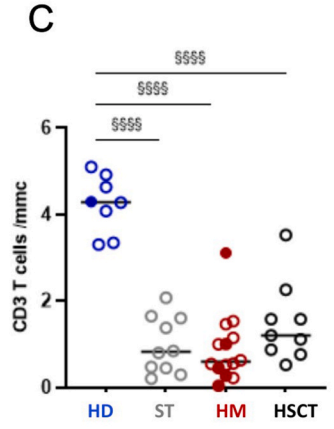
(B–D) The full dot represents the subjects who had previously reported SARS-CoV-2 infection.

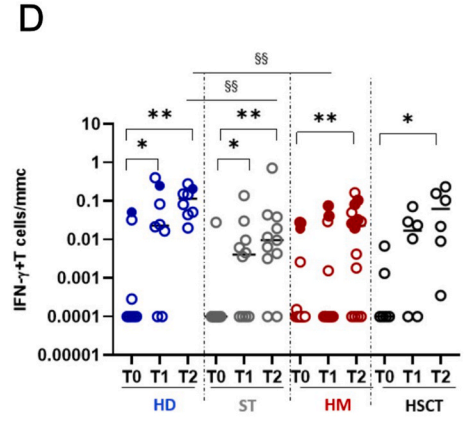
Abbreviations: S++ MBC, High-affinity Spike-specific memory B cells; HD, healthy donors; ST, solid tumors; HM, hematological malignancies; HSCT, Hematopoietic stem-cell transplantation; IQR, interquartile range; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome 2 virus; T0, before vaccination; T1, after 3–4 weeks from T0; T2, 5–8 weeks from T0. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



Chi-Square test	T1 p value (vs HD)	T2 p value (vs HD)
ST	0.9493	0.2315
HM	0.1888	**0.0417**
HSCT	0.9999	0.2994







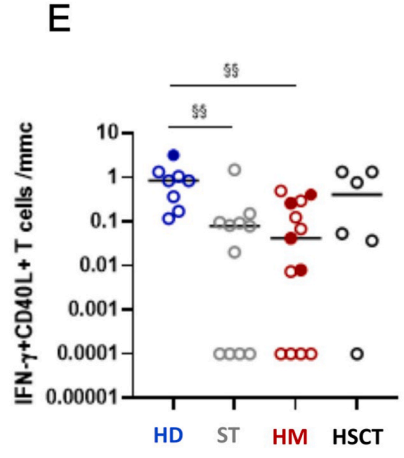


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Fig. 3. Kinetic of Spike specific T cells response in different group
A, The percentage of HD (blue dots, n = 7); ST (grey dots, n = 11); HM (red dot, n = 9); HSCT (black dots, n = 6) presenting a positive S-specific T cells response at each time point (T0, T1, and T2) is shown. The S-specific T cell response was evaluated as T cells producing IFN-γ. The table below the graph reports statistical data (chi square test) percentage of positive S-specific T cell response between each frail group and HD.
B. Kinetics of S-specific T cells response before and after vaccination in HD (blue dots, n = 8), ST (grey dots, n = 11), HM (red dot, n = 14), HSCT (black dots, n = 6) is shown. Differences were evaluated by Wilcoxon paired test. *$p < 0.05$, **$p < 0.01$.
C, The absolute number of $CD3^+$ T cells in HD (blue dots, n = 8), ST (grey dots, n = 11), HM (red dot, n = 11), HSCT (black dots, n = 6) at T0 is shown. Difference between groups were evaluated with Mann-Whitney *t*-test $^{§§§§}p < 0.0001$.
D, The absolute number of S-specific T cells in HD (blue dots, n = 8), ST (grey dots, n = 11), HM (red dot, n = 11), HSCT (black dots, n = 6) at each time point (T0, T1, and T2) is shown. Differences were evaluated by Wilcoxon paired test. *$p < 0.05$; **$p < 0.01$. The absolute number of S-specific T cells at T2 was compared among groups (Mann-Whitney t-tes); $^{§§}p < 0.01$) and was expressed as IFN-γ+$CD3^+$ cells/mmc.
E, The absolute numbers of IFN-g-producing CD40L + T cells in all groups. Difference were evaluated with Mann-Whitney *t*-test $^{§§}p < 0.01$.
(B–E) The full dot shows HD and patients with a previously reported SARS-CoV-2 infection.
Abbreviations: HD, healthy donors; ST, solid tumors; HM, hematological malignancies; HSCT, hematopoietic stem-cell transplantation; IQR, interquartile range; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome 2 virus; T0, before vaccination; T1, after 3–4 weeks from T0; T2, 5–8 weeks from T0. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

