Defective T cell response to COVID-19 vaccination in Acute Myeloid Leukemia and Myelodysplastic syndromes

Running title:
Deficient T-cell response to COVID-19 vaccination in AML

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Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) infection is associated with an increased risk of life-threatening disease in patients with hematological diseases [1]. Vaccinations play an important role in reducing the risk of severe infection, however initial trials of anti-SARS-COV-2 vaccines excluded patients with hematological diseases [2]. Even later studies investigating the immune response to vaccination recruited limited numbers of patients with acute myeloid leukemia (AML) or myelodysplasia with excess blasts (MDS-EB2) [3]. Due to a combination of disease biology and treatment, patients with AML/MDS-EB2 are profoundly myelosuppressed, even compared to patients with other hematological malignancies [4,5].

Recent studies have assessed antibody titers [6] and their neutralization capacity[7] in patients with AML/MDS but have not examined T-cell function in this cohort. An independent role for T-cell memory following vaccination, beyond that provided by humoral immunity has been established [8]. We therefore set up a prospective study, PACE (Patients with AML and COVID-19 Epidemiology) to determine the impact of COVID-19, infections and cancer treatment on patients with AML/MDS-EB2. Here, we report the analysis to date of T-cell responses, viral neutralization, and antibody titers in patients with AML/MDS-EB2 post COVID-19 vaccination.

Patients with a diagnosis of AML or MDS-EB2 aged 16 or over, who were planning to or had already received a COVID-19 vaccine, were recruited between June 2021 to April 2022. Patients with acute promyelocytic leukemia were excluded from the study, alongside patients who had received an allogeneic stem cell transplant prior to vaccination. Informed consent was obtained and REC approval was granted from the North of Scotland Research Ethics Committee (20/NS/0059). The study was conducted according to the declaration of Helsinki. 133 samples were available in total as some patients provided more than one study sample.

Antibody levels were measured using the Roche serum Spike antibody assay (Elecsys® Anti-SARS-CoV-2 S, Roche). The limit of detection was 0.4 U/mL and a level of ≥0.8 U/mL was interpreted as positive. Samples which reached the maximum threshold of the test (>2500 U/mL) were diluted 10-fold with phosphate buffered saline (PBS) and repeated. A SARS-CoV-2 neutralization assay was performed in accordance with the protocol described previously.[9] T-cell reactivity to SARS-COV-2 spike antigen (S1) was assayed using a COVID T.SPOT (Oxford Immunotec) assay. Results are reported here as spot forming units (SFU) per 10^6 cells but were provided as SFU per 250,000 cells; the raw result was therefore multiplied by 4. A result of >16 (≥20) was interpreted as 'adequate' (i.e. including borderline results) and a result of >28 (≥32) was interpreted as a clear positive. Patients with chronic lymphocytic leukemia (CLL) were recruited as part of a study from the University of Birmingham, UK.[10] Antibody titers and T cell responses were measured using the same methodology.

We used frequentist regression models with stepwise regression to identify any significant covariates, exploring the effect of age, sex, treatment intensity, time from vaccination to sample, and disease response (CR/CRi vs not). Analysis presented incorporated treatment at the relevant time as a covariate. This only included patients for whom their treatment was able to be ascertained and where there was greater than 1 patient per treatment group. Statistical analyses were performed in Stata version 17 and R version 4.1.0.

105 patients were registered to this part of the PACE study. This report describes data post-vaccine 2 and 3 (PV2 and PV3). 93/105 had at least one available sample (Table 1), at the time of analysis, and 30/93 (32%) of patients were in complete remission (including CRi (incomplete count recovery)) at study entry. The median time since hematological diagnosis was 2.1 months (Range 0-199.5 months). 56 patients provided 66 samples PV2 (54% received Pfizer/BioNtech vaccine, 41% received AstraZeneca), whilst 57 patients provided 64 samples PV3 (86% received Pfizer/BioNtech vaccine, 12% Moderna) (Supplementary Table 1). No samples were taken following allogeneic stem cell transplantation in these cohorts.

Antibodies against SARS-COV-2 spike antigen were measured at a median of 23.1 weeks PV2. All results remained over the threshold for positivity of 0.8 U/mL and were consistent over time. There was minimal variation in antibody levels collected within or after 6 months PV2 (Figure 1A); levels remained above the threshold for positivity in individuals sampled more than once (Figure 1B). Antibody levels PV3 were on average higher than PV2: median levels PV2 were 867.0 U/ml (IQR:298.0-1619.0 U/ml), and PV3 were 6340.0U/ml (IQR:1332.0-25000.0U/ml).

We compared our results to a cohort of patients with chronic lymphocytic leukemia (CLL) [10], employing identical immune response assays, and observed that antibody responses in patients with AML/MDS-EB2 undergoing anti-leukemic treatment appeared more robust. Despite the comparable median age of the two cohorts (67 years), only 63/100 (63%) of CLL patients had a detectable antibody response after 2 vaccinations using the same assay and at a similar time point (Figure 1C).

Although antibody titers appear adequate, this does not evaluate the ability to neutralize variants of concern. Therefore, a pseudovirus-based neutralization assay [9] was performed (n=74). Neutralization of the omicron variant was poorer than wild-type (ancestral virus) and delta variant but improved markedly at PV3 (Figure 1D). Most samples achieved measurable neutralization with titers in a similar range to those of convalescent immunocompetent patients following infection [9]. Neutralizing antibody titers against all tested variants correlated positively with total S-antibody titer (Fig 1E), suggesting that the antibody measured in patient serum post-vaccination on the Roche assay is functionally relevant.

Given this apparently preserved ability to generate an antibody response to SARS-COV-2, we postulated that prior infection, as evidenced by the presence of anti-nucleocapsid antibody (N-antibody (present:29/111, 26%), would be associated with greater S-antibody responses. As anticipated, patients with positive N-antibody results exhibited higher S-antibody response, at least PV2 (Figure 1F). N-antibody positivity may identify patients who have not only had recent infection but are also able to mount an immune response.

T-cell reactivity was assayed in 120 samples (COVID T.SPOT (Oxford Immunotec)). In contrast to the antibody responses, after a second vaccine, an adequate T cell response ($\geq$20 spot forming units (SFU)/10^6 cells) was seen in only 16/47 (34%) patients, at a median of 23 weeks post-

vaccination. This appeared to be lower than in healthy individuals at a similar time point (Supplementary Table 2, Figure 1C) and in 96 patients with primary/secondary antibody deficiency [11]. Only 14/47 (29.8%) patients had a clear positive T-cell response of ≥32 SFU/10^6 cells. Post-vaccine 3, this increased marginally: 23/52 (44.2%) had an adequate T-cell response (≥20 SFU/10^6 cells), with 21/52 (40.4%) above ≥32 SFU/10^6 cells (Figure 1G). In 19 patients who had multiple samples, some spanning vaccinations, 12 had an inadequate first T-cell response, of which 9 remained suboptimal at a median of 7.7 weeks (range 3.1-14.1) by the time of the second sample (Figure 1H). Unlike S-antibody responses, presence of N-antibody did not seem to impact on T-cell response.

Given the poor T-cell response in our cohort of patients, we sought to identify which factors influenced this. We used frequentist regression models with stepwise regression to identify any significant covariates, exploring the effect of age, sex, treatment intensity, time from vaccination to sample, and disease response (CR/CRi vs not). PV2 we noted that disease response for those not in CR/CRi, and age were significant covariates (-159.11 SFU/10^6 cells (95%CI: -341.92, 23.7) and -4.14 SFU/10^6 cells per year of age from median (95%CI: -9.5, 1.22) respectively), whilst at PV3, time from vaccine to sample (-17.15 SFU/10^6 cells per week (95%CI: -40.01, 5.71)) was the only significant covariate.

In summary, our prospective study of immune response to COVID-19 vaccination in patients with AML/MDS-EB2, has identified a striking defect in T-cell responses to vaccination that appears to persist PV3 and is independent of receipt of an allogenic stem-cell transplant. One limitation of our study was that samples were taken from patients only at routine clinic appointments. However, our antibody data are consistent with smaller studies of an adequate serological response to SARS-COV2 vaccination [6] in patients with AML/MDS-EB2. In serial samples (n=19), S-antibody responses appeared relatively consistent over time. This was similar in both our healthy and CLL comparator cohort, suggesting only a limited waning effect in S-antibody response on this assay in the first few months following vaccination. The poor T-cell response in AML [12] mediated by alteration in T-cell phenotypes is likely to exacerbate the deