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Peripheral lymphocyte subset counts predict antibody response after SARS-CoV-2 mRNA-BNT162b2 vaccine in cancer patients: Results from the Vax-On-Profile study

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ABSTRACT

Background: The adaptive immune response following COVID-19 vaccination is essential for humoral immunogenicity and clinical protection against symptomatic infections. We present the results of circulating lymphocyte profiling and their correlation with antibody response in cancer patients tested serologically six months after receiving a two-dose schedule of mRNA-BNT162b2 vaccine.

Methods: Absolute counts of lymphocyte subsets were determined using peripheral blood immunophenotyping. We collected samples for flow cytometry analysis alongside quantitative detection of IgG antibodies against the receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein (S1). An IgG titer ≥ 50 AU/mL defined a positive seroconversion response.

Results: 311 patients were evaluable for lymphocyte profiling and serologic testing. A preliminary multivariate analysis revealed that cytotoxic chemotherapy was the most consistent factor associated with lower counts of all lymphocyte subpopulations. T helper and B cells were found to be useful in predicting the occurrence of a positive seroconversion response using ROC curve analysis. A significant positive linear correlation was shown when anti-RBD-S1 IgG titers were compared to these lymphocyte subset counts. Univariate analysis indicated that antibody titers and seroconversion rates were significantly improved in the high-level T and B cell subgroups. Multivariate analysis confirmed these significant interactions, as well as the negative predictive value of immunosuppressive corticosteroid therapy.

Conclusions: These findings suggest that simple and widely available peripheral counts of T helper and B cells correlate with humoral response to mRNA-BNT162b2 vaccine in actively treated cancer patients. Upon validation, our results could provide additional insights into the predictive assessment of vaccination efficacy.

1. Introduction

The vaccination coverage against COVID-19 pandemic prioritized cancer patients on active treatment because of the increased morbidity and mortality rates associated with this immunocompromising

condition [1]. Even without evidence from randomized controlled trials, several observational studies have consistently indicated that the two-dose schedule of mRNA-based vaccines is safe and effective in recipients with solid malignancies [2]. Identifying reliable biomarkers of immunogenicity to develop personalized vaccination strategies is a

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

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research priority [3]. Although adaptive immunity is considered essential for humoral response and clinical protection in high-risk conditions [4–6], its relevance has not been thoroughly characterized in cancer patients undergoing active treatments. In this context, relatively few studies have evaluated SARS-CoV-2-specific T and B cell responses after full-dose vaccination [7–10]. Recent data suggest that absolute counts of circulating lymphocyte subpopulations correlate with specific cell-mediated immunity and vaccine efficacy in a highly vulnerable population of patients undergoing CD20 B-cell depletion treatments [11–12]. The interaction of these immune parameters with the immunogenicity of SARS-CoV-2 vaccines has never been investigated in patients with solid tumors receiving active treatments.

The Vax-On study provided data on antibody response to the first and second doses of the mRNA-BNT162b2 (tozinameran) vaccine in actively treated cancer patients. According to preliminary findings, the second dose induced an exponential increase in anti-Spike protein IgG titers and seroconversion rates of up to 90% [13]. We also reported a significant decrease in antibody titers over time and a seroconversion rate that remained adequate even five months after the second dose [14]. Herein, we aimed to describe the absolute counts of peripheral lymphocyte subsets in this population and their correlation with humoral immunogenicity six months after beginning tozinameran vaccination.

2. Methods

2.1. Study design and participants

The Vax-On-Profile is a prospective, single-center, observational study approved by the referring Ethics Committee (Protocol Number: 1407/CE Lazio1; clinical trial identifier: EudraCT Number 2021–002611-54). The study protocol follows the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) reporting guidelines. The main eligibility requirements were age ≥ 18 years, histological diagnosis of solid malignancy, ongoing active cancer treatment or its conclusion within the previous six months, and completion of the two-dose vaccination schedule of tozinameran by at least 22 weeks. Key exclusion criteria comprised life expectancy < 12 weeks, active and concomitant hematological malignancy, documented COVID-19 infection at any time, and pregnancy. All participants signed a written informed consent before enrollment. The procedures used in this study comply with the tenets of the 1964 Declaration of Helsinki and its subsequent amendments. The primary endpoint was the correlation of absolute counts of peripheral blood lymphocyte subpopulations with serum IgG antibody titers against the receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein (S1) and seroconversion rates. The secondary endpoint was to determine whether lymphocyte subset counts could predict humoral responses. The cut-off date for conclusive analysis was November 10, 2021.

2.2. Serological test

Whole blood draws (3 mL/subject) were collected in standard tubes and separated by immediate centrifugation. Serum specimens were analyzed through chemiluminescent microparticle immunoassay technology at each sampling. The SARS-CoV-2 IgG II Quant assay on the ARCHITECT i2000sr automated platform (Abbott Laboratories, Diagnostics Division, Sligo, Ireland) was used to detect anti-RBD-S1 IgG antibodies according to the manufacturer's instructions [15]. The results were reported as arbitrary units (AU)/mL, with a cut-point ≥ 50 AU/mL indicating a positive seroconversion response.

2.3. Immunophenotyping

At the same time as serological testing, whole blood draws for flow cytometry analysis (3 mL/subject) were collected in ethylenediaminetetraacetic acid (EDTA) tubes. The BD Multitest 6-color

TBANK reagent was used to determine absolute counts of B and NK, as well as CD4 and CD8 subpopulations of T cells. The panel for staining included the following monoclonal antibodies: CD3 FITC, CD4 PE-Cy7, CD8 APC-Cy7, CD19 APC, CD45 PerCP-Cy5.5, and CD16 PE + CD56 PE; BD Biosciences, San Jose, CA). The BD Trucount tubes (BD Biosciences, San Jose, CA) were filled with 20 μ L of BD Multitest 6-color TBANK reagent and 50 μ L aliquots of EDTA-anticoagulated whole blood. The mixture was incubated at room temperature in the dark for 20 min before being lysed with 2 mL of FACS Lysis Solution (BD Biosciences, San Jose, CA). After an additional 15 min of incubation, the erythrocyte-lysed, unwashed, and stained samples were analyzed. The data were acquired using the BD FACSCanto II system and BD FACS-Canto clinical software (BD Biosciences, San Jose, CA). The calibration of the instrument with BD FACS 7-color setup beads was confirmed before each running process according to the manufacturer's instructions [16]. The results for each lymphocyte subset were reported as absolute cell counts/ μ L.

2.4. Statistical analysis

A mean with standard deviation (SD) was used to describe normally distributed variables, while a median with 95% confidence interval (CI) or interquartile range (IQR) was reported for skewed variables. Comparative assessments were performed by applying Pearson's χ^2 test for categorical data and Mann-Whitney U test for continuous variables. A preliminary multivariate analysis was performed by adjusting a generalized linear model on the logarithmic (log) values of each subset of lymphocytes as a function of predefined covariates, including sex, age, Eastern Cooperative Oncology Group Performance Status (ECOG PS), treatment setting, corticosteroid therapy, granulocyte colony-stimulating factor (G-CSF) therapy, type and timing of active treatment. Receiver operating characteristic (ROC) curves were calculated to determine the sensitivity and specificity of candidate peripheral blood lymphocyte subsets related to positive seroconversion response. An area under the curve (AUC) > 0.65 was considered meaningful for subsequent evaluations. The Youden index was applied to identify the optimal cut-point. The Spearman method was used to assess the correlation between the log values of anti-RBD S1 IgG titers and significant lymphocyte subset counts. A secondary multivariate analysis was performed by fitting the same model to anti-RBD-S1 IgG log titers and seroconversion response as a function of significant lymphocyte levels in addition to the independent covariates described above. All tests performed were two-sided, and a P value < 0.05 was considered significant. SPSS (IBM SPSS Statistics for Windows, version 23.0, Armonk, NY) and Prism (GraphPad, version 9) software were used for statistical evaluations and figure rendering, respectively.

