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# Multiple COVID-19 vaccine doses in CLL and MBL improve immune responses with progressive and high seroconversion

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**Abstract:**
Chronic lymphocytic Leukemia (CLL) and Monoclonal B-Lymphocytosis (MBL) patients have impaired response to COVID-19 vaccination. A total 258 patients (215 CLL and 43 MBL) had anti-spike levels evaluable for statistical analysis. The overall seroconversion rate for CLL was 94.2% (anti-spike ³50AU/mL Abbott Diagnostics) and for MBL 100%. After 3 doses (post-D3) in 167 CLL patients, 73.7% were seropositive, 17.4% had anti-spike levels 50-999AU/mL, and 56.3% ≥1000AU/mL with a median rise from 144.6AU/mL to 1800.7AU/mL. Of patients seronegative post-D2, 39.7% seroconverted post-D3. For those who then remained seronegative after their prior dose, seroconversion occurred in 40.6% post-D4, 46.2% post-D5, 16.7% post-D6, and 0% after D7 or D8. Following seroconversion, most had a progressive increment in anti-spike antibody level: in CLL after the latest dose, 70.2% achieved anti-spike level ≥1,000AU/mL, 48.1% ≥5,000AU/mL, and 30.3% ≥10,000AU/mL. Neutralization was associated with higher anti-spike levels, more vaccines and earlier COVID variants; 65.3% detected neutralizing antibody against early clade D614G, 52.0% against Delta, and 36.5% against Omicron. COVID-specific T-cell production of IFN-γ occurred in 73.9% and IL-2 in 60.9% of 23 tested, and more consistently with higher anti-spike levels. After multiple vaccine doses, by multivariate analysis, IgM ≥0.53g/L (OR=2.90, p=0.0314), IgG3 ≥0.22g/L (OR=3.26, p=0.0057), and lack of current CLL therapy (OR=2.48, p=0.0574) were independent predictors of positive serological responses. Strong neutralization and T-cell responses had high concordance with high anti-spike levels. Multiple sequential COVID-19 vaccination significantly increased seroconversion and anti-spike antibody levels in CLL and MBL.

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**Agreement to Share Publication-Related Data and Data Sharing Statement:** There were 24 of 98 post-dose-2 samples tested for neutralization against D614G and Delta variants presented in our previous report "Shen Y, Freeman JA, Holland J, et al. COVID-19 vaccine failure in chronic lymphocytic leukaemia and monoclonal B-lymphocytosis; humoural and cellular immunity. British Journal of Haematology. 2022;197(1):41-51." Data was retained in this study to enable comparison and statistical evaluation with subsequent vaccine doses.

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**Title**

# Multiple COVID-19 vaccine doses in CLL and MBL improve immune responses with progressive and high seroconversion

**Running title**

Multiple COVID-19 vaccine doses in CLL and MBL

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**Key Points**

- Multiple doses result in high rates of seroconversion in CLL (94.2%) and MBL (100%), with progressively higher anti-spike antibody levels

- Neutralization against recent COVID-19 variants and strong specific T-cell responses requires higher anti-spike levels

**Explanation of Novelty**

Novel points include i) first longitudinal evaluation of a large cohort CLL, and MBL, patients after third, fourth, and up to eighth dose of COVID-19 vaccination, with ii) all clinical parameters, treatment groups, including naïve, statistically correlated with iii) seroconversion rates, iv) anti-spike antibody levels, v) neutralization against D614G, Delta and Omicron (live virus, not "pseudotyping") and vi) COVID-19 specific T-cell responses. The data is consequential and globally applicable.

**Abstract**

Chronic lymphocytic Leukemia (CLL) and Monoclonal B-Lymphocytosis (MBL) patients have impaired response to COVID-19 vaccination. A total 258 patients (215 CLL and 43 MBL) had anti-spike levels evaluable for statistical analysis. The overall seroconversion rate for CLL was 94.2% (anti-spike ≥50AU/mL Abbott Diagnostics) and for MBL 100%. After 3 doses (post-D3) in 167 CLL patients, 73.7% were seropositive, 17.4% had anti-spike levels 50-999AU/mL, and 56.3% ≥1000AU/mL with a median rise from 144.6AU/mL to 1800.7AU/mL. Of patients seronegative post-D2, 39.7% seroconverted post-D3. For those who then remained seronegative after their prior dose, seroconversion occurred in 40.6% post-D4, 46.2% post-D5, 16.7% post-D6, and 0% after D7 or D8. Following seroconversion, most had a progressive increment in anti-spike antibody level: in CLL after the latest dose, 70.2% achieved anti-spike level ≥1,000AU/mL, 48.1% ≥5,000AU/mL, and 30.3% ≥10,000AU/mL. Neutralization was associated with higher anti-spike levels, more vaccines and earlier COVID variants; 65.3% detected neutralizing antibody against early clade D614G, 52.0% against Delta, and 36.5% against Omicron. COVID-specific T-cell production of IFN-γ occurred in 73.9% and IL-2 in 60.9% of 23 tested, and more consistently with higher anti-spike levels. After multiple vaccine doses, by multivariate analysis, IgM ≥0.53g/L (OR=2.90, p=0.0314), IgG3 ≥0.22g/L (OR=3.26, p=0.0057), and lack of current CLL therapy (OR=2.48, p=0.0574) were independent predictors of positive serological responses. Strong neutralization and T-cell responses had high concordance with high anti-spike levels. Multiple sequential COVID-19 vaccination significantly increased seroconversion and anti-spike antibody levels in CLL and MBL.

## Introduction

The Severe Acute Respiratory Syndrome Corona-virus-type 2 (SARS-CoV-2) 2019 (COVID-19) pandemic is well into its third year and the emergence of variants now account for most infections across the globe. Patients with chronic lymphocytic Leukemia (CLL) have immune failure with hypogammaglobulinemia,[1] higher infection risk,[2-4] and higher risk of severe COVID-19 infection.[5-8] We[9] and others[10-15] have documented impaired response after 2 initial doses (post-D2) of COVID-19 vaccination with 44% of CLL patients and 9.5% of monoclonal B-Lymphocytosis (MBL) patients failing seroconversion (Abbott Diagnostics assay level ≥50AU/mL). Furthermore, post-D2, 75% of CLL and 50% of MBL have no or minimal neutralizing activity to early viral clades and the Delta variant[9].

Immunocompromised CLL patients unable to generate their own antibody response remain vulnerable with a limited range of imperfect options. Continued social distancing equates to ongoing social isolation and for many is not possible; anti-viral therapy targets only recent onset COVID-19 infection; and the prophylactic antibody combination tixagevimab and cilgavimab (Evusheld, AstraZeneca) remains difficult to access for many CLL patients globally and evidence of viral resistance is emerging, especially for BA.4 and BA.5.[16-19] A study by the Leukemia and Lymphoma Society presented at the Scientific Symposium of the American Society of Hematology 2021 reported that many seronegative patients "took matters into their own hands" and sought additional vaccine doses.[20] In our patient population, we also noted higher rates of seroconversion and higher anti-spike antibody levels in most patients who accessed third, fourth and subsequent vaccine doses. MBL patients also have an immune defect.[9,21-23] The prevalence of MBL in the community in those aged >65 years is extremely high at ~10%[21,24], and many will have suboptimal vaccine response yet be

unaware of their status and risk. Adequate protection against COVID-19 for the elderly population therefore remains a major public health issue.

Internationally, many governments and health systems have launched third and sometimes fourth vaccine dose programs in the general population or selected groups deemed immunocompromised or vulnerable. Recent Israeli, United Kingdom, French and Danish data showed a third dose (D3) in CLL patients seronegative post-D2 seroconverted 23.7%, ~14%, 35% and 30% respectively.[25-28] Generally, this supports the hypothesis that multiple doses boost the anti-spike antibody level, and that additional doses may result in successful seroconversion.

In this prospective observational study, we evaluated responses in CLL and MBL patients after third, fourth, and up to eighth doses of COVID-19 vaccination and report, i) seroconversion rates, ii) anti-spike antibody levels, iii) neutralizing antibody activity levels against the early viral clade D614G, and the later variants Delta and Omicron, and iv) COVID-19 specific T-cell responses.

## Methods

The study was approved by the Northern Sydney Local Health District Human Research Ethics Committee (approval number: LNR/14/HAWKE/181). The diagnosis of CLL and MBL were according to iwCLL guidelines.[29] Vaccination occurred through the Australian Government program (www.health.gov.au) with Vaxzevria (ChAdOx1 nCoV-19, adenoviral vector-based vaccine; AstraZeneca AZ), Cominarty (Pfizer-BioNTech, mRNA based vaccine; Pfizer), Spikevax (mRNA-1273, mRNA based vaccine; Moderna) and Nuvaxovid (NVX-

CoV2373, spike protein subunit vaccine; Novavax).[30] Most patients had an initial 2 doses with Vaxzevria, then subsequent doses mostly with Cominarty vaccine, and some with Spikevax. Vaccine doses were accessed through vaccine hubs, pharmacies, or local medical practitioners, distributed by the Australian Government with Australian Technical Advisory Group on Immunization ("ATAGI") issued guidelines regarding vaccine type, number and schedule that evolved through the pandemic. Australia had very low COVID case numbers until December 2021 (Supplementary Figure 1). While many patients were able to access additional doses, some were not, as detailed in Supplementary Figure 2. The current study is an expansion of our 2-dose cohort[9] but focuses on outcomes after 3, and up to 8 multiple sequential vaccine doses. The reported "ultimate positivity rate" was calculated based on the latest anti-spike level from post-D2 onwards. Blood samples were collected approximately 4 weeks after each sequential vaccine dose. The SARS-CoV-2 IgG II Quant assay® (Abbott Diagnostics, Macquarie Park, NSW) was performed in accordance with the manufacturer's instructions. An anti-spike antibody level of ≥50AU/mL is defined as "positive", and the maximum reported level is >25,000AU/mL.

*SARS-CoV-2 live virus neutralization assay*

HEK-ACE2/TMPRSS cells (Clone 24)[31] were performed as previously described[18], for viral variants Omicron (BA.1), Delta (B.1.617.2), as well as 'wild-type' control virus (B.1.319/D614G strain) from an early circuiting 2020 clade (B.1).

*IFN-γ/IL-2 Fluorospot Assay*

Peripheral blood mononuclear cells were seeded in T-cell interferon gamma (IFN-γ)/interleukin-2 (IL-2) dual color Fluorospot plates (Mabtech) and incubated with overlapping SARS-CoV-2 peptide pool spanning the complete S-protein (1µg/mL; Miltenyi) as previously described.[9,32] Reported data are represented as the mean of the duplicates expressed as spot forming units (SFU) per $10^6$ cells. The threshold for a positive response was set based on the average number of SFU per $10^6$ cells across all negative controls plus 3 standard deviations. The mean number of responding cells in negative controls ('no stimulation' controls, i.e. cells incubated in media with no peptides) were subtracted from stimulated samples to account for background responses.

*Statistical analysis*

Statistical analyses were conducted using SAS V9.4. A generalized mixed model was fitted (using SAS PROC GLIMMIX) where neutralization antibody response (positive/negative) was the outcome. COVID variants and anti-spike levels (≥/<1000) were included as factors, together with an interaction term. Number of doses was also included as a factor with values 2,3,4 or ≥5. To acknowledge the dependence in the data (i.e., each patient provided results for up to 3 variants), variants were included as a random term with subject as the repeating term using a compound symmetry variance/covariance matrix. Univariate logistic regression models, modelling the odds of adequate/positive response (anti-spike≥1000AU/mL), fitted with conditional exact methods to obtain exact P-values and 95% confidence limits. Exact methods were employed due to the small patient numbers in some categories. Multivariate logistic regression models were subsequently fitted including

multiple clinical variables found to be significant in univariate models. If two clinical variables were highly correlated only one variable was included. Any analyses of IgG levels (or IgG subclasses) excluded patients on IgG replacement therapy.

## Results

### *Patient characteristics*

From 1st March 2021 through to 24th June 2022, a total of 258 patients, 215 CLL and 43 MBL, with anti-spike antibody measured after at least 2 doses of vaccination were included in the analysis. The median age for CLL and MBL patients were both 72 years. The proportion of males for CLL and MBL was 55.8% and 32.6% respectively. Patient characteristics and baseline demographics are summarized in Table 1. In this cohort post-D2, there were 229 patients whose anti-spike level was assessed, seroconversion rates were 55.5% in CLL (106 of 191) and 92.1% in MBL (35 of 38) (Supplementary Table 1 and Supplementary Figure 2). At the time of D2, there was no reported COVID-19 infection, and all had negative anti-nucleocapsid assays. Since December 2021, 32 patients (28 CLL and 4 MBL) have reported COVID-19 infection of whom there has been 30 with relatively mild disease and 2 severely ill with 1 fatal outcome. Both hospitalized patients had CLL with their latest anti-spike levels prior to infection of 1.0AU/mL and 2462.3AU/mL (Supplementary Figure 3). After COVID-19 infection, no further analysis is reported on these patients as this will reflect response to infection and not only vaccination.

***Serological response of anti-spike seroconversion rate and antibody levels in CLL***

The ultimate seroconversion rate for CLL was 94.2% (180 of 191 i.e., 215 total CLL patients, excluding 13 and 11 patients missing post-D2 or post-D3 data respectively but with later time points recorded) defined as an anti-spike ≥50AU/mL (Abbott Diagnostics). No patient seroconverted if they remained seronegative after 6 doses.

After 3 doses, the seroconversion rate was 73.7% (123 of 167) (Figure 1 and Figure 2). For patients seronegative after D2 (85 of 191), 68 received D3, and 27 of those 68 (39.7%) seroconverted. The median anti-spike level post-D3 rose from 144.6AU/mL to 1800.7AU/mL. To assess heterologous versus (vs) homologous vaccine doses, we analyzed a subset of 75 patients; 64 with Vaxzevria (AZ) as D1+2, then an mRNA vaccine for D3 (heterologous) vs 11 patients who received mRNA for D1+2+3 (all Cominarty - homologous). The anti-spike antibody increase in the heterologous cohort was 1.6 times higher than in the homologous cohort (95% confidence limits 0.5-5.28), adjusted for D2 response, but was not statistically significant (p=0.42) (Supplementary Figure 4A).

In CLL after D4, 81.7% seroconverted (>50AU/mL), but only 35.6% achieved a level >5000AU/mL capable of neutralization for Omicron; i.e. 64.4% of CLL patients are inadequately protected with 4 doses (Figure 2). After 4 and more doses, the number and proportion of CLL patients who seroconverted or achieved a higher anti-spike level increased, and therefore fewer patients proceeded with additional doses; hence the denominator of patients at risk progressively fell with multiple sequential doses (Supplementary Table 1 and Supplementary Figure 2). Sequentially for those who remained seronegative after their prior dose, seroconversion occurred in 40.6% (13/32) post D4, 46.2%

(6/13) post D5, 16.7% after D6 (1/6), and none after D7 (0/1) or D8 (0/1) (Supplementary Table 1).

However importantly, and in contrast to those with no detectable anti-spike antibody, CLL patients with detectable but low-level anti-spike antibodies frequently achieved a higher level of antibody with subsequent doses and the level typically progressively incremented with each sequential vaccine dose (Figure 1A and 1C). Hence the ultimate seroconversion rate for CLL was 94.2%, of whom 79.1% achieved anti-spike level ≥1,000AU/mL, 54.1% ≥5,000AU/mL, and 34.1% ≥10,000AU/mL. The later and higher anti-spike levels were commonly associated with neutralization activity against all major COVID-19 variants including Delta and Omicron. Seroconversion rates post each dose are summarized in Supplementary Table 1. Patients with only qualitative (i.e., positive or negative) results were not included in Figure 2.

***Anti-spike protein seroconversion and antibody levels following doses 3 to 5 in MBL patients***

In MBL, the seroconversion rate after D3 was 100% (29 of 29), including the 3 patients failed to seroconvert after D2 (Table 1). There were 11 MBL patients received a fourth dose and 3 received a fifth dose (Figure 2). After D4, while all achieve ≥50AU/mL, only 72.7% achieve ≥5000AU/mL; i.e. 27.3% of MBLs remain vulnerable. The median anti-spike level in MBL patients increased from 904.9AU/mL post-D2, to 6,188.2AU/mL post-D3, to 19,016.2AU/mL post-D4, and >25,000AU/mL post-D5 (Figure 1B). Hence, in MBL, multiple sequential vaccine doses progressively increment to higher anti-spike antibody levels. No significant vaccine adverse events were reported in MBL patients.

***Adverse events***

Adverse events were mainly mild and related to localized injection site or short-term systemic inflammatory responses. There were no serious adverse events reported with the Vaxzevria (AZ) vaccine, and specifically no episodes of Vaccine-induced thrombosis thrombocytopenia (VITT) syndrome. One patient required hospital admission for bradycardia following a first dose of Cominarty (mRNA, Pfizer) as his third total dose after initial two Vaxzevria; this did not recur with the next fourth total vaccine dose which was with Nuvaxovid (Novovax).

***Neutralization titers against D614G, Delta and Omicron variants following doses 2 to 8***

Detection of neutralizing antibody against the D614G, Delta and Omicron variants was performed on a total of 98 CLL and 13 MBL samples, including sequential samples collected ~4 weeks after each dose from the same patient in 50 CLL and 12 MBL patients. There were 27 samples collected post-D2, 34 post-D3, 21 post-D4, 10 post-D5, 5 post-D6, and 1 post-D7. Detection of neutralizing antibody against the three COVID-19 variants is summarized in Figure 3A and 3B.

Of the 98 CLL tested, anti-spike antibody levels did not reach the positive/negative threshold of ≥50AU/mL in 9 patients, and no neutralizing antibodies against any variant was detected in these 9. Of the remainder, 37 were 50-1000AU/mL, and 52 were ≥1000AU/mL.

There were 24 of 98 post-D2 samples that pre-dated emergence of the Omicron strain and previously reported[9] but retained in this study to enable comparison and statistical evaluation with subsequent vaccine doses.

Of all 98 CLL samples, 65.3% detect neutralizing antibody against early clade D614G, 52.0% against Delta, and 36.5% against Omicron (excluding the 24 samples that pre-dated Omicron). Detection of neutralizing antibody against all three COVID-19 variants demonstrated a moderate, non-linear, but statistically significant correlation (Spearman's definition) with anti-spike levels (Supplementary Figure 5). Correlation coefficients to the three variants are D614G 0.65, $p<0.0001$; Delta 0.53, $p=0.0029$; and Omicron 0.78, $p<0.0001$.

For all variants, the odds of a positive neutralizing response with anti-spike <1000 is markedly reduced compared with patients with anti-spike ≥1000. The ORs are similar for all variants at 0.012-0.017 (Figure 3C). We then evaluated differences between variants for those with an anti-spike level ≥1000AU/mL and also accounted for the number of doses received by the patients when calculating the odds of positive neutralization. The adjusted ORs ranked the likelihood of positive neutralization for a patient with anti-spike level ≥1000AU/mL as D614G>Delta>Omicron (Figure 3C).

**COVID-specific, T-cell responses following sequential doses**

IFN-γ and IL-2 responses specific to the SARS-CoV-2 peptide pool (Miltenyi) were measured in 23 patients by the FluoroSpot assay. Patients were selected to evaluate a broad spectrum of anti-spike levels and different number of doses. Of those 23 patients, 17 (73.9%) were positive for IFN-γ, 14 (60.9%) were positive for IL-2, and 14 (60.9%) positive for both IFN-γ

and IL-2. Based on the latest anti-spike levels of those 23 patients, we observed strong correlation between positive T-cells responses and high anti-spike levels (>5000AU/mL) (Figure 4A). All 7 patients who had an anti-spike level >25,000AU/mL showed consistent positive T-cell responses against the stimulatory SARS-CoV-2 peptide pool. Conversely, no correlation was found in patients with an anti-spike level <5000AU/mL. No correlation was found between T-cell responses and number of doses administered (Supplementary Figure 5).

Neutralizing activities was measured in 14 of the 23 patients assessed for T-cell responsiveness. Strong neutralizing activities against all three D614G, Delta and Omicron variants were observed in 7 patients, and were highly consistent with their positive T-cell responses in 6 out of those 7 patients (85.7%). The one patient with positive neutralizing activities but not an adequate T-cell response, had an anti-spike level of 1072AU/mL.

***Statistical analysis for persistent vaccine failure after multiple doses***

The association between best serological response post multiple doses and clinical variables was estimated using univariate logistic regression models. Immunoglobulin levels and treatments for CLL are shown in Supplementary Figures 6 and 7 and Supplementary Table 2, respectively. Statistically significant association was identified between the positive/adequate (anti-spike ≥1000AU/mL) serological responses and IgM ≥0.53g/L (OR=2.71, p=0.0209), IgG3 ≥0.22g/L (OR=4.06, p=0.0031, only assessed in patients without IgG replacement therapy [IgRT]), lack of CLL treatment in the last 12 months (OR=2.96, p=0.0048), and lack of current CLL therapy (OR=3.18, p=0.0081), including CD20 based therapy (OR=9.22, p=0.0501). Ibrutinib and venetoclax were associated with negative

response (ORs 2.33 and 1.73 respectively) but did not achieve a low p-value due to the small numbers of patients.

We then fitted a multivariate logistic regression model, modelling the odds of a positive/adequate (anti-spike ≥1000AU/mL) response based on clinical and biological variables identified in the univariate analysis described above. Variables statistically associated with an adequate anti-spike response include IgM ≥0.53g/L (OR=2.90, p=0.0314) and IgG3 ≥0.22g/L (OR=3.26, p=0.0057), and the lack of any current CLL therapy (OR=2.48, p=0.0574). Interesting, patients who received IgRT at any time (currently or previously) appeared to have a trend to a better anti-spike response (Figure 5B), possibly due to the emergence of anti-spike antibodies in the vaccinated IgRT source population[33].

***Statistical predictors and associations with vaccine responses***

Vaccine responses after multiple doses were highly variable ranging from 0AU/mL to >25,000AU/mL (upper reported limit of the Abbott assay). To enable evaluation of biological or clinical predictors of anti-spike levels, we categorized CLL patients into three cohorts based on their serological responses, also considering the number of doses administered. Univariate models were constructed for i) patients who had more than four doses but remain anti-spike <50AU/mL ("zero-responders"); ii) patients with anti-spike <1000AU/mL post-D3 but rose ≥1000AU/mL post-D4 or more ("late-responders"); and iii) patients who reached ≥20,000AU/mL at any point post-D1 ("high-responders"). Using these definitions, there were 11 patients identified as "zero-responders" and 204 as responders to any "positive" anti-spike level (i.e., ≥50AU/mL), while 28 patients were defined as "late-

responders” versus (vs) 67 who were not, and finally 36 patients were “high-responders” and 179 not.

There were large sample size discrepancies between the comparator groups, hence we focused on large or small OR values and used a p-value cut-off of $p<0.2$ for this analysis. Using the approach described above, being a “zero-responder” vs any response was highly associated with reduced IgA (OR=3.08, p=0.1659), reduced IgG2 (OR=5.86, p=0.1443), reduced IgG3 (OR=8.21, p=0.0233), treatment in the last 12 months (OR=7.31, p=0.0048), and current ibrutinib treatment (OR=4.43, p=0.1259). Factors associated with being a “late-responder" vs other response patterns included reduced IgM (OR=3.09, p=0.1396), and reduced IgM as a sole immunoglobulin abnormality (OR=3.38, p=0.0875). Current ibrutinib treatment (OR=2.41, p=0.2149) was not statistically significant but may be clinically relevant. Failure to become a “high-responder” was associated with reduced IgM (OR=0.21, p=0.0013), reduced IgA (OR=0.36, p=0.1573), reduced IgG3 (OR=0.23, p=0.0521), treatment in the last 12 months (OR=0.14, p=0.04), current ibrutinib treatment (OR=0.20, p=0.0475), and female sex (OR=0.47, p=0.1250) (Figure 5). All BTKi treated patients received ibrutinib.

## Discussion

Immune impairment in CLL, and also MBL, leads to a significantly impaired response to COVID-19 vaccination with lower and slower rates of both seroconversion and anti-spike antibodies levels generated compared to normal individuals.[9-12,34-36] The Israeli group recently documented that a third dose in CLL seroconverts 23.8% of those negative after D2.[25] We found a somewhat higher rate of 39.7% D2 to D3 seroconversion, very similar to the French study of 35% where all 3 doses were mRNA vaccine but used the same Abbott assay for anti-spike levels.[27] These differences may reflect the CLL population under study (higher or lower numbers with advanced disease), different vaccine use (all Comirnaty in Israel, vs mainly Vaxzevria followed by Comirnaty in Australia), and possibly the assay used to measure for anti-spike antibody (Roche vs Abbott) that have different non-interchangeable numerical values and assay kinetics.[37,38] In any event, it highlights the principal that additional doses provide seroconversion in a significant proportion of patients.

The key messages from this study are that multiple sequential COVID-19 vaccine doses will ultimately seroconvert a very high proportion of 94.2% with CLL, more than doubling the rate after 2 doses, and all patients with MBL and generate progressive rises in the anti-spike antibody level in most patients, thereby providing in some anti-spike levels necessary for neutralization of the Delta and Omicron variants of SARS-CoV-2. High anti-spike levels achieved by multiple vaccine are associated with stronger COVID-specific T-cell responses.

There are compelling arguments to support strategies to achieve an endogenous immune response to COVID-19 vaccination. Access to prophylactic monoclonal antibody therapies that had high neutralization activity to early variants such as tixagevimab and cilgavimab (Evusheld) remains difficult in many areas of the globe, including Australia at the time of the

study for patients with CLL (Supplementary Table 3), despite being the hematological malignancy with the highest rate of vaccine failure.[35,39] Furthermore, there is already evidence that the Omicron family of variants BA.1, 2 and 4, 5 are less sensitive to tixagevimab and cilgavimab (Evusheld)[17,19,40] and Sotrovimab[17] than prior COVID-19 variants, and hence there is an ongoing risk that future COVID-19 mutations, strains and variants may further escape its effect rendering this therapy potentially ineffective. For example, whilst tixagevimab and cilgavimab (Evusheld) had high neutralizing activity against early variants (20ng/mL), its activity is now 14-fold lower at around 300ng/mL and effectively a monotherapy as tixagevimab has no activity to BA.2 and BA.5.[17] BA.5 drops the potency for tixagevimab and cilgavimab (Evusheld) and Sotrovimab by 14.3-fold and 16.8-fold respectively.[17] Therefore, immunocompromised patients are left with Paxlovid, Molnupiravir or the less potent Remdesivir as their remaining effective options. Vaccination also produces a COVID-specific T-cell response that may be very important in long-term protection from severe COVID disease and mortality[41], and a T-cell response cannot develop from passive antibody therapy such as tixagevimab and cilgavimab (Evusheld) or pooled, convalescent immunoglobulin.

The number of individuals at risk is considerable. CLL is the most common hematological malignancy with a high prevalence arising from prolonged survival with current treatment options. By comparison however, MBL is extraordinarily common with a clone detectable in ~10% of the adult population over aged >60 years[42-44]; we recently postulated that unrecognized MBL with its attendant immune impairment may be one factor leading to higher risk of severe COVID disease in those >60 years.[21] Vaccination strategies of 2 to 4 doses based on data from immunocompetent normal individuals leave many immunocompromised patients with CLL and MBL, including unrecognized MBL,

inadequately protected. After D4 only 35.6% of CLL and 73.7% of MBL had anti-spike levels ≥5,000AU/mL, the lowest threshold for Omicron neutralizing antibodies. If provided with vaccine access, over 94% of CLL patients will seroconvert, and most achieve higher anti-spike levels. Hence, strategies for achieving seroconversion, with higher endogenous antibody production remains an important unmet need. The results of this and other recent studies showing improved humoral responses after a third vaccine dose in CLL patients provides support for tailored vaccine strategies incorporating serological testing and multiple sequential vaccine dosing.[20,25,26,45]

It remains unclear whether the progressive seroconversion or the progressive rise in anti-spike levels result primarily from the total vaccine dosage, sequencing, interval spacing between doses, or the combination. Multiple vaccine options are universally available, and relatively inexpensive in most countries. Furthermore, at the time of writing, Pfizer[46] and Moderna[47] have announced bivalent mRNA vaccines combining high viral neutralizing activity against both earlier viral clade and Omicron BA.4 and BA.5.

Neutralization data in this study demonstrates that seroconversion *per se* with a low anti-spike level (i.e. ≥50 but <1000AU/mL) is insufficient to provide viral neutralization protection[9,48] and worse against later variants (Figure 3). Indeed, neutralization against Omicron was not observed until the anti-spike level surpassed 5000AU/mL, and consistent neutralization of Omicron was only observed with anti-spike level ≥20,000AU/mL. Similar data was reported in the literature[13], in particular Parry *et al.*[49] demonstrated a 28% reduction in neutralization capacity against Delta compared to the Wuhan variant. We fitted a generalized mixed model (using SAS PROC GLIMMIX) to identify determinants of the neutralization antibody response against D614G, Delta and Omicron (positive/negative as

the outcome). Our analysis, consistent with others[40,49] showed that novel variants, anti-spike level <1000AU/mL and fewer doses are contributing factors for poor neutralization with statistical significance (Figure 3D). Anti-spike levels interpreted together with neutralizing data show the majority of CLL patients lack sufficient neutralizing activity, especially against the latest COVID variants.[50]

COVID-19-specific T-cell function also showed a strong association with higher serological responses (anti-spike ≥5000AU/mL) after multiple doses. Less than half (5/12, 41.7%) the patients with anti-spike <5000AU/mL had normal levels of both IFN-γ and IL-2, compared to 81.8% (9/11) with anti-spike levels ≥5000AU/mL. Although a strong T-cell response was almost always associated with high anti-spike antibody level, good T-cell responses were not necessarily accompanied by high anti-spike levels with neutralizing activity. Similar results were shown in the literature demonstrating a heterogeneous mix of T-cell responses amongst all CLL patients[51], while weak correlation between anti-spike level and IFN-γ was observed.[52] It remains unclear to what extent the B-cell vs the T-cell response contribute to protection against severe COVID disease, especially pneumonitis.[41]

Univariate analysis for parameters associated with positive/adequate (anti-spike ≥1000AU/mL) serological responses showed correlations that included IgM ≥0.53g/L, IgG3 ≥0.22g/L, lack of treatment in the last 12 months, lack of current CLL therapy, including CD20 containing therapy, all consistent with our previous study[9], except for IgG2 (OR=1.85, p=0.1763). While the OR for ibrutinib and venetoclax of 2.33 and 1.73 respectively did not achieve a low p-value due to the small numbers of patients it is possible that these reflect clinically significant risks for failure to seroconvert. The association between vaccine

response and IgG3 ≥0.22g/L again suggests that the IgG3 subclass is important in COVID-19 vaccine efficacy.[53]

Reduced IgM is again identified as a major contributing factor to poor serological responses and with being a "late-responder". IgM deficiency is extremely common in early-stage CLL[3], 72.2% in the current cohort, and almost half (49.3%) of MBL in our center (unpublished data). The importance of IgM in the primary immune response was apparent in serological responses after initial 2 doses, and this study reinforces this finding with multiple vaccine doses. Total IgG levels, as opposed to adequate IgG2 or IgG3 subclass levels, were not associated with positive vaccine response, in keeping with not being an important determinant of COVID-19 vaccine response.

Multivariate analysis confirmed IgM ≥0.53g/L (OR=2.90, p=0.0314), IgG3 ≥0.22g/L (OR=3.26, p=0.0057) and the lack of current CLL therapy (OR=2.48, p=0.0574) as key statistical associations with adequate serological responses (anti-spike ≥1000AU/mL), consistent with our previous report and the literature.[9-12,25-27] Unlike others[10,11,25], reduced IgA was associated with either complete vaccine failure or failure to achieve anti-spike ≥20,000AU/mL after multiple doses, in univariate analyses. Differences in the diagnostic anti-spike antibody assay performance[54,55] (Abbott[9,12] vs Roche[10,11]) may contribute to discrepancies in the identification of statistically significant predictors of responses.

