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Antibody Response to Breakthrough SARS-CoV-2 Infection in "Booster" Vaccinated Patients with Multiple Myeloma According to B/T/NK Lymphocyte Absolute Counts and anti-CD38 Treatments

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To the editor.

Lymphopenia (particularly low CD19+ B-lymphocyte count) and current treatment with either anti-CD38 or anti-BCMA monoclonal antibodies (MoAbs) have been reported to significantly correlate with poor antibody response to conventional doses of anti-SARS-CoV-2 vaccines in patients with multiple myeloma (MM).[1-3] Notably, "booster" doses have been shown to enhance the humoral response of these patients.[4-7] We recently reported the greatly improved clinical outcome of breakthrough SARS-CoV-2 infection in MM patients who had received three or more doses of anti-SARS-CoV-2 vaccines during the different phases of the pandemic COVID-19 including the most recent viral variants of concern (VOCs);[8,9] most of the tested patients had developed an adequate antibody response (anti-spike IgG) to the virus.[9] Due to the scarcity of data about the role of different lymphocyte subsets in this specific population of patients, in the present study, we aimed to evaluate a possible relationship between antibody response after SARS-CoV-2 infection in "booster" vaccinated (at least 3 doses) MM patients and main circulating lymphocyte subpopulations. We also investigated the possible correlation between antibody titer and current treatments, including anti-CD38 MoAbs (daratumumab and isatuximab), in the same patient population. Sixty-two MM patients with breakthrough SARS-CoV-2 infection (men, 58.1%; median age, 65.5 years) followed at our Institution were included in this study between January 2022 and April 2023, when prevailing VOCs were Omicron BA.1, BA.2 and BA.5. Their main baseline characteristics of are listed in **Table 1**. All patients had been previously vaccinated against SARS-CoV-2 infection with at least three doses. Acquisition of informed consent and collection of serum samples were performed at the first outpatient visit after a median number of 22 days (range: 9-162) from SARS-CoV-2 infection. Determination of anti-spike IgG antibodies was performed using the SARS-CoV-2 IgG II Quant ABBOTT assay, an automated, two-step immunoassay (Chemiluminescent Microparticle ImmunoAssay technology) for the qualitative and quantitative determination of immunoglobulin class G (IgG) antibodies, including neutralizing antibodies to the receptor binding domain of the S1 subunit of the spike protein of SARS-CoV-2 in human serum and plasma. It utilizes a four Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration and results. The chemiluminescent reaction is measured as a relative light unit (RLU). There is a direct relationship between the amount of IgG antibodies to SARS-CoV-2 in the sample and the RLU detected by the system optic. Results were reported as arbitrary units (AU), with a positivity cut-off of ≥ 50 AU/mL as an arbitrary threshold for "adequate" response. Flow cytometric analyses were performed for assessment of the patients' lymphocyte status. Briefly, 50 µl of EDTA whole blood was stained with 20 µl of BD Multitest™ CD3/CD16+CD56/CD45/CD4/CD19/CD8 (FITC-labeled CD3, clone SK7; PE-labeled CD16, clone B73.1, and CD56, clone NCAM 16.2; PerCP-labeled CD45, clone 2D1 (HLe-1); PE-Cy7-labeled CD4, clone SK3; APC-labeled CD19, clone SJ25C1; and APC-Cy7-labeled CD8, clone SK1) in BD Trucount tubes after 15 minutes in the dark at room temperature 450 µL of lysing solution were added to the tube. After 10-15 minutes, samples were analyzed on the BD FACSCanto II flow cytometer. Absolute counts of T cell subsets, B cells, and NK cells were determined by the software BD FACSCanto™. Correlation between different lymphocyte subpopulations and anti-SARS-CoV-2 antibody titers was investigated using Spearman's Rho criterion, while comparisons between groups were performed by the Mann–Whitney U test. Statistical analyses were carried out using Jamovi

Table 1. Clinical and laboratory characteristics of MM patients with breakthrough SARS-CoV-2 Infection after at least three anti-SARS-CoV-2 vaccine doses.