3. Results

3.1. Patient characteristics

This current study included 311 consecutive patients who met the inclusion criteria and had received the first dose of tozinameran between March 9 and April 12, 2021. A total of 203 patients (65.3%) were on active treatment, while the remaining 108 cases (34.7%) had discontinued it for at least 28 days. The majority of patients were female (58.8%), had ECOG PS 0–1 (97.1%) and metastatic extent of disease (63.3%). Cytotoxic chemotherapy (32.2%) and targeted therapy (33.8%) were the most frequent active treatments. Table 1 summarizes in detail the baseline characteristics of enrolled patients. All serologically tested patients were also evaluable for peripheral blood immunophenotyping (Table 2). Preliminary multivariate analysis indicated cytotoxic chemotherapy as the only variable significantly associated with lower counts in all lymphocyte subsets (Supplementary Table S1).

Table 1
Baseline patient characteristics.

Characteristic	All patients, N = 311 (100%)
Mean age, years (SD)	64.3 (10.4)
Sex	
- Female	183 (58.8%)
- Male	128 (41.2%)
ECOG PS	
- 0	189 (60.8%)
- 1	113 (36.3%)
- 2	9 (2.9%)
Tumor type	
- Breast	93 (29.9%)
- Lung	51 (16.4%)
- Kidney	10 (3.2%)
- Prostate	8 (2.6%)
- Colorectal	61 (19.6%)
- Urothelial	31 (10.0%)
- Pancreatic	9 (2.9%)
- Gastric	9 (2.9%)
- Skin (Melanoma, Merkel-cell)	6 (1.9%)
- Gynaecological	10 (3.2%)
- Head and Neck	5 (1.6%)
- Brain	8 (2.6%)
- Other ^a	10 (3.2%)
Extent of disease	
- Local	114 (36.7%)
- Metastatic	197 (63.3%)
Treatment setting	
- adjuvant or neoadjuvant	120 (38.6%)
- metastatic, first line	126 (40.5%)
- metastatic, second or later line	65 (20.9%)
Type of last active treatment	
- Cytotoxic chemotherapy	100 (32.2%)
- Targeted therapy	105 (33.8%)
- Immune checkpoint inhibitors	32 (10.3%)
- Hormonal therapy	29 (9.3%)
- Cytotoxic chemotherapy and biological agent	18 (5.8%)
- Intravesical therapy	27 (8.7%)
Time from last active treatment	
- > 28 days	108 (34.7%)
- ≤ 28 days	203 (65.3%)
Reasons for discontinuation of active treatment	
- treatment completion	92 (29.6%)
- disease progression	13 (4.2%)
- refusal to continue	2 (0.6%)
- other reasons	1 (0.3%)
Time (days) from first vaccine dose, median (IQR)	183 (181–195)
Time (days) from second vaccine dose, median (IQR)	163 (160–174)
Corticosteroid therapy ^b	38 (12.2%)
G-CSF therapy ^c	26 (8.4%)

Abbreviations: SD, standard deviation; ECOG PS, Eastern Cooperative Oncology Group Performance Status; IQR, interquartile range; G-CSF, granulocyte colony-stimulating factor

^a Other cancer types included soft-tissue sarcoma, thymoma, anal cancer, testicular cancer and thyroid anaplastic carcinoma; ^b corticosteroid therapy indicates ≥ 10 mg prednisone equivalent daily for at least 7 days in the 28 days preceding the first dose of vaccine; ^c G-CSF therapy indicates administration of granulocyte-colony stimulating factor in the 28 days preceding the first dose of vaccine

3.2. ROC curve and correlation analysis

To investigate the predictive potential of peripheral lymphocyte subsets on antibody response, a preliminary ROC curve was computed to establish the critical values with the highest sensitivity and specificity. The AUCs of T helper (0.68) and B cells (0.74) were considered useful in predicting the incidence of a positive seroconversion response (Fig. 1). With a cut-off value of 505/μL for T helper and 75/μL for B cells, the sensitivity and specificity were 0.68 and 0.63, and 0.72 and 0.73, respectively. Evaluating anti-RBD-S1 log IgG titers as a function of lymphocyte log counts demonstrated a significant positive linear correlation for either T helper [$\rho = 0.31$ (95% CI 0.20–0.41), $P < 0.001$, Fig. 2A] and B cell subpopulation [$\rho = 0.30$ (95% CI 0.20–0.40), $P <$

Table 2
Baseline immune parameters.