In summary, multiple sequential COVID-19 vaccine doses significantly increase serological responses, both seroconversion and higher anti-spike antibody levels, and of neutralizing activities, and furthermore still resulted in stronger COVID-19-specific T-cell response in a high proportion of CLL and virtually all MBL patients. This data is consequential and globally applicable for immunocompromised CLL and MBL patients. COVID-19 vaccination policies

must support the specific needs of these patients and afford them an optimal level of protection as the pandemic proceeds.

**Author contribution**

YS, JF, JH, KN, AS, PD, IK, LW, NB, SM collected the data. SM, JF, PD, IK confirmed the accuracy of the clinical data. VM, AA, GG, CD, KS, AC, ST, TC performed neutralization and Elispot experiments and interpreted the data. YS compiled the data for statistical analysis. AS performed the statistical analysis. YS and SM prepared the final manuscript, which all authors reviewed and approved.

**Conflict of Interest Statement**

There is no conflict of interest to report.

## Reference


1. Andersen MA, Vojdeman FJ, Andersen MK, et al. Hypogammaglobulinemia in newly diagnosed chronic lymphocytic leukemia is a predictor of early death. *Leuk Lymphoma*. 2016;57(7):1592-1599.
2. Crassini KR, Zhang E, Balendran S, et al. Humoral immune failure defined by immunoglobulin class and immunoglobulin G subclass deficiency is associated with shorter treatment-free and overall survival in Chronic Lymphocytic Leukaemia. *Br J Haematol*. 2018;181(1):97-101.
3. Freeman JA, Crassini KR, Best OG, et al. Immunoglobulin G subclass deficiency and infection risk in 150 patients with chronic lymphocytic leukemia. *Leuk Lymphoma*. 2013;54(1):99-104.
4. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. *Blood*. 2015;126(5):573-581.
5. Scarfò L, Chatzikonstantinou T, Rigolin GM, et al. COVID-19 severity and mortality in patients with chronic lymphocytic leukemia: a joint study by ERIC, the European Research Initiative on CLL, and CLL Campus. *Leukemia*. 2020;34(9):2354-2363.
6. Mato AR, Roeker LE, Lamanna N, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. *Blood*. 2020;136(10):1134-1143.
7. Roeker LE, Eyre TA, Thompson MC, et al. COVID-19 in patients with CLL: improved survival outcomes and update on management strategies. *Blood*. 2021;138(18):1768-1773.
8. Langerbeins P, Hallek M. COVID-19 in patients with hematologic malignancy. *Blood*. 2022.
9. Shen Y, Freeman JA, Holland J, et al. COVID-19 vaccine failure in chronic lymphocytic leukaemia and monoclonal B-lymphocytosis; humoural and cellular immunity. *British Journal of Haematology*. 2022;197(1):41-51.
10. Parry H, McIlroy G, Bruton R, et al. Antibody responses after first and second Covid-19 vaccination in patients with chronic lymphocytic leukaemia. *Blood Cancer Journal*. 2021;11(7):136.
11. Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood*. 2021;137(23):3165-3173.
12. Benjamini O, Rokach L, Itchaki G, et al. Safety and efficacy of the BNT162b mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Haematologica*. 2022;107(3):625-634.
13. Mellinghoff SC, Robrecht S, Mayer L, et al. SARS-CoV-2 specific cellular response following COVID-19 vaccination in patients with chronic lymphocytic leukemia. *Leukemia*. 2022;36(2):562-565.
14. Stefano M, Francesca RM, Diana G, et al. Differentiating chronic lymphocytic leukemia from monoclonal B-lymphocytosis according to clinical outcome: on behalf of the GIMEMA chronic lymphoproliferative diseases working group. *Haematologica*. 2011;96(2):277-283.
15. Chatzikonstantinou T, Herishanu Y, Montserrat E, et al. COVID-19 and Chronic Lymphocytic Leukemia: Where We Stand Now. *Cancer J*. 2021;27(4):328-333.
16. Planas D, Saunders N, Maes P, et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature*. 2022;602(7898):671-675.
17. Aggarwal A, Akerman A, Milogiannakis V, et al. SARS-CoV-2 Omicron BA.5: Evolving tropism and evasion of potent humoral responses and resistance to clinical immunotherapeutics relative to viral variants of concern. *medRxiv*. 2022:2022.2007.2007.22277128.
18. Aggarwal A, Stella AO, Walker G, et al. Platform for isolation and characterization of SARS-CoV-2 variants enables rapid characterization of Omicron in Australia. *Nat Microbiol*. 2022;7(6):896-908.
19. Tao K, Tzou PL, Kosakovsky Pond SL, Ioannidis JPA, Shafer RW. Susceptibility of SARS-CoV-2 Omicron Variants to Therapeutic Monoclonal Antibodies: Systematic Review and Meta-analysis. *Microbiol Spectr*. 2022:e0092622.
20. Greenberger LM, Saltzman LA, Senefeld JW, Johnson PW, DeGennaro LJ, Nichols GL. Sars-Cov-2 Antibody Levels in Blood Cancer Patients after a Third Sars-Cov-2 "Booster" Vaccination - Observational Data from the LLS National Registry. *Blood*. 2021;138(Supplement 1):185-185.

21. Tang C, Shen Y, Soosapilla A, Mulligan SP. Monoclonal B-cell Lymphocytosis - a review of diagnostic criteria, biology, natural history, and clinical management. *Leuk Lymphoma*. 2022:1-12.
22. Whitaker JA, Parikh SA, Shanafelt TD, et al. The humoral immune response to high-dose influenza vaccine in persons with monoclonal B-cell lymphocytosis (MBL) and chronic lymphocytic leukemia (CLL). *Vaccine*. 2021;39(7):1122-1130.
23. Shanafelt TD, Kay NE, Parikh SA, et al. Risk of serious infection among individuals with and without low count monoclonal B-cell lymphocytosis (MBL). *Leukemia*. 2021;35(1):239-244.
24. Shim YK, Rachel JM, Ghia P, et al. Monoclonal B-cell lymphocytosis in healthy blood donors: an unexpectedly common finding. *Blood*. 2014;123(9):1319-1326.
25. Herishanu Y, Rahav G, Levi S, et al. Efficacy of a third BNT162b2 mRNA COVID-19 vaccine dose in patients with CLL who failed standard 2-dose vaccination. *Blood*. 2022;139(5):678-685.
26. Parry H, Bruton R, Roberts T, et al. COVID-19 vaccines elicit robust cellular immunity and clinical protection in chronic lymphocytic leukemia. *Cancer Cell*. 2022;40(6):584-586.
27. Bagacean C, Letestu R, Al-Nawakil C, et al. Humoral response to mRNA anti-COVID-19 vaccines BNT162b2 and mRNA-1273 in patients with chronic lymphocytic leukemia. *Blood Adv*. 2022;6(1):207-211.
28. Ehmsen S, Asmussen A, Jeppesen SS, et al. Antibody responses following third mRNA COVID-19 vaccination in patients with cancer and potential timing of a fourth vaccination. *Cancer cell*. 2022;40(4):338-339.
29. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*. 2018;131(25):2745-2760.
30. McCaughan G, Di Ciaccio P, Ananda-Rajah M, et al. COVID-19 vaccination in haematology patients: an Australian and New Zealand consensus position statement. *Internal Medicine Journal*. 2021;51(5):763-768.
31. Tea F, Ospina Stella A, Aggarwal A, et al. SARS-CoV-2 neutralizing antibodies: Longevity, breadth, and evasion by emerging viral variants. *PLoS Med*. 2021;18(7):e1003656.
32. Lee E, Sandgren K, Duette G, et al. Identification of SARS-CoV-2 Nucleocapsid and Spike T-Cell Epitopes for Assessing T-Cell Immunity. *J Virol*. 2021;95(6).
33. Seroprevalence of SARS-CoV-2-specific antibodies among Australian blood donors, February–March 2022. https://kirby.unsw.edu.au/report/seroprevalence-sars-cov-2-specific-antibodies-among-australian-blood-donors-february-march: The Australian COVID-19 Serosurveillance Network 2022.
34. Roeker LE, Knorr DA, Thompson MC, et al. COVID-19 vaccine efficacy in patients with chronic lymphocytic leukemia. *Leukemia*. 2021;35(9):2703-2705.
35. Herzog Tzarfati K, Gutwein O, Apel A, et al. BNT162b2 COVID-19 vaccine is significantly less effective in patients with hematologic malignancies. *Am J Hematol*. 2021;96(10):1195-1203.
36. Roeker LE, Knorr DA, Pessin MS, et al. Anti-SARS-CoV-2 antibody response in patients with chronic lymphocytic leukemia. *Leukemia*. 2020;34(11):3047-3049.
37. Perkmann T, Mucher P, Perkmann-Nagele N, et al. The Comparability of Anti-Spike SARS-CoV-2 Antibody Tests is Time-Dependent: a Prospective Observational Study. *Microbiol Spectr*. 2022;10(1):e0140221.
38. Perkmann T, Perkmann-Nagele N, Koller T, et al. Anti-Spike Protein Assays to Determine SARS-CoV-2 Antibody Levels: a Head-to-Head Comparison of Five Quantitative Assays. *Microbiol Spectr*. 2021;9(1):e0024721.
39. Teh JSK, Coussement J, Neoh ZCF, et al. Immunogenicity of COVID-19 vaccines in patients with hematologic malignancies: a systematic review and meta-analysis. *Blood Advances*. 2022;6(7):2014-2034.
40. Wang Q, Guo Y, Iketani S, et al. Antibody evasion by SARS-CoV-2 Omicron subvariants BA.2.12.1, BA.4, & BA.5. *Nature*. 2022.

41. Moss P. The T cell immune response against SARS-CoV-2. *Nature Immunology*. 2022;23(2):186-193.
42. Rawstron AC, Green MJ, Kuzmicki A, et al. Monoclonal B lymphocytes with the characteristics of "indolent" chronic lymphocytic leukemia are present in 3.5% of adults with normal blood counts. *Blood*. 2002;100(2):635-639.
43. Nieto WG, Almeida J, Romero A, et al. Increased frequency (12%) of circulating chronic lymphocytic leukemia–like B-cell clones in healthy subjects using a highly sensitive multicolor flow cytometry approach. *Blood*. 2009;114(1):33-37.
44. Ghia P, Prato G, Scielzo C, et al. Monoclonal CD5+ and CD5- B-lymphocyte expansions are frequent in the peripheral blood of the elderly. *Blood*. 2004;103(6):2337-2342.
45. Re D, Seitz-Polski B, Brglez V, et al. Humoral and cellular responses after a third dose of SARS-CoV-2 BNT162b2 vaccine in patients with lymphoid malignancies. *Nature Communications*. 2022;13(1):864.
46. Pfizer. Pfizer and BioNTech Announce Omicron-Adapted COVID-19 Vaccine Candidates Demonstrate High Immune Response Against Omicron. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-announce-omicron-adapted-covid-19; June 25, 2022.
47. Moderna. Moderna announces Omicron-containing bivalent bosster candidate mRNA-1273.214 demonstrates superior antibody response against Omicron https://investors.modernatx.com/news/news-details/2022/Moderna-Announces-Omicron-Containing-Bivalent-Booster-Candidate-mRNA-1273.214-Demonstrates-Superior-Antibody-Response-Against-Omicron/default.aspx; June, 08, 2022.
48. Sun C. COVID-19 vaccine response in chronic lymphocytic leukaemia is more than just seroconversion. *British Journal of Haematology*. 2022;197(1):11-12.
49. Parry H, McIlroy G, Bruton R, et al. Impaired neutralisation of SARS-CoV-2 delta variant in vaccinated patients with B cell chronic lymphocytic leukaemia. *Journal of Hematology & Oncology*. 2022;15(1):3.
50. Lyke KE, Atmar RL, Islas CD, et al. Rapid decline in vaccine-boosted neutralizing antibodies against SARS-CoV-2 Omicron variant. *Cell Rep Med*. 2022:100679.
51. Liebers N, Speer C, Benning L, et al. Humoral and cellular responses after COVID-19 vaccination in anti-CD20-treated lymphoma patients. *Blood*. 2022;139(1):142-147.
52. Blixt L, Wullimann D, Aleman S, et al. T-cell immune responses following vaccination with mRNA BNT162b2 against SARS-CoV-2 in patients with chronic lymphocytic leukemia: results from a prospective open-label clinical trial. *Haematologica*. 2022;107(4):1000-1003.
53. Kallolimath S, Sun L, Palt R, et al. Highly active engineered IgG3 antibodies against SARS-CoV-2. *Proceedings of the National Academy of Sciences*. 2021;118(42):e2107249118.
54. Narasimhan M, Mahimainathan L, Araj E, et al. Clinical Evaluation of the Abbott Alinity SARS-CoV-2 Spike-Specific Quantitative IgG and IgM Assays among Infected, Recovered, and Vaccinated Groups. *Journal of Clinical Microbiology*. 2021;59(7):e00388-00321.
55. Wehrhahn MC, Brown SJ, Newcombe JP, et al. An evaluation of 4 commercial assays for the detection of SARS-CoV-2 antibodies in a predominantly mildly symptomatic low prevalence Australian population. *J Clin Virol*. 2021;138:104797.
56. Di Ciaccio P, McCaughan G, Trotman J, et al. Australian and New Zealand consensus statement on the management of lymphoma, chronic lymphocytic leukaemia and myeloma during the COVID-19 pandemic. *Internal Medicine Journal*. 2020;50(6):667-679.
57. Weinkove R, McQuilten ZK, Adler J, et al. Managing haematology and oncology patients during the COVID-19 pandemic: interim consensus guidance. *Med J Aust*. 2020;212(10):481-489.

**Figure and table legends**

**Table 1. General patient information**

**Figure 1. Sequential post vaccination anti-spike protein IgG levels in CLL (A) and MBL (B) patients and treatment history (C).** An anti-spike level >50AU/mL is classified as positive response and an anti-spike level ≥1000AU/mL is classified as strong positive response. Red bars in panel C indicate the median anti-spike levels in each group. An anti-spike level of 0 could not be displayed in this figure. Although Australia had low COVID case numbers, consensus guidelines developed at the beginning of the pandemic recommended deferral of CLL therapy where possible during COVID outbreaks.[56,57] Hence the number of patients on therapy (Panel C, box 4) are relatively low.

**Figure 2. Anti-SARS-Cov2 Spike antibody level stratification in CLL and MBL patients post vaccination.** Only patients with quantitative anti-spike antibody level available was included.

**Figure 3. Detection of neutralization antibodies against D614G, Delta and Omicron variants of SARS-Cov-2.** (A) Heatmap summarizes the distribution of anti-spike levels of each sample tested and their corresponding neutralizing activities against D614G, Delta and Omicron. Red, yellow, and green each indicates low, medium, and high anti-spike levels, respectively. (B) Bar chart shows the proportion of samples with positive or negative neutralization and were split into four stratifications of anti-spike levels. (C) Odds ratio (OR)

indicates the differences between patients with anti-spike levels <1000AU/mL and ≥1000AU/mL (top), or the differences between strains for those with an anti-spike protein level greater than 1000AU/mL (bottom), i.e., the odds of a positive neutralizing antibody response against Delta was only 0.186 times the odds of that against D614G. The OR was adjusted for number of doses. (D) Determinants of neutralization response (positive/negative). The overall p-values accounted for different variants, number of doses, serological responses (positive/negative) and anti-spike levels. The anti-spike per variant was not significant. I.e., for all three variants, the patients with anti-S≥1000AU/mL are always consistently better than those with anti-S<1000AU/mL. All other terms are statistically significant (having 'adjusted' for the others).

**Figure 4. T-cell function analysis on CLL patients post multiple vaccination.** The IFN-γ and IL-2 responses were tested against the Miltenyi Spike peptide pool. The mean number of responding cells in negative controls were subtracted from stimulated samples to account for background responses and results were expressed as spot forming units (SFU) per 10^6 cells. The positivity threshold (dotted line) was set based on the average number of responding cells across all negative controls in the cohort + 3x standard deviation. Median (red) and 95% confidence interval was plotted for each anti-spike protein antibody level strata.

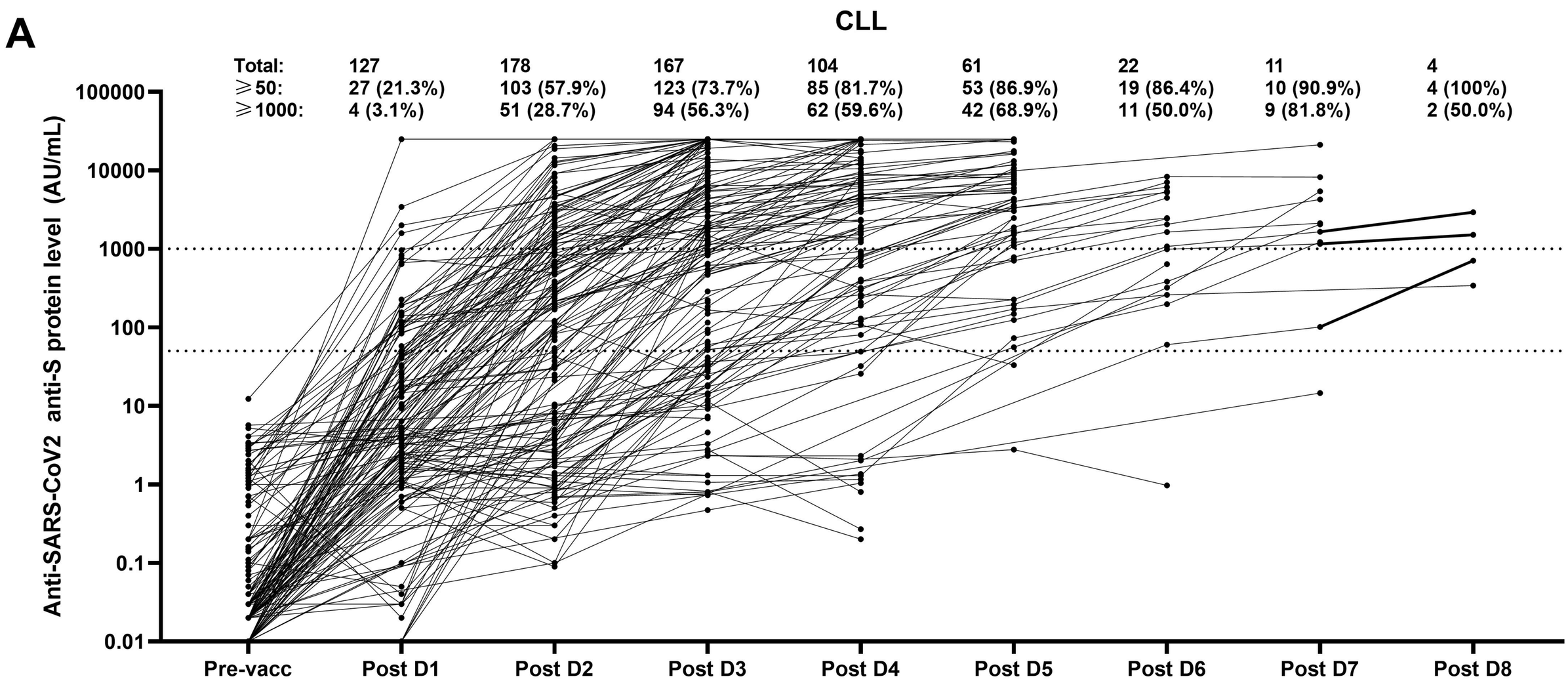
**Figure 5. Univariate and multivariate analysis of serological responses**. Odds ratio (OR) for positive serological response (anti-spike≥1000AU/mL) to COVID vaccine calculated by univariate (A) and multivariate (B) logistic regression models. Biological and clinical factors,

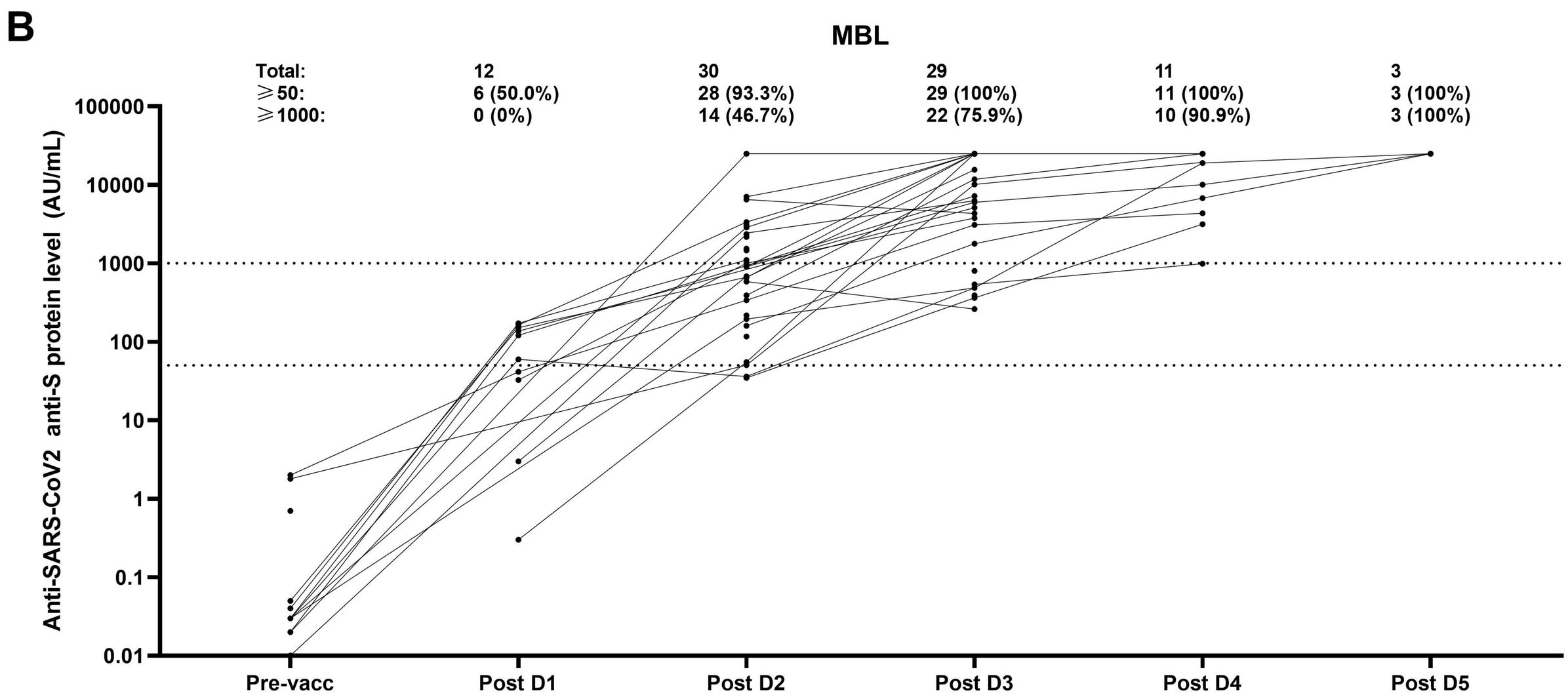
using univariate models, associated with “zero-responders” (C) (more than four doses but remain anti-spike<50AU/mL); “late-responders” (D) (anti-spike<1000AU/mL after three doses but rose over 1000AU/mL after the fourth dose); “high-responders” (E) (anti-spike≥20000AU/mL at any point after their first dose). Any analyses of IgG levels (or IgG subclasses) excluded patients on IgG replacement therapy. All BTKi treated patients received ibrutinib. $p<0.2$ was used as the cutoff value for the above models.

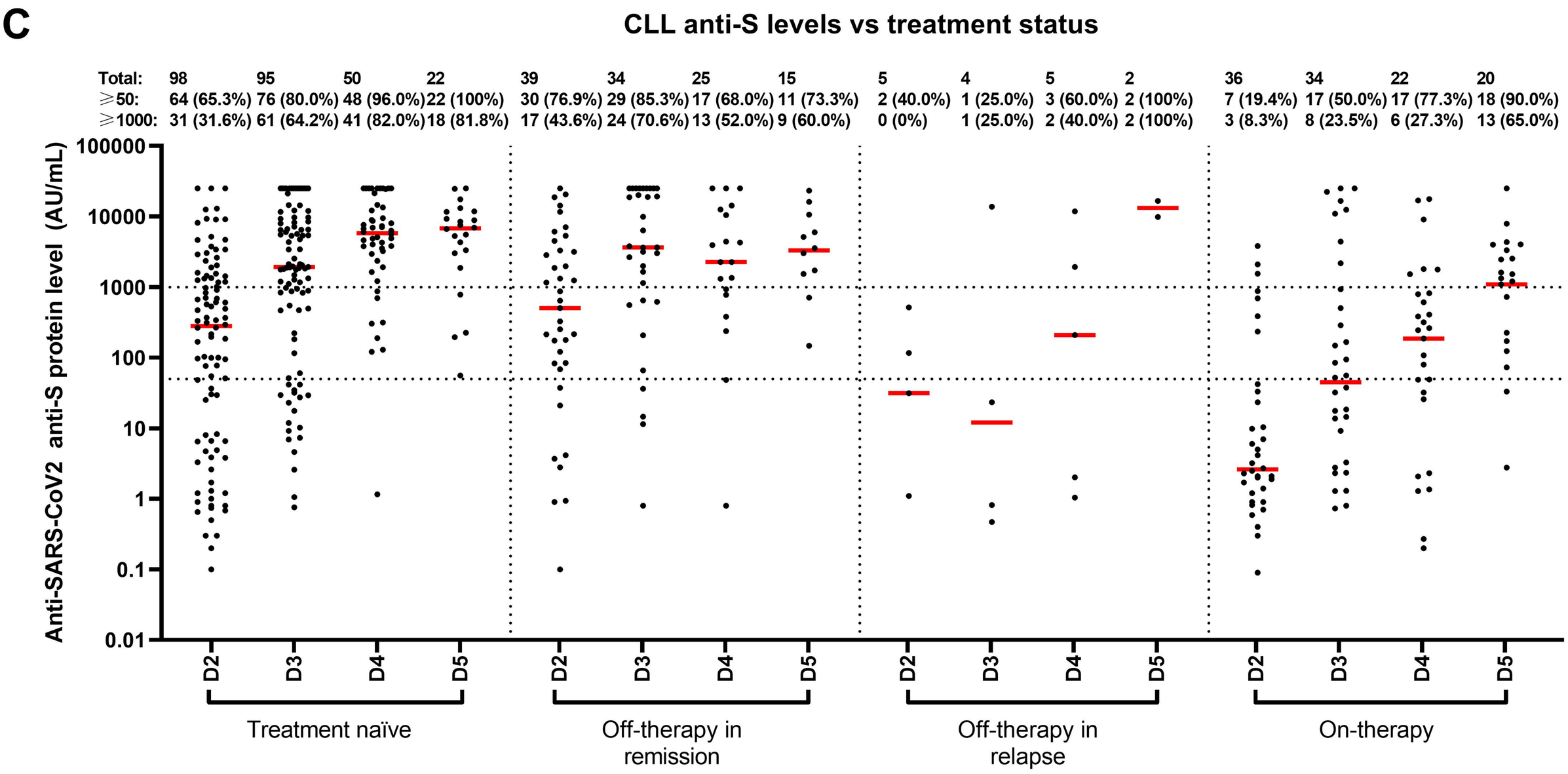
**Table 1. General patient information**

| **Total number of patients (n=258)** | CLL (n=215) | MBL (n=43) |
|---|---|---|
| Males | 120 (55.8%) | 14 (32.6%) |
| Median age (years) | 72 (22-95) | 72 (49-88) |
| Median age at diagnosis (years) | 60 (18-85) | 67.5 (27-84) |
| **Seroconversion (at the time of analysis)** | | |
| Positive | 180 (94.2%) | 43 (100%) |
| Negative | 11 (5.8%) | 0 (0%) |
| Lost contact | 24 | 0 |
| **Cytogenetics** | | |
| Del 13q | 92/145 (63.4%) | 6/10 (60.0%) |
| Del 11q | 16/145 (11.0%) | 0/10 |
| Del 17p | 10/145 (6.9%) | 0/10 |
| Trisomy 12 | 23/145 (15.9%) | 1/10 (10.0%) |
| Unknown | 70 | 36 |
| **IGHV** | | |
| Mutated | 27 | - |
| Unmutated | 7 | - |
| Unknown | 181 | 43 |
| **Treatment status at vaccination** | | |
| Treatment naïve | 119 (55.3%) | 43 (100%) |
| Off-therapy in remission (CR or PR) | 46 (21.4%) | 0 |
| Off-therapy in relapse | 6 (2.8%) | 0 |
| On-therapy | 44 (20.5%) | 0 |
| **Ig replacement therapy** | | |
| Currently on | 27 (12.6%) | 0 |
| Prior but not current | 10 (4.7%) | 0 |
| Never on Ig replacement therapy | 149 (69.3%) | 43 (100%) |
| Unknown | 29 (13.5%) | - |
| **Anti-CD20 inhibitor in the last 12 months** | | |
| Yes | 14 (6.5%) | 0 |
| No | 201 (93.5%) | 43 |