analyses were performed using GraphPad Prism v8.0 (GraphPad Software, Inc., San Diego, CA). To visualize a correlation matrix in R, we used the corrplot function and generate a Heatmap object using correlation coefficients (computed using the Spearman) as input to the Heatmap. The heatmap was produced with the R package heatmap3.

3. Results

3.1. Patient characteristics

Thirty-five pediatric patients aged >5 and < 12 years who received the BNT162b2 vaccine between January and March 2022. The patients were divided into three groups, namely: ST (n = 11), HM (n = 14), or recipients of HSCT (n = 10). The control group HD (n = 12) included children matched for age and sex. Patient characteristics are described in Table I. No major side effects were observed, while local side effects were reported in less than 50 % of the vaccines. No fever was reported. All patients in ST and HM received chemotherapy, only one patient with non-Hodgkin lymphoma received rituximab while none received blinatumomab or inotuzumab.

3.2. Humoral response to vaccination

The first dose of vaccine is able to induce the anti-RBD seroconversion in all HD (100 %) and in the large majority of HSCT recipients (88.9 %). By contrast, a significantly lower percentage of ST (36.4 %, $p = 0.0020$) and HM patients (22.2 %, $p = 0.0004$) seroconverted after one single vaccine dose. After the second dose, all HD and children recipients of HSCT seroconverted (100 %), whereas ST and HM patients maintained a lower seroconversion rate (72.7 % and 66.6 %, respectively), although not statistically different from HD (Fig. 1A).

We also measured the concentration of anti-RBD Abs in the different groups, also including patients with a previously reported SARS-CoV-2 infection (identified in the figure with full dots, Fig. 1B). All frail patients showed a significant increase in anti-RBD Abs levels after two vaccine doses, but the anti-RBD titer measured at T2 in each patient group was significantly lower than that observed in HD ($p < 0.05$ for all comparisons).

3.3. Spike-specific memory B cells

Spike-specific memory B cells (S++ MBCs) were present in 90 % of the HD after the first dose and in all of them after the second dose (Fig. 2A). By contrast, patients had a significantly reduced response rate after the first dose (18.2 % for ST, $p = 0.0010$; 10 % for HSCT, $p = 0.0001$) or no response at all, as in the case of HM patients ($p < 0.0001$). The second dose increased the B-cell response rate of patients to 45.5 % in ST and to 55.5 % in HSCT (Fig. 2A). The percentage of response of HM patients remained significantly lower that of HD ($p = 0.0177$) with only 22 % of the patients producing S++ MBCs (Fig. 2A).

In more detail, we found that S++ MBCs were detectable at T0 in two HD reflecting the immune response to a previous natural infection as also confirmed by the presence of anti-N-Abs in the serum. In the rest of the group, the frequency of S++ MBCs increased significantly after the first dose ($p = 0.001$) and further after the second (Fig. 2B). The increase of S++ MBCs after the second dose was close to significance in patients from all groups ($p = 0.06$, Fig. 2B). However, at T2 the frequency of S++ MBCs remained significantly lower in the patients than in the HD (Fig. 2B; all $p < 0.05$). As the underlying disease and the ongoing treatments may affect the development and survival of B lymphocytes in all patients group (Fig. 2C), we also calculated the absolute number of S++ MBCs cells before and after vaccination, which were significantly lower than in the HD in all groups of patients (Fig. 2D; all $p < 0.05$).