Parameter	All patients, N = 311 (100%)
Anti-RBD-S1 IgG titer, AU/mL, median (95% CI)	367 (296–432)
Seroconversion response [†] , N (%)	274 (88.1%)
Total T cells (CD3 +), cell count/μL, median (95% CI)	1124 (1024–1216)
T helper cells (CD3 + CD4 +), cell count/μL, median (95% CI)	658 (602–723)
T cytotoxic cells (CD3 + CD8 +), cell count/μL, median (95% CI)	381 (343–430)
B cells (CD19 +), cell count/μL, median (95% CI)	105 (98–117)
NK cells (CD16 + CD56 +), cell count/μL, median (95% CI)	256 (235–274)
CD3 + CD4+/CD3 + CD8 + ratio, median (range)	2 (1–5)

Abbreviations: RBD-S1, receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein (S1); AU, arbitrary unit; CI, confidence interval; NK, Natural Killer
[†] Seroconversion response at cut-off ≥ 50 AU/mL

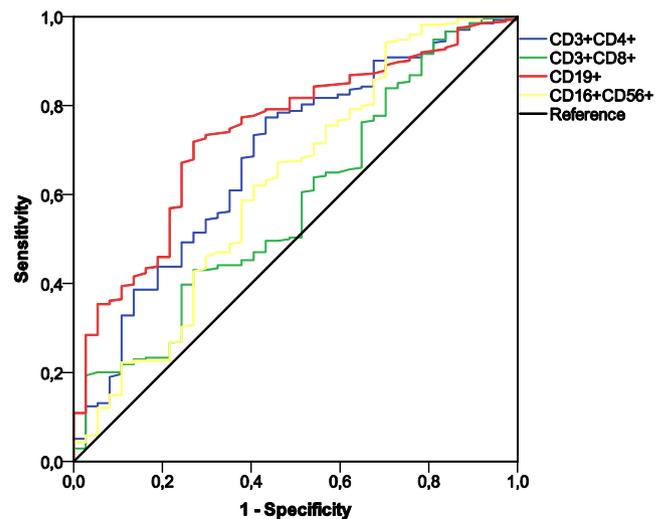


Fig. 1. ROC curve analysis of peripheral lymphocyte subpopulation counts on positive seroconversion response. AUC relative values for lymphocyte subpopulations: T helper cells (CD3 + CD4 +): 0.68 (95% CI 0.59 to 0.77), $P < 0.001$; T cytotoxic cells (CD3 + CD8 +): 0.58 (95% CI 0.48 to 0.67), $P = 0.11$; B cells (CD19 +): 0.74 (95% CI 0.66 to 0.82), $P < 0.001$; NK cells (CD16 + CD56 +): 0.62 (95% CI 0.51 to 0.72), $P = 0.015$. Abbreviations: ROC, receiver operating characteristic; AUC, area under the curve; NK, Natural Killer; CI, confidence interval.

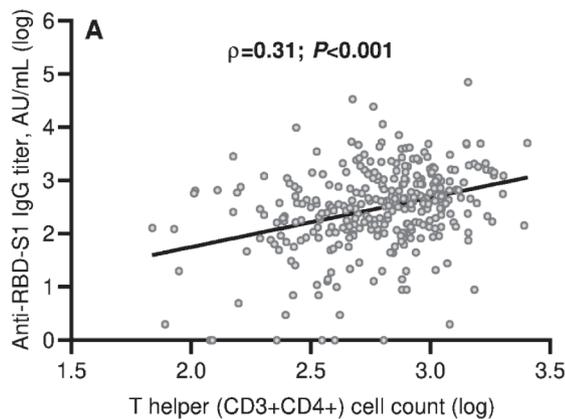
0.001; Fig. 2B]. The same assessment performed according to the different types of active treatment confirmed a significant positive linear correlation for targeted and hormonal therapies in both T helper (Supplementary Figure S1) and B cell subsets (Supplementary Figure S2).