# Figure 1. Sequential post vaccination anti-spike protein IgG levels.



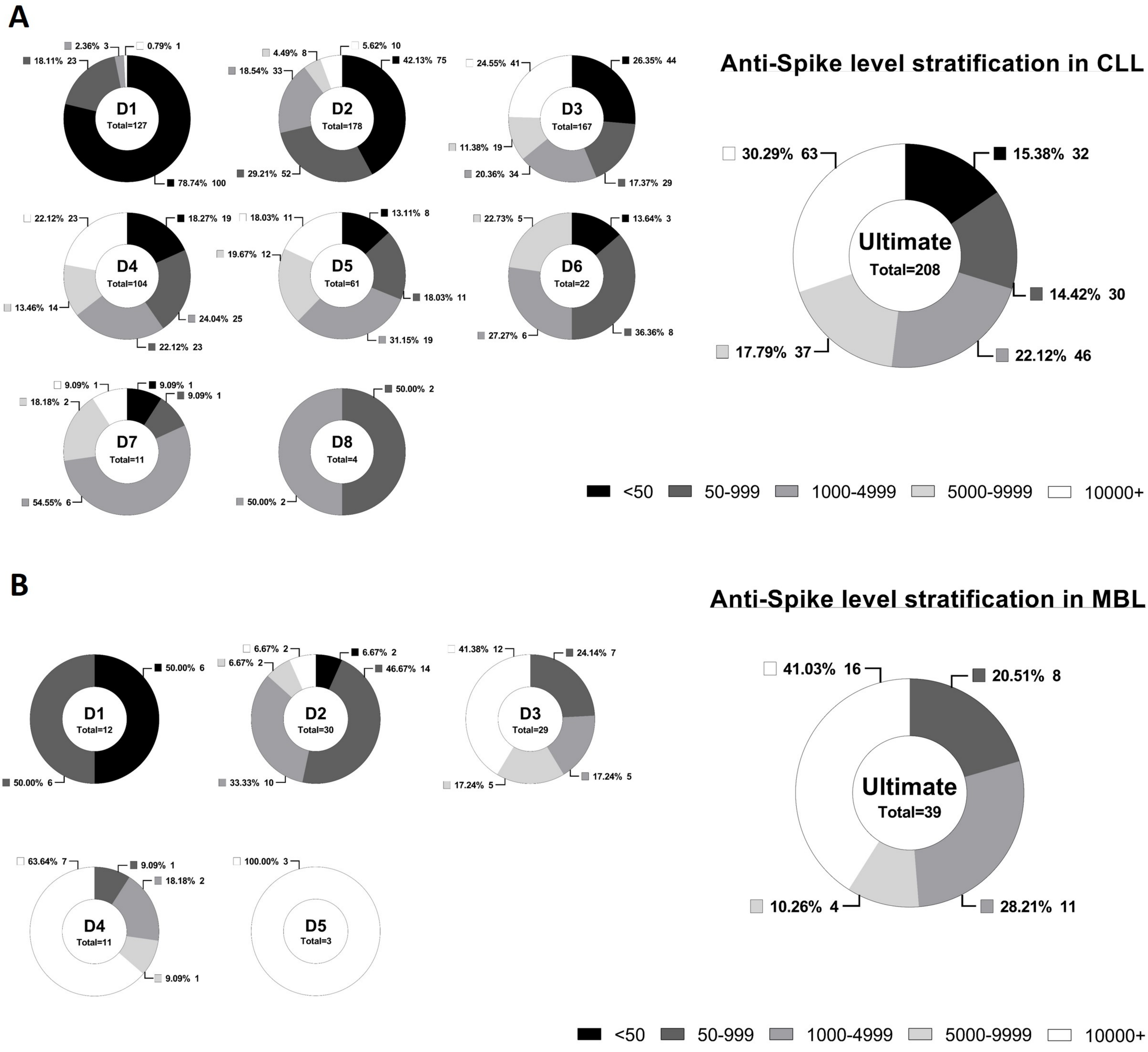


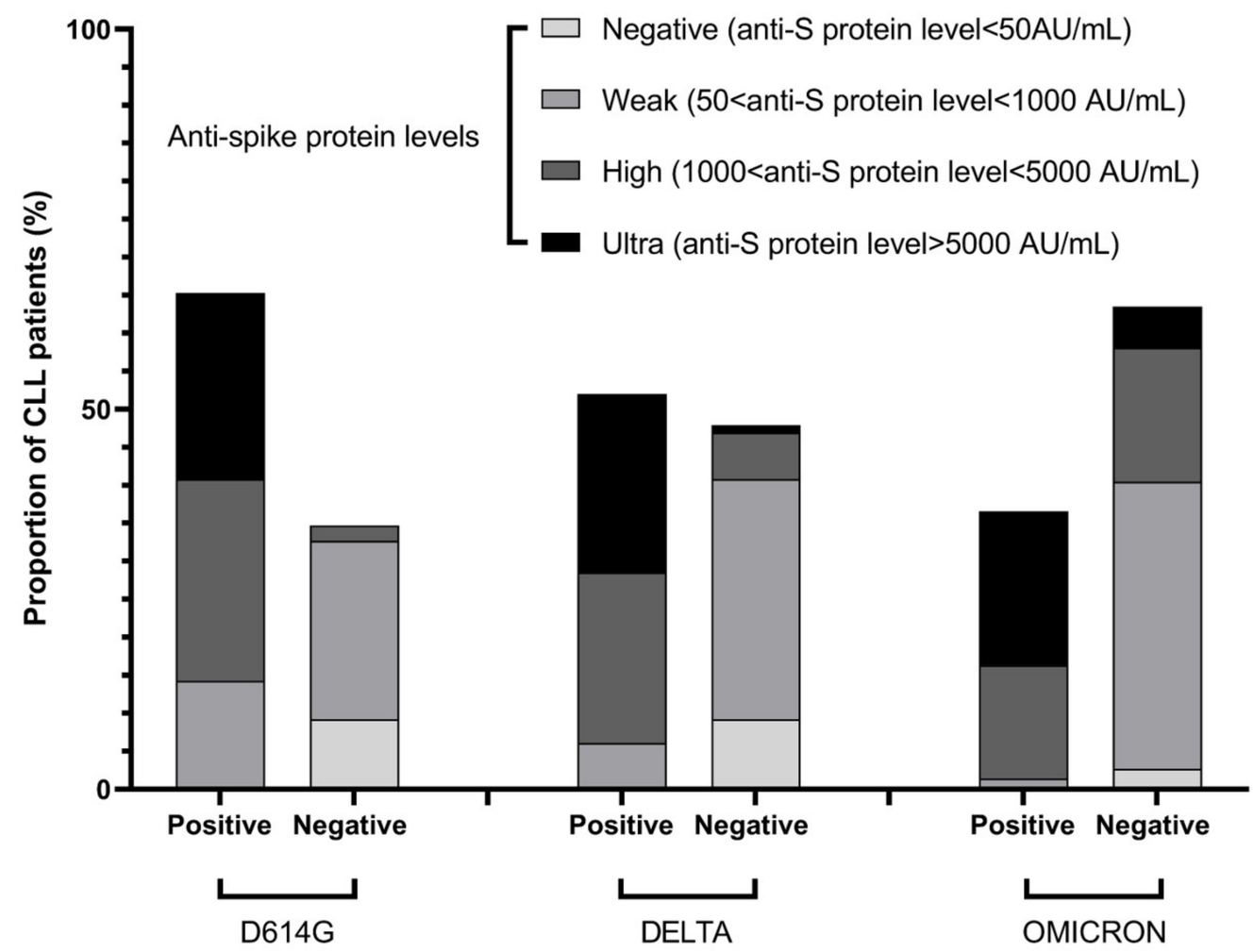

**Figure 2. Anti-SARS-Cov2 Spike antibody level stratification in CLL and MBL patients post vaccination.**

# Figure 3. Detection of neutralization antibodies against D614G, Delta and Omicron variants of SARS-Cov-2.

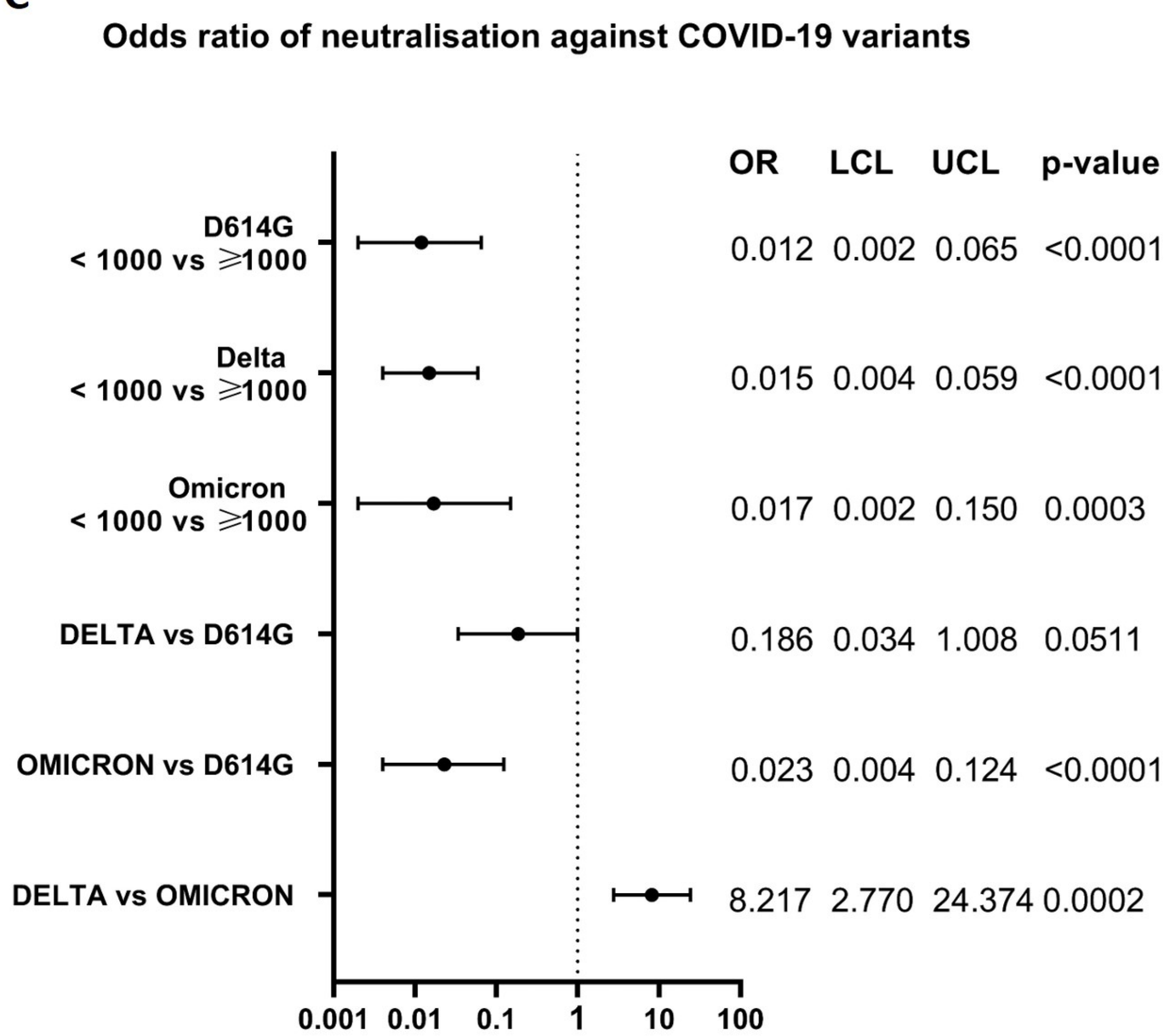
**A**

| | Anti-Spike protein levels | Neutralising antibody against D614G | DELTA | OMICRON |
|---|---|---|---|---|
| 0-50AU/mL | 2.1 | 0 | 0 | |
| | 5 | 0 | 0 | |
| | 9.8 | 0 | 0 | |
| | 10.4 | 0 | 0 | |
| | 23.4 | 0 | 0 | |
| | 30.2 | 0 | 0 | |
| | 37.4 | 0 | 0 | |
| | 48.69 | 0 | 0 | 0 |
| | 49.01 | 0 | 0 | 0 |
| 50-1000AU/mL | 52.29 | 0 | 0 | 0 |
| | 60.2 | 0 | 0 | 0 |
| | 60.21 | 40 | 0 | 0 |
| | 80.05 | 0 | 0 | 0 |
| | 84 | 0 | 0 | |
| | 84.55 | 0 | 0 | 0 |
| | 94 | 0 | 0 | 0 |
| | 95.2 | 40 | 0 | |
| | 101.12 | 40 | 0 | 0 |
| | 103.4 | 0 | 0 | 0 |
| | 120.68 | 0 | 0 | 0 |
| | 124.08 | 40 | 0 | 0 |
| | 129.71 | 160 | 160 | 0 |
| | 141.9 | 0 | 0 | |
| | 166.85 | 40 | 80 | 0 |
| | 177.6 | 0 | 0 | |
| | 189.5 | 0 | 0 | 0 |
| | 195.1 | 40 | 0 | 0 |
| | 214.7 | 0 | 0 | |
| | 225.77 | 640 | 160 | 0 |
| | 259.72 | 0 | 0 | 0 |
| | 302.04 | 0 | 0 | 0 |
| | 333 | 0 | 0 | 0 |
| | 381.95 | 0 | 0 | 0 |
| | 382.32 | 20 | 0 | 0 |
| | 466.65 | 0 | 0 | 0 |
| | 497.44 | 0 | 0 | 0 |
| | 548.44 | 40 | 0 | 0 |
| | 647 | 40 | 0 | 0 |
| | 664.8 | 0 | 0 | |
| | 703.2 | 0 | 0 | |
| | 775.07 | 0 | 0 | 0 |
| | 821.23 | 0 | 0 | 0 |
| | 830.61 | 640 | 80 | 20 |
| | 869.45 | 5120 | 5120 | 0 |
| | 875 | 0 | 40 | |
| | 992.2 | 40 | 0 | 0 |
| 1000-5000AU/mL | 1072.03 | 1280 | 320 | 20 |
| | 1105.5 | 160 | 80 | 20 |
| | 1146.41 | 160 | 160 | 80 |
| | 1157.12 | 80 | 40 | 20 |
| | 1192.92 | 20 | 0 | 0 |
| | 1208.1 | 40 | 0 | 20 |