Age at COVID-19 diagnosis, years	
Median (IQR)	65.5 (60.25-76.75)
Range	39-84
Gender, n. (%)	
Male	36 (58.1)
Female	26 (41.9)
MM isotype, n. (%)	
IgG	36 (58.1)
IgA	14 (22.6)
Light chain	9 (14.5)
Other	3 (4.8)
Current MM treatment regimen, n. (%)	
Contains proteosome inhibitor	**17 (27.4)**
Bortezomib	12
Carfilzomib	3
Ixazomib	2
Contains IMiDs	**48 (77.4)**
Thalidomide	8
Lenalidomide	36
Pomalidomide	5
Contains CD38 mAbs	**31 (50)**
Daratumumab	28
Isatuximab	3
Contains others	**6 (9.7)**
Elotuzumab	3
Belantamab	1
Melphalan	2
Contains steroids	**43 (69.3)**
Dexamethasone	41
Prednisone	2
Vaccine sequence pts, n. (%)	
Three doses	**50 (80.6)**
BNT162b2 mRNA x 3	41
ChAdOx1 nCoV-19 x 2/mRNA-1273	1
BNT162b2 mRNA x 2/mRNA-1273	3
ChAdOx1 nCoV-19 x 2/BNT162b2 mRNA	4
mRNA-1273 x 2/ BNT162b2 mRNA	2
Four doses	**9 (14.5)**
BNT162b2 mRNA x 4	7
mRNA-1273 x 2/ BNT162b2 mRNA x 2	2
Five doses	**3 (4.8)**
BNT162b2 mRNA x 5	3
Anti-spike IgG* post infection, median (range)	31,641 (10.7-219,255)
Days from first positive swab, median (range)	22 (9-162)
Total lymphocyte count, median (range), cells/μL	1220 (329-3710)
Lymphopenia (< 1000 cells/μL), n. (%)	
Yes	20 (32.3)
No	42 (67.7)
Lymphocyte subsets after SARS-CoV-2 infection, median (range), cells/μL	
CD4+	390 (86-1688)
CD8+	527 (119-1966)
CD3-/CD16+/CD56+	47 (0-886)
CD19+	40 (0-467)

* Chemiluminescent microparticle immunoassay (CMIA) technology-ABBOTT: results are reported as arbitrary units (AU), with a positivity cut-off of ≥50 AU/mL (patients above cut-off level were considered as "responders", and those below as "non responders").

(version 2.4.7) and GraphPad Prism (version 8.3.0). The favorable clinical outcome of breakthrough SARS-CoV-2 infection in this cohort of patients has been previously reported;[9] in particular, only 4 hospitalizations (6.4%) were observed, but none in an intensive cure unit. After a median number of 22 days (range 9-162) from positive swabs for SARS-CoV-2, almost all patients (60/62, 96.8%) achieved a titer greater than 50 AU/mL. Only two patients showed a lower titer after 5 and 3 vaccine doses, respectively: a 79-year-old female, receiving isatuximab, pomalidomide, and dexamethasone as 4th line therapy, and an 82-year-old female, receiving elotuzumab, pomalidomide, and dexamethasone as 3rd line treatment. At the time of SARS-CoV-2 infection, these patients were respectively in partial response and very

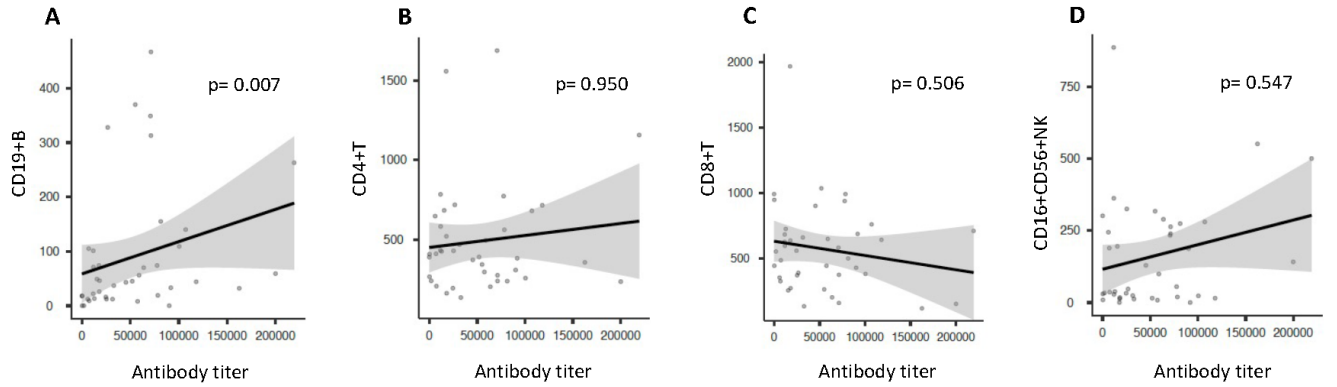

Figure 1. Correlation between antibody titer and absolute count of (**A**) CD19+B-lymphocytes, (**B**) CD4+T-lymphocytes, (**C**) CD8+T-lymphocytes, **(D)** and CD16+CD56+ NK-lymphocytes.

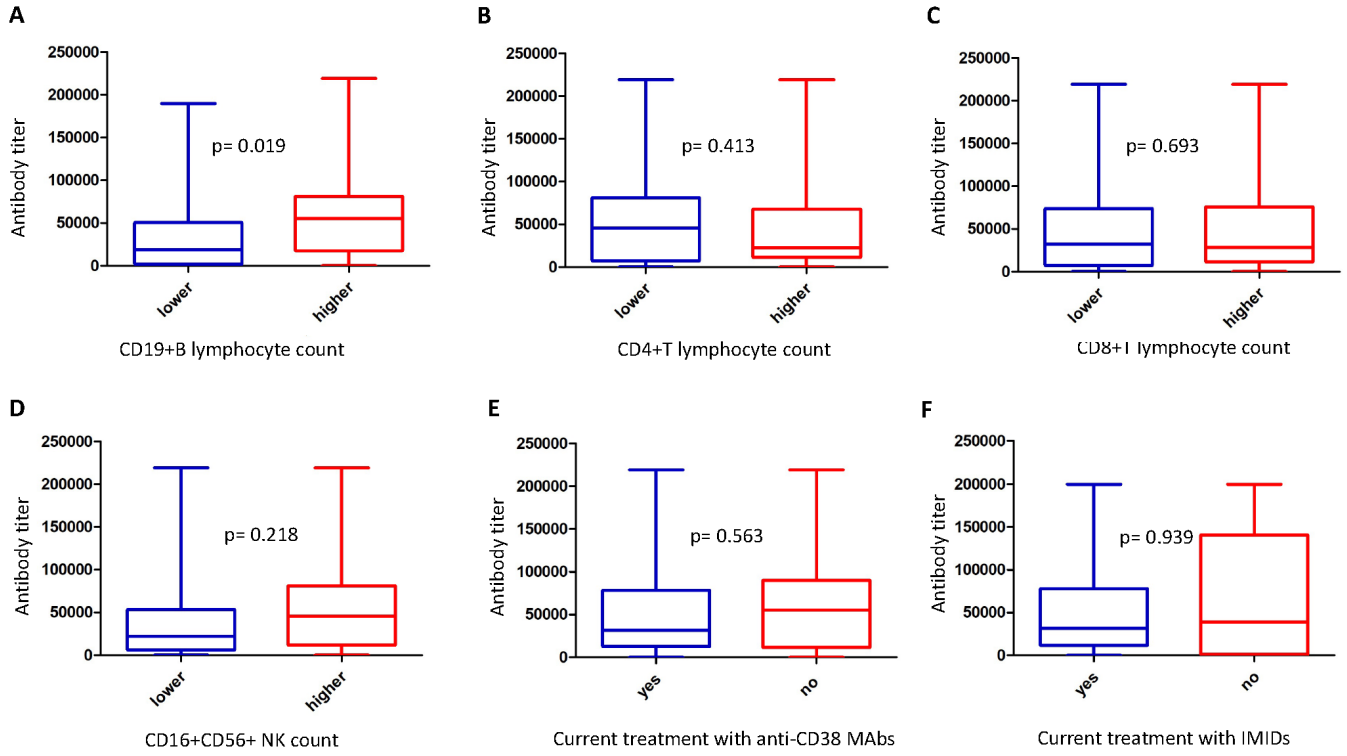

Figure 2. Comparison of antibody titer according to the median (lower vs higher) absolute count of (**A**) CD19+B-lymphocytes, (**B**) CD4+T-lymphocytes, (**C**) CD8+T-lymphocytes, **(D)** CD16+CD56+ NK-lymphocytes and (**E**) the use of anti-CD38 MAbs (yes vs. no) or (**F**) IMIDs (yes vs. no).

good partial response, according to the International Myeloma Working Group (IMWG) criteria. Notably, both these patients showed a low count of CD19+ B-lymphocytes and the concomitant use of pomalidomide. Regarding the antibody response according to the absolute count of CD19+ B-Lymphocytes, the presence of a direct correlation between the two variables (Spearman: 0.417; p= 0.007) (**Figure 1 A**), and a significant difference according to the median value used as a cut-off level (**Figure 2 A**) were observed. By contrast, assessing the impact of the absolute count of other lymphocyte populations on the development of antibody titer, no correlation was found (CD4+: Spearman: -0.010, p = 0.950; CD8+: Spearman: -0.108, p = 0.506; CD16+CD56+: Spearman: 0.098, p = 0.547) (**Figure 1, B-D**). Likewise, no statistically significant differences in terms of antibody titer emerged comparing patients with lower versus higher median CD4, CD8, and CD16/CD56 positive lymphocyte absolute values (**Figure 2, B-D**). Finally, evaluating the antibody response according to current treatment with anti-CD38 Mo-abs, no statistically significant correlation was identified between 31 patients undergoing these treatments and 31 patients who did not (**Figure 2 E**). Overall, our study suggests that, in patients who have previously received three or more doses of anti-SARS-CoV-2 vaccines, the absolute number of CD19+ B cells may marginally reduce the production of specific antibodies after breakthrough SARS-CoV-2 infections without significantly

decreasing; however, the percentage of patients with potentially "protective" titers. In this setting, the absolute number of T and NK populations, as well as the use of anti-CD38 antibodies for the treatment of MM, did not show significant effects on humoral response to viral infection. Curiously, the only two patients with suboptimal humoral response after breakthrough SARS-CoV-2 infection were both receiving pomalidomide; the possible detrimental effect of this drug on antibody production would warrant further investigation. However, evaluating the antibody response according to current treatment with IMIDs, no statistically significant correlation was identified between 48 patients undergoing these treatments and 14 patients who did not (**Figure 2 F**). The study has several limitations, particularly the limited number of patients tested, the lack of information about serological response and lymphocyte counts before infection, the heterogeneous timing of blood collection, the different types of vaccine employed, and the lack of a control group. Furthermore, the important role of specific functional aspects of T and NK-cell responses to breakthrough SARS-CoV-2 infection in fully vaccinated MM patients[10-12] was not investigated. Notwithstanding, our observation is in line with the generally favorable clinical outcome of COVID-19 we observed in these patients and would seem to reflect the independence of clinical and serological response upon quantitative amounts of different lymphocyte sub-populations present at the time of viral infection, including patients receiving anti-CD38 therapies after booster vaccinations and infected by novel SARS-CoV-2 VOCs that represent the current epidemiological scenario.

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References:

1. Ghandili S, Schönlein M, Lütgehetmann M, Schulze Zur Wiesch J, Becher H, Bokemeyer C, Sinn M, Weisel KC, Leypoldt LB. Post-Vaccination Anti-SARS-CoV-2-Antibody Response in Patients with Multiple Myeloma Correlates with Low CD19+ B-Lymphocyte Count and Anti-CD38 Treatment. Cancers (Basel). 2021 Jul 28;13(15):3800. https://doi.org/10.3390/cancers13153800 PMid:34359701 PMCid:PMC8345197
2. Van Oekelen O, Gleason CR, Agte S, Srivastava K, Beach KF, Aleman A, Kappes K; PVI/Seronet team; Mouhieddine TH, Wang B, Chari A, Cordon-Cardo C, Krammer F, Jagannath S, Simon V, Wajnberg A, Parekh S. Highly variable SARS-CoV-2 spike antibody responses to two doses of COVID-19 RNA vaccination in patients with multiple myeloma. Cancer Cell. 2021 Aug 9;39(8):1028-1030. https://doi.org/10.1016/j.ccell.2021.06.014 PMid:34242572 PMCid:PMC8238657
3. Terpos E, Gavriatopoulou M, Ntanasis-Stathopoulos I, Briasoulis A, Gumeni S, Malandrakis P, Fotiou D, Papanagnou ED, Migkou M, Theodorakakou F, Roussou M, Eleutherakis-Papaiakovou E, Kanellias N, Trougakos IP, Kastritis E, Dimopoulos MA. The neutralizing antibody response post-COVID-19 vaccination in patients with myeloma is highly dependent on the type of anti-myeloma treatment. Blood Cancer J. 2021 Aug 2;11(8):138. https://doi.org/10.1038/s41408-021-00530-3 PMid:34341335 PMCid:PMC8327056
4. Faustini SE, Hall A, Brown S, Roberts S, Hill H, Stamataki Z; (PITCH) consortium; Jenner MW, Owen RG, Pratt G, Cook G, Richter A, Drayson MT, Kaiser MF, Heaney JLJ. Immune responses to COVID-19 booster vaccinations in intensively anti-CD38 antibody-treated patients with ultra-high-risk multiple myeloma: results from the Myeloma UK (MUK) nine OPTIMUM trial. Br J Haematol. 2023 Jun;201(5):845-850. https://doi.org/10.1111/bjh.18714 PMid:36895158
5. Terpos E, Gavriatopoulou M, Ntanasis-Stathopoulos I, Briasoulis A, Gumeni S, Malandrakis P, Papanagnou ED, Migkou M, Kanellias N, Kastritis E, Trougakos IP, Dimopoulos MA. Booster BNT162b2 optimizes SARS-CoV-2 humoral response in patients with myeloma: the negative effect of anti-BCMA therapy. Blood. 2022 Mar 3;139(9):1409-1412. https://doi.org/10.1182/blood.2021014989 PMid:34986251 PMCid:PMC8736278
6. Aleman A, Van Oekelen O, Upadhyaya B, Beach K, Kogan Zajdman A, Alshammary H, Serebryakova K, Agte S, Kappes K, Gleason CR, Srivastava K; PVI/MM/Seronet Study Group; Almo S, Cordon-Cardo C, Krammer F, Merad M, Jagannath S, Wajnberg A, Simon V, Parekh S. Augmentation of humoral and cellular immune responses after third-dose SARS-CoV-2 vaccination and viral neutralization in myeloma patients. Cancer Cell. 2022 May 9;40(5):441-443. https://doi.org/10.1016/j.ccell.2022.03.013 PMid: 35390296; PMCid: PMC8983835
7. Šušol O, Hájková B, Zelená H, Hájek R. Third dose of COVID-19 vaccine restores immune response in patients with haematological malignancies after loss of protective antibody titres. Br J Haematol. 2022 May;197(3):302-305. https://doi.org/10.1111/bjh.18073 PMid:35076937
8. Musto P, Salmanton-García J, Sgherza N, Bergantim R, Farina F, Glenthøj A, Cengiz Seval G, Weinbergerová B, Bonuomo V, Bilgin YM, van Doesum J, Jaksic O, Víšek B, Falces-Romero I, Marchetti M, Dávila-

Valls J, Martín-Pérez S, Nucci M, López-García A, Itri F, Buquicchio C, Verga L, Piukovics K, Navrátil M, Collins GP, Jiménez M, Fracchiolla NS, Labrador J, Prezioso L, Rossi E, Čolović N, Meers S, Kulasekararaj A, Cuccaro A, Blennow O, Valković T, Sili U, Ledoux MP, Batinić J, Passamonti F, Machado M, Duarte RF, Poulsen CB, Méndez GA, Espigado I, Demirkan F, Čerňan M, Cattaneo C, Petzer V, Magliano G, Garcia-Vidal C, El-Ashwah S, Gomes-Da-Silva M, Vena A, Ormazabal-Vélez I, van Praet J, Dargenio M, De-Ramón C, Del Principe MI, Marques-De-Almeida J, Wolf D, Szotkowski T, Obr A, Çolak GM, Nordlander A, Izuzquiza M, Cabirta A, Zambrotta GPM, Cordoba R, Žák P, Ammatuna E, Mayer J, Ilhan O, García-Sanz R, Quattrone M, Arellano E, Nunes-Rodrigues R, Emarah Z, Aiello TF, Hanakova M, Ráčil Z, Bavastro M, Limongelli A, Rahimli L, Marchesi F, Cornely OA, Pagano L. Survival in multiple myeloma and SARS-COV-2 infection through the COVID-19 pandemic: Results from the epicovideha registry. Hematol Oncol. 2023 Dec 4.
https://doi.org/10.1002/hon.3240
PMid:38050405

9. Sgherza N, Curci P, Rizzi R, Battisti O, Perfetto A, Weigl S, Larocca AMV, Chironna M, Tafuri S, Musto P. Clinical outcome of breakthrough COVID-19 in multiple myeloma patients after three or more anti-SARS-CoV-2 vaccine doses: a single center analysis of 64 cases. Ann Hematol. 2024 Jan;103(1):351-355.
https://doi.org/10.1007/s00277-023-05484-z
PMid:37782371
10. Aleman A, Upadhyaya B, Tuballes K, Kappes K, Gleason CR, Beach K, Agte S, Srivastava K; PVI/Seronet Study Group; Van Oekelen O, Barcessat V, Bhardwaj N, Kim-Schulze S, Gnjatic S, Brown B, Cordon-Cardo C, Krammer F, Merad M, Jagannath S, Wajnberg A, Simon V, Parekh S. Variable cellular responses to SARS-CoV-2 in fully vaccinated patients with multiple myeloma. Cancer Cell. 2021 Nov 8;39(11):1442-1444.
https://doi.org/10.1016/j.ccell.2021.09.015
PMid:34706273 PMCid:PMC8523488
11. Azeem MI, Nooka AK, Shanmugasundaram U, Cheedarla N, Potdar S, Manalo RJ, Moreno A, Switchenko JM, Cheedarla S, Doxie DB, Radzievski R, Ellis ML, Manning KE, Wali B, Valanparambil RM, Maples KT, Baymon E, Kaufman JL, Hofmeister CC, Joseph NS, Lonial S, Roback JD, Sette A, Ahmed R, Suthar MS, Neish AS, Dhodapkar MV, Dhodapkar KM. Impaired SARS-CoV-2 Variant Neutralization and CD8+ T-cell Responses Following 3 Doses of mRNA Vaccines in Myeloma: Correlation with Breakthrough Infections. Blood Cancer Discov. 2023 Mar 1;4(2):106-117.
https://doi.org/10.1158/2643-3230.BCD-22-0173
PMid:36511813 PMCid:PMC9975771
12. Enssle JC, Campe J, Moter A, Voit I, Gessner A, Yu W, Wolf S, Steffen B, Serve H, Bremm M, Huenecke S, Lohoff M, Vehreschild M, Rabenau HF, Widera M, Ciesek S, Oellerich T, Imkeller K, Rieger MA, von Metzler I, Ullrich E. Cytokine-responsive T- and NK-cells portray SARS-CoV-2 vaccine-responders and infection in multiple myeloma patients. Leukemia. 2023 Dec 4.
https://doi.org/10.1038/s41375-023-02070-0
PMid:38049509 PMCid:PMC10776400