3.3. Analysis of antibody response

ROC curve analyses established cut-point values that allowed the subpopulations of T helper and B cells to be divided into low- and high-level subgroups. On univariate analysis, both high-level T helper [483 AU/mL (95% CI 418–590) vs 179 AU/mL (95% CI 127–265), $P < 0.001$] and B cell counts [471 AU/mL (95% CI 378–580) vs 183 AU/mL (95% CI 120–258), $P < 0.001$] resulted in a significant increase of antibody titers (Fig. 3A). Accordingly, seroconversion rates improved significantly in both the high-level T helper (93.0% vs 79.1%, $P < 0.001$) and B cell (95.2% vs 75.0%, $P < 0.001$) subsets (Fig. 3B).

The subgroups defined by different T helper and B cell counts were included in the multivariate testing as independent covariates. Lower levels of T helper and B cells correlated significantly with decreased antibody titers, but only the latter feature was also associated with an

A. T helper cells



B. B cells

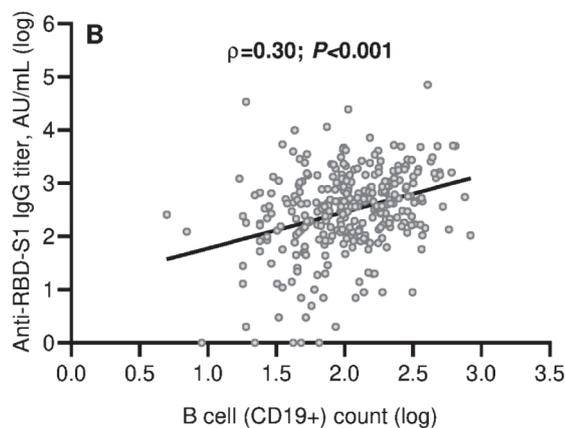


Fig. 2. Scatter plot of antibody titers by peripheral lymphocyte subpopulation counts. Abbreviations: RBD-S1, receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein (S1); AU, arbitrary unit; log, logarithmic values.

impaired seroconversion response. Only prolonged immunosuppressive corticosteroid dosing before the first dose of vaccine also predicted a harmed antibody and seroconversion response (Table 3).

4. Discussion

The current is a longitudinal study investigating the predictive potential of peripheral lymphocytes profiling on humoral immunity after complete vaccination with tozinameran. The positive correlation of absolute T helper and B cell counts with anti-RBD-S1 IgG titers indicates a direct interaction between these immune parameters. As expected, the cut-points defined by ROC curve analysis resulted in subgroups characterized by a differentiated humoral response. A noteworthy observation was the strong correlation between the level of circulating T helper and B cells and antibody and seroconversion responses on multivariate analysis. These findings suggest that peripheral T helper and B cell counts could predict humoral immunogenicity six months after starting vaccination. The fact that they are being reported for the first time in patients with solid tumors on active treatment requires a critical evaluation in terms of methodology and clinical significance.

The choice of absolute counts of circulating lymphocyte subpopulations as a correlate of vaccine-induced adaptive immunity might represent a controversial issue. In vaccinated cancer patients, T cell-

mediated immunity was characterized less comprehensively than the humoral response and only through enzyme-linked immune adsorbent spot (ELISpot) assays to quantify interferon-gamma (IFN γ) - producing SARS-CoV-2-specific T cells. Preliminary data indicated that patients with a poor humoral immune response may be protected by a viable cellular immunity [7–9,17]. However, the same methodology in a different study suggested that most seronegative patients with this condition did not elicit CD8 $^{+}$ /CD4 $^{+}$ T cell responses [10]. In contrast to patients with hematologic malignancies, only one study profiled the B cell-mediated response in patients with solid tumors using a high-resolution flow cytometry assay incorporating multiple cytokines and activation markers. In this research, RBD-S1-specific memory B cells were observed to correlate with neutralizing antibody titers after the second and third immunization [7]. Although the methods used in these studies allow for highly selective assessment of the cell-mediated immune response induced by anti-SARS-CoV-2 vaccination, the lack of harmonization among published studies and undefined reference standards continue to prevent regulatory agencies from approving these assays for widespread use [18–19]. The immunophenotypic characterization of circulatory lymphocytes in the present study provides a nonspecific description of lymphocyte responses after tozinameran vaccination. This approach has inherent weaknesses and strengths. Peripheral lymphocyte subset counts likely reflect the adaptive immune response to anti-SARS-CoV-2 vaccination, but may also be influenced by factors unrelated to the vaccine, the most relevant of which are the effects or timing of cancer treatments [20–21]. Preliminary multivariate analysis ruled out selection bias due to direct interaction with treatment timing but showed a significant effect of cytotoxic chemotherapy and corticosteroid therapy on T helper and B lymphocyte counts. Since it is commonly used for the diagnosis and monitoring of hematologic malignancies and is available in most facilities, the main strength of this methodology is the high level of procedure standardization and reproducibility of the results [22]. The lack of comparable studies in patients with solid malignancies makes determining the clinical significance of these findings challenging. Nonetheless, several studies have demonstrated a direct correlation between vaccine-induced absolute T helper and B cell counts and anti-spike IgG antibody titers in vulnerable patients receiving CD20 B-cell-depleting treatments [11–12,23]. Despite the fact that we were unable to perform antigen-specific cellular immunity tests, a condition representing a major limitation of this research, there was also high concordance between SARS-CoV-2-specific T and B cell reaction tests and circulating lymphocyte immunophenotyping results in the aforementioned studies. Given the differences between patients with hematologic and solid malignancies, the described experimental evidence is consistent with our findings, thereby confirming the validity of this methodological approach to adaptive immunity associated with tozinameran vaccination. Another noteworthy similarity was the impairment of both the vaccine-induced humoral and adaptive immune responses with immunosuppressive corticosteroid dosing [11].

The present study acknowledges several shortcomings that are not limited to the issues addressed above. The sample size did not rely on statistical assumptions. Our “all-comer” design skewed adequate participant stratification and may have made the study susceptible to selection bias. This flaw increased the possibility of false-positive results from multivariable statistical comparisons, the significance of which should thus be regarded as hypothesis-generating. At the same time, this unselected enrollment has produced a highly heterogeneous population that can adequately represent a real-world setting. Unlike antibody titers, we did not perform measurements of circulating lymphocyte counts before vaccination or after the first or second dose of vaccine that would allow an analysis of their dynamic variations. Finally, we did not report the incidence of breakthrough infections because of the low rate (<1%) even six months after vaccine priming. This finding may support vaccine efficacy itself or, more likely, reflects the effects of non-pharmaceutical interventions.

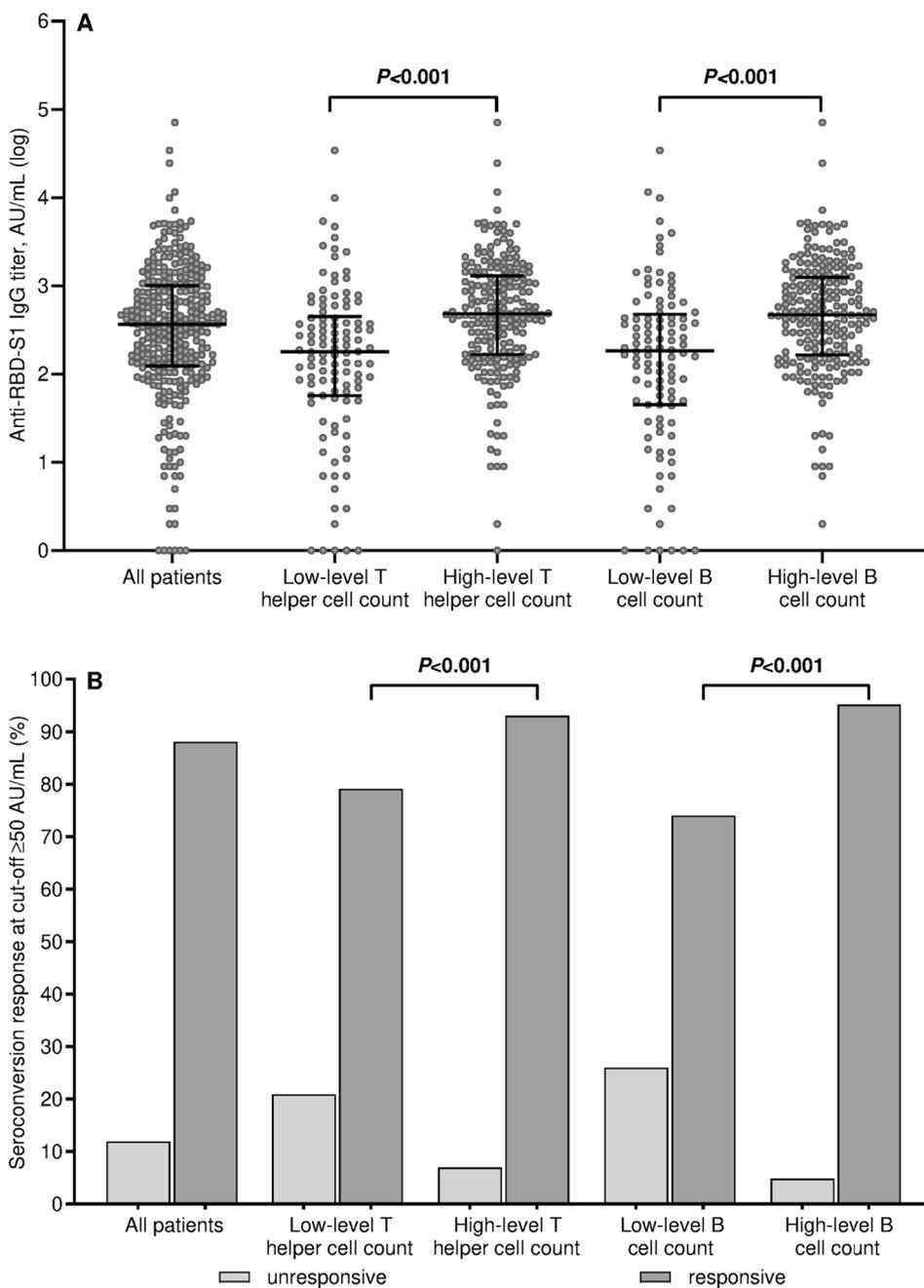


Fig. 3. Antibody response six months after the first dose of vaccine. A. Comparison of scatter plot distributions and medians of antibody titers by level of peripheral lymphocyte subpopulation counts (logarithmic values). Bars represent median values with 95% confidence interval. B. Comparison of seroconversion response rates by level of peripheral lymphocyte subpopulation counts. Abbreviations: RBD-S1, receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein (S1); AU, arbitrary unit.

5. Conclusions

Although the threshold of antibody titer for clinical protection remains ill-defined [24], higher levels have been predictive of a lower risk of symptomatic infection [25]. The waning of humoral response after the second dose of tozinameran [26], as well as SARS-CoV-2 variants of concern breakthrough infections [27], indicates a decrease in efficacy over time and the need for biomarkers of vaccination response. Our data suggest that widely available peripheral counts of T helper and B cells could predict humoral responses in actively treated cancer patients. We would emphasize the role of low-level B cells associated with an impaired seroconversion response, a high-risk condition for severe outcomes in immunocompromised recipients [28]. Assuming a valid concordance between the SARS-CoV-2-specific cell-mediated response and the generic adaptive immunity profile based on the immunophenotype of peripheral lymphocytes, our findings are consistent with

observations suggesting T and B cell responses as a prevalent long-term protective mechanism following vaccination [29–30]. The limitations of the current research warrant confirmation by independent prospective cohorts. Upon validation, our results could provide insights into the predictive assessment of vaccination efficacy concerning additional measures, such as booster dosing, maintenance of safety precautions, and passive antibody treatments.