| | 1340.5 | 40 | 0 | |
| | 1424.6 | 0 | 0 | 20 |
| | 1540.14 | 40 | 40 | 0 |
| | 1638.42 | 80 | 40 | 20 |
| | 1870.5 | 0 | 0 | |
| | 1878.26 | 40 | 0 | 0 |
| | 1932.78 | 40 | 20 | 0 |
| | 2056.76 | 40 | 20 | 0 |
| | 2116.61 | 80 | 40 | 20 |
| | 2181.82 | 80 | 80 | 40 |
| | 2339 | 80 | 40 | 0 |
| | 2462.3 | 40 | 40 | 0 |
| | 2534.67 | 80 | 40 | 40 |
| | 2646.72 | 160 | 160 | 0 |
| | 3015.21 | 80 | 80 | 40 |
| | 3771.1 | 160 | 80 | |
| | 3992.83 | 40 | 80 | 0 |
| | 4020.55 | 80 | 80 | 0 |
| | 4267.88 | 320 | 160 | 0 |
| | 4357.78 | 160 | 80 | 0 |
| | 4515.6 | 160 | 80 | |
| | 4869.67 | 640 | 80 | 0 |
| 5000-25000AU/mL | 5350 | 160 | 80 | |
| | 5684.38 | 160 | 80 | 20 |
| | 8285.48 | 160 | 80 | 40 |
| | 8640.02 | 160 | 80 | 40 |
| | 9081.4 | 80 | 0 | |
| | 9676.95 | 80 | 160 | 0 |
| | 9844.75 | 160 | 80 | 0 |
| | 10510.1 | 320 | 320 | 20 |
| | 12196.1 | 320 | 320 | 40 |
| | 12493.4 | 320 | 320 | 0 |
| | 18781.3 | 320 | 160 | |
| | 20105 | 320 | 160 | 40 |
| | 20548 | 320 | 160 | |
| | 21313.6 | 1280 | 640 | 320 |
| | 24512.2 | 40 | 20 | 0 |
| | 25000 | 640 | 320 | 160 |
| | 25000 | 1280 | 640 | 160 |
| | 25000 | 2560 | 1280 | 640 |
| | 25000 | 320 | 160 | |
| | 25000 | 640 | 160 | 160 |
| | 25000 | 1280 | 640 | 640 |
| | 25000 | 2560 | 1280 | 160 |
| | 25000 | 5120 | 2560 | 1280 |
| | 25000 | 1280 | 640 | 640 |

**B**



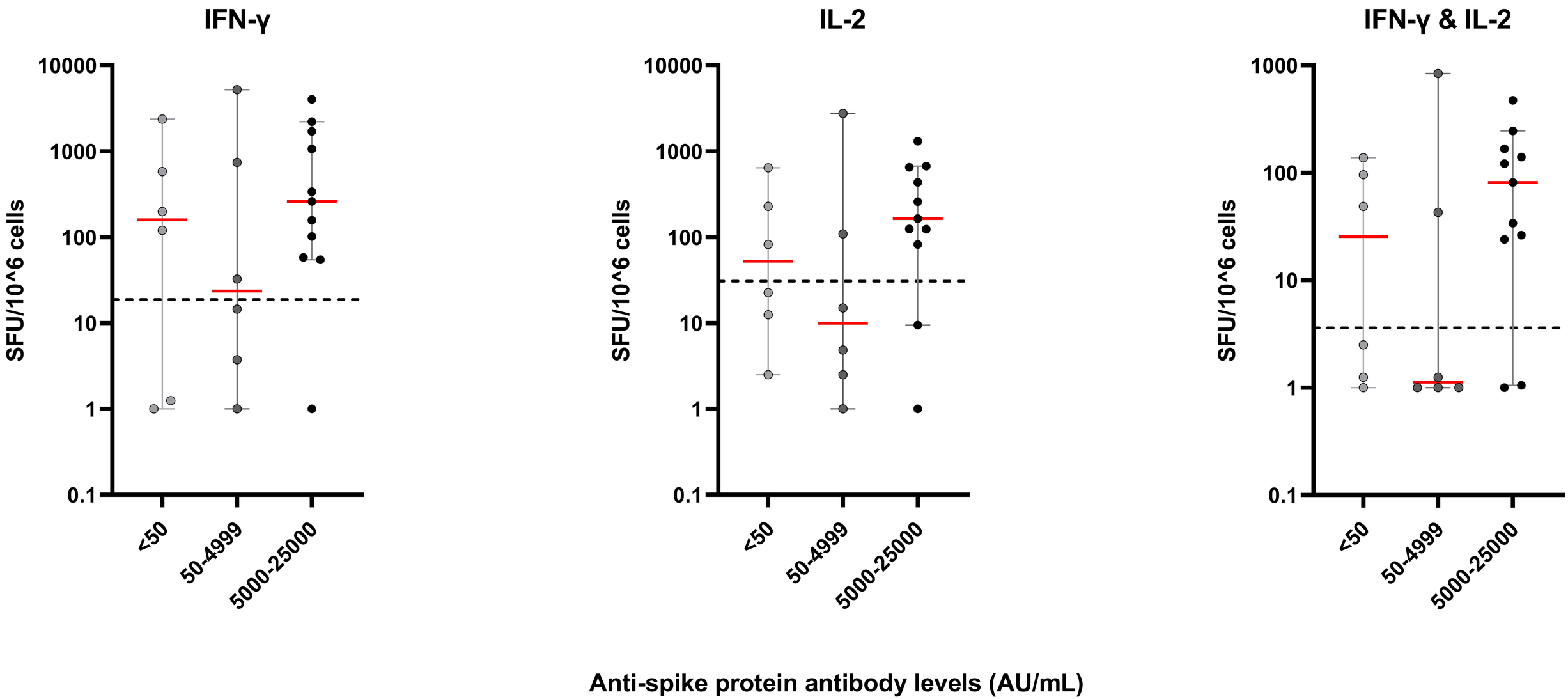
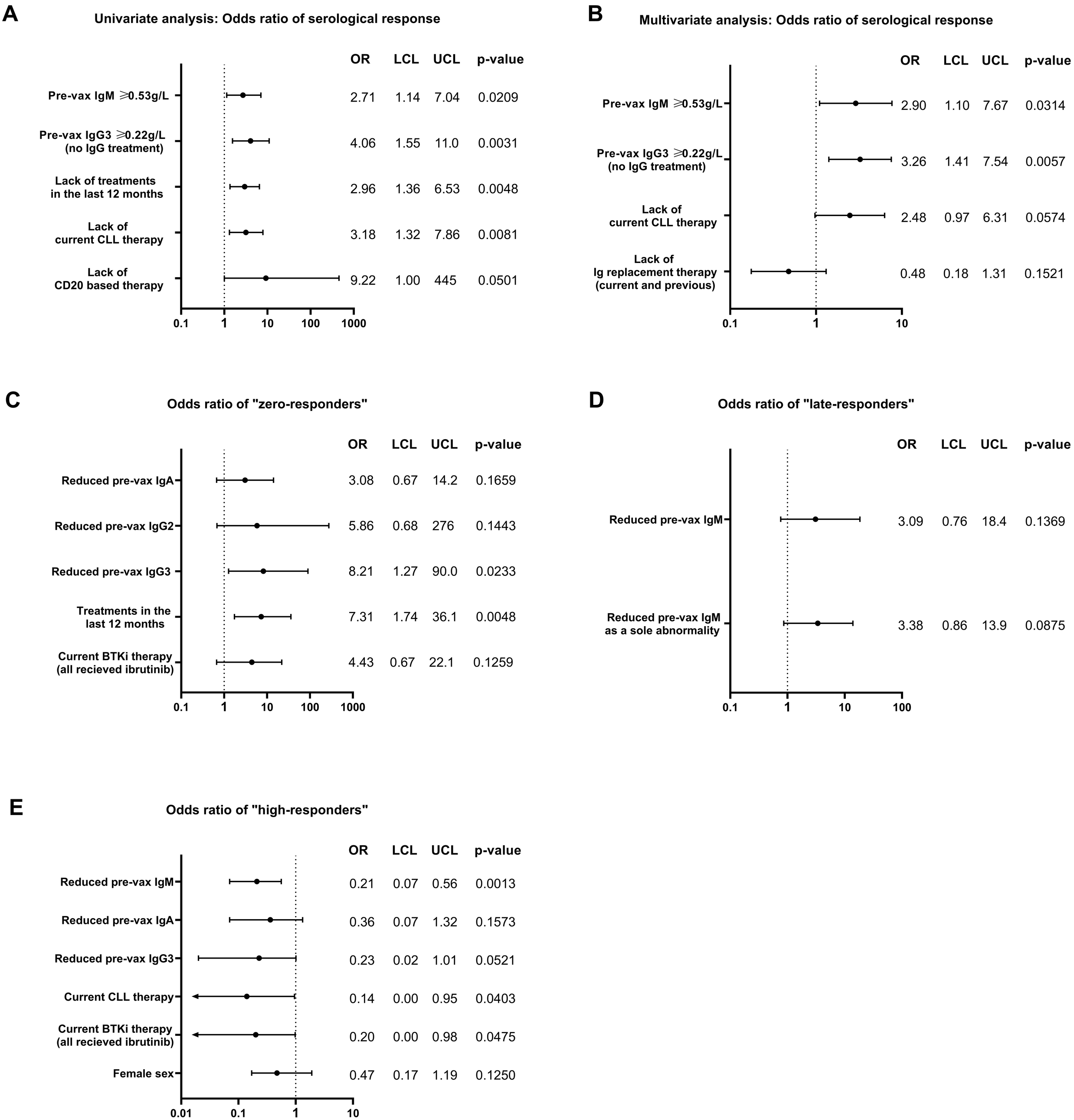

**C**




**D**

| Determinants of neutralization response | |
|---|---|
| **Effect** | **P value** |
| **Variants** | <.0001 |
| **Anti spike protein (<1000/>=1000)** | <.0001 |
| **Anti spike protein per varients** | 0.9500 |
| **Number of doses** | 0.0031 |

**Figure 4. T-cell function analysis on CLL patients post multiple vaccination.**



# Figure 5. Univariate and multivariate analysis of serological responses.