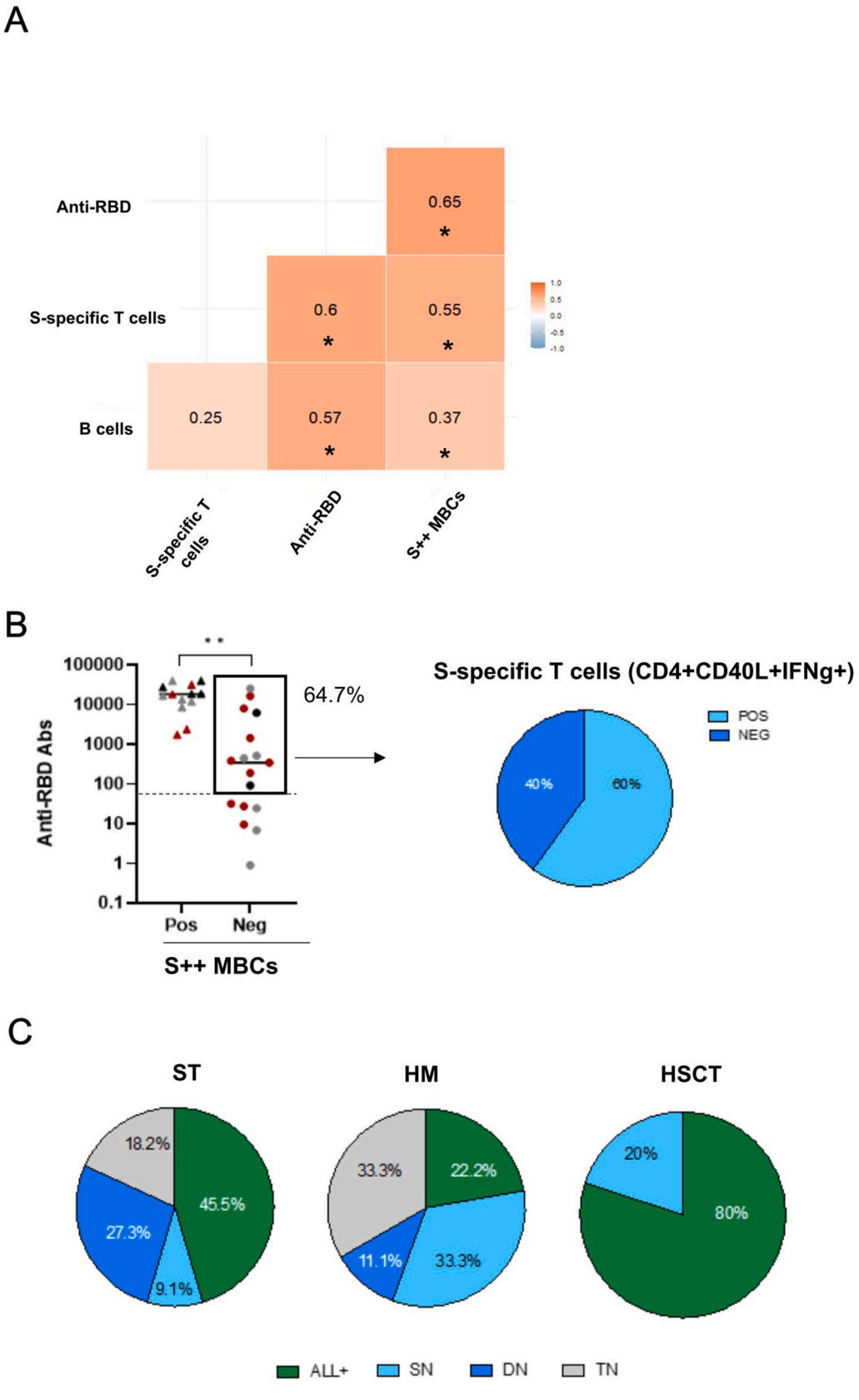

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Fig. 4. Interplay between the humoral and cell-mediated arms.
A, Correlation matrix across the frequency of CD19^{+} B cells, S++ MBCs, Spike specific T cells (IFN-γ+CD40L + T cells), and anti-RBD Abs titer from ST, HM, and HSCT at T2 are shown (n = 30). The significant correlations (<0.05) are indicated with an asterisk. Spearman correlation coefficients are reported.
B, Frequency of frail patients (n = 30) with S++ MBCs response at T2 in relationship with anti-RBD titer (left panel). The frequency of Spike-specific T cells in patients with a positive anti-RBD and negative S++ MBCs response is shown in the right panel.
C, Pie charts illustrating the proportion of triple-negative (negative for anti-RBD, B and T cell response, TN, grey slice), double negative (positive for anti-RBD and negative for B and T cell response, DN, blue slice), single negative (positive for anti-RBD and negative for B cell response, SN, light blue slice) and positive for all response (anti-RBD, B and T cell response, green slice) are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.4. Specific T-cell response to vaccination

To study the impact of different frailties on T-cell immunity, the kinetics of the spike-specific T-cell response before and after vaccination were evaluated in HD (n = 8), ST (n = 11), HM (n = 14) and HSCT (n = 6). The PBMC yield of the other enrolled subjects was too low and did not allow this analysis. Spike-specific IFNγ-producing T cells were detectable in 71.4 % of the HD after the first dose and in 100 % after the second dose (Fig. 3A). A similar rate of T-cell response was observed in ST (60 %, p = 0.9493) and in HSCT (71.4 %, p = 0.9999), while a lower frequency of HM children with a positive T-cell response was observed (37.5 %, p = 0.1888). The second dose increased the response rate of patients to 81.8 % in ST (p = 0.2315) and 85.7 % in HSCT (p = 0.2994), while the percentage of response of HM patients persisted to be significantly lower than HD (55.5 %, p = 0.0417) (Fig. 3A).