CRediT authorship contribution statement

Fabrizio Nelli: Conceptualization, Project administration, Supervision, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Agnese Fabbri:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Valentina Panichi:** Formal analysis, Investigation, Writing – review & editing. **Diana Giannarelli:** Formal analysis,

Table 3
Multivariate analysis of antibody response six months after the first dose of vaccine.

Covariate	Effect on anti-RDB-S1 logarithmic IgG titer		Effect on seroconversion response at cut-off \geq 50 AU/mL	
	Beta (95% CI)	P value	Beta (95% CI)	P value
Sex - male vs female	-0.24 (-0.41; -0.06)	0.007	-0.65 (-1.49; 0.18)	0.12
Age (years) - > 55 vs \leq 55	-0.08 (-0.27; 0.11)	0.41	-0.94 (-2.18; 0.28)	0.13
ECOG PS				
-0†	-	-	-	-
-1	-0.08 (-0.27; 0.09)	0.35	0.41 (-0.46; 1.29)	0.35
-2	-0.67 (-1.17; -0.17)	0.008	-1.72 (-3.52; 0.07)	0.06
Treatment setting				
- adjuvant or neoadjuvant†	-	-	-	-
- metastatic, first line	0.06 (-0.14; 0.27)	0.55	-0.12 (-1.19; 0.93)	0.81
- metastatic, second or later line	-0.03 (-0.28; 0.22)	0.79	-1.67 (-1.67; 0.67)	0.40
Corticosteroid therapy - yes vs no	-0.33 (-0.59; -0.08)	0.009	-1.00 (-1.96; -0.05)	0.039
G-CSF therapy - yes vs no	-0.16 (-0.45; 0.12)	0.26	-0.36 (-1.75; 1.02)	0.60
Type of active treatment				
- active surveillance†	-	-	-	-
- cytotoxic chemotherapy	0.24 (-0.04; 0.52)	0.09	1.08 (-0.37; 2.55)	0.14
- targeted therapy	0.11 (-0.10; 0.34)	0.29	0.14 (-0.91; 1.20)	0.79
- immune checkpoint inhibitors	0.02 (-0.29; 0.34)	0.88	0.53 (-0.90; 1.97)	0.47
- hormonal therapy	0.14 (-0.29; 0.58)	0.52	0.34 (-2.02; 2.72)	0.77
- chemotherapy and biological agents	-0.20 (-0.60; 0.19)	0.31	-0.10 (-1.79; 1.58)	0.90
- intravesical therapy	0.15 (-0.33; 0.63)	0.53	19.64 (NA)	0.99
Time from last active treatment - \leq 28 days vs > 28 days	0.11 (-0.07; 0.30)	0.24	0.43 (-0.48; 1.35)	0.35
T helper cell subset - low vs high-level	-0.26 (-0.46; -0.06)	0.009	-0.52 (-1.39; 0.34)	0.24
B cell subset - low vs high-level	-0.32 (-0.51; -0.12)	0.001	-1.67 (-2.57; -0.77)	< 0.001

Abbreviations: RBD-S1, receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein (S1); CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; G-CSF, granulocyte colony-stimulating factor; AU, arbitrary unit; NA, not applicable.

†Reference category.

Methodology, Validation, Writing – review & editing. **Giuseppe Topini**: Investigation, Writing – review & editing. **Julio R. Giron Berrios**: Data curation, Investigation, Writing – review & editing. **Antonella Virtuoso**: Data curation, Investigation, Writing – review & editing. **Eleonora Marrucci**: Data curation, Investigation, Writing – review & editing. **Marco Mazzotta**: Data curation, Investigation, Writing – review & editing. **Marta Schirripa**: Data curation, Investigation, Writing – review & editing. **Carlo Signorelli**: Data curation, Investigation, Writing –

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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 material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2022.108774>.

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