Before vaccination, we reported the presence of Spike-specific T-cell response in two HD, three ST, four HM and two HSCT. This pre-vaccination response may be due to previous infections (full dots) and/or to a cross-immunity against other respiratory coronaviruses as well described. In HD, the frequency of S-specific T cells significantly increased after the first dose (p = 0.031) and expanded again after the second dose (Fig. 3B). ST patients exhibits a similar kinetics of vaccine response than the one of HD. In HM and HSCT groups, one single dose was not able to induce the increase of S-specific T-cell frequency. After the second dose, an increase of S-specific T-cell frequency was observed in HM patients (p = 0.004) and, at lesser extent in HSCT (p = 0.06, Fig. 3B).

As all groups of patients showed a significantly lower CD3^{+} T cell count (Fig. 3C), we also analyzed the absolute number of S-specific CD3+IFNγ+ cells. Results confirmed a significant lower number of specific T cells in ST and HM than in HD (Fig. 3D).

The adaptive immune response to vaccination requires the collaboration of B cells with T cells through the CD40L in the germinal centers which allow class-switching and affinity maturation. Moreover, the frequency of CD4 T cells expressing IFN-γ is associated with the persistence of MBCs specific to the spike protein and with high levels of SARS-CoV-2-neutralizing antibodies. Thus, we analyzed the absolute number of IFN-γ-producing CD40L + T cells. Results showed a significant reduction of these population in ST and HM compared to HD (p = 0.002 for both) (Fig. 3E).

Overall, these results showed that, although with large variability, two doses of vaccine were able to significantly increase the frequency of S-specific T cells in all patient groups that, however, persisted lower than in HD.

3.5. Coordinated immune response

In order to define the interplay between the humoral and cell-mediated arms of the immune response after vaccination in frail patients, we performed a correlation analysis using immunological data (anti-RBD Abs, S++ MBCs and IFN-γ+CD40L + T cells) from ST, HM and HSCT at T2 for whom we had all measurements and were naïve for SARS-CoV-2 infection. As shown in Fig. 4A, the frequency of CD19^{+} B cells correlated with anti-RBD Abs titer and with the frequency of S++ MBCs. Moreover, the titer of anti-RBD Abs correlated with the frequency of S++ MBCs, suggesting good coordination between plasma cells and memory B cells after vaccination. Of note, a positive correlation was also observed between S-specific T cell response and anti-RBD and S++ MBCs, highlighting an effective cross-talk between T and B cell compartments. The generation of high-affinity MBCs is essential to ensure a long-lasting immunity to SARS-CoV-2, and patients who failed to generate S++ MBCs cells may represent a subset more susceptible to further infections. We found that 56.7 % of fragile patients (17 out of 30 patients) were negative for S++ MBCs at T2, independently from the disease. These patients (S++ MBCs negative) had a lower titer of anti-RBD than the S++ MBCs positive patients Fig. 4B, p < 0.01), confirming the correlation described in Fig. 4A. Of note, 64.7 % of S++MBCs negative patients were still positive for anti-RBD Abs, suggesting that antibody production is not always paralleled by memory B-cell differentiation[31]. Moreover, 40 % of the S++MBCs negative patients anti-RBD positive patients also failed to expand S-specific T cells.

Based on the combination of antibodies, memory B cells and T cell measurements, we can identify triple negative (negative for anti-RBD, B and T-cell response, TN, grey slice) patients, but also double negative (positive for anti-RBD and negative for B and T-cell response, DN, blue slices) individuals and a group of patients that only lacks B cell memory (SN, light blue slices). As shown in Fig. 4C, the large majority of HSCT had a complete response to vaccination (All+, green slice); in contrast, a fraction of HM and to a lesser extent ST, showed a null response (TN, grey slice). By measuring only serum antibodies we would have identified the TN individuals (grey slice), but we would have ignored 44.4 % of HM and 36.4 % of ST patients lacking a memory T and/or B-cell response (blue + light blue slices).

Our data demonstrate that the anti-RBD antibody measurement alone cannot identify all patients who failed to generate immunological memory after vaccination.

4. Discussion

In this prospective observational study, after two BNT162b2 we observed a seroconversion rate of 76.7 %. This response is similar to that recently reported in older children with cancer, but lower compared to healthy children and adolescents [20,26–28]. All patients with HSCT produced specific Abs (100 % response rate), demonstrating that vaccination early after transplantation is highly effective in this group. A lower humoral response was measured in ST (72.7 %), and HM (66.6 %), as previously observed [17,26].

The effectiveness of vaccination depends not only on serum Abs, but also relies on the establishment of immune memory, embodied by persistent memory T and B cells and long-lived plasma cells [11]. Upon exposure to the pathogen, Abs produced by long-lived plasma cells neutralize the virus thus limiting infectivity, memory B-cells migrate to the site of infection and locally increase antibody concentration, whereas memory T-cells kill infected cells. Several evidence supports the role of T cells in protecting against severe COVID-19 in healthy and in fragile patients, including children [29,30]. Polyfunctional specific T cells were associated with rapid viral clearance and mild/asymptomatic infection [29,31] and cross-recognize different viral strains [32]. Thanks to immune B- and T-cell memory, vaccines prevent infection and severe disease [33].

We measured Spike-specific B and T memory cells in all the patients. After the second dose, S++ MBCs could be detected only in 42.8 % of patients included in our study, similar to what was reported in older cancer patients [20]. B-cell memory was most severely impaired in the HM patients (22 % response rate) mostly because of the impact of the underlying disease and the treatments on B-cell numbers. Among patients with ST, only 48 % developed S++ MBCs, suggesting that the anti-neoplastic treatments may reduce the proliferative expansion of B cells in the germinal centers that is indispensable for the generation of antigen-specific memory B cells [34]. The best response (55 %) was obtained in children with HSCT. The proportion of patients showing a positive T-cell response to vaccination was 66.7 %, with a significantly lower number of circulating Spike-specific T cells. Moreover, as observed for the B cell response, the more vulnerable patients were HM with a percentage of response of 55.5 %.

The strength of this study is the availability of different immunological data (anti-RBD Abs, Spike-specific memory B, and T cells), allowing us to define the impact of different frailties on all immunological arms. We found a positive correlation among anti-RBD, B, and T cell response, suggesting good coordination between the humoral and cell-mediated arms of the immune system. The differentiation of specific memory B cells was the most affected process in frail patients, occurring in 42.8 % of patients, whereas anti-RBD seroconversion was observed in 76.6 % and differentiation of T cells response in 66.7 %.

Of note, 64.7 % of patients who failed to induce S++ MBCs showed a positive anti-RBD response (although with a low titer), and 40 % of these patients also lacked the specific T cell response, demonstrating that anti-RBD antibody quantification is not sufficient to identify patients who may remain at risk for severe diseases. Abs induced by vaccination decline in time [35] but immune memory exerts a rapid response in case of infection by producing new Abs, migrating at the site of viral invasion, and killing infected cells [25]. In the absence of memory T and B cells, vaccinated patients will be unable to generate a recall response in the case of infection.

One/third of HM patients completely failed to respond to vaccination (TN, triple negative subjects), providing the immunological evidence of the deep frailty of these subjects and their high vulnerability to severe COVID-19. In contrast, children with HSCT showed the best immune function, with an 80 % complete response and an optimal T-cell function.

Few works have focused on the immune response to the COVID-19 vaccine in immunocompromised children. Many of them are limited to the study of the antibody response [21,22], and others present data in which different types of immunocompromision are analyzed as a single group [23]. Our study is the first comparing the whole vaccine immunity (anti-RBD and B and T cell response) among pediatric patients with different types of frailties.

However, our study has important limitations due to the small number of patients enrolled and to the heterogeneity. Nevertheless, our data are unique for this age group and demonstrate that both humoral and cellular immune responses can be elicited in a significant proportion of HSCT recipients and, to a lesser extent to ST ad HM subjects. The additional boosters in the vaccine schedule could improve the immunization in these frail patients, in particular in view of the emerging variants of SARS-CoV-2. Moreover, the experience with vaccinations against COVID-19 opens new horizon in term of immunization strategy in cancer patient on treatment.

5. Conclusion

In conclusion, we demonstrated an overall seroconversion rate of 76.6 % with a 100 % seroconversion in HSCT. HM represents the most vulnerable group, with 33.3 % who failed to induce any immunity. Finally, anti-RBD Abs quantification is not able to mirror the induction of a memory B- and T-cell immune response, essential for long-lasting protection. A combined immunological evaluation is necessary in frail population to identify subject at higher risk of severe COVID-19.

Data availability statement

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

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki and was ap-proved by the Institutional Review Board of Bambino Gesù Children's Hospital. Number: 638. Date of approval: May 30, 2023.

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CRediT authorship contribution statement

Angela Mastronuzzi: Writing – review & editing, Supervision, Conceptualization. **Rita Carsetti:** Writing – original draft, Data curation, Conceptualization. **Maria Antonietta De Ioris:** Writing – original draft, Data curation, Conceptualization. **Chiara Agrati:** Writing – original draft, Data curation, Conceptualization. **Giada Del Baldo:** Writing – review & editing, Visualization. **Cristina Russo:** Investigation, Data curation. **Maria Giuseppina Cefalo:** Project administration, Methodology. **Pietro Merli:** Methodology, Formal analysis. **Carlo Federico Perno:** Visualization, Supervision. **Vito Andrea dell'Anna:** Investigation, Data curation. **Annalisa Serra:** Visualization, Data curation. **Veronica Bordoni:** Data curation. **Eva Piano Mortari:** Data curation. **Valentina Marcellini:** Data curation. **Christian Albano:** Data curation. **Giulia Linardos:** Data curation. **Valentino Costabile:** Data curation. **Matilde Sinibaldi:** Data curation. **Marika Guercio:** Data curation. **Stefano di Cecca:** Data curation. **Concetta Quintarelli:** Writing – review & editing, Supervision, Data curation, Conceptualization. **Franco Locatelli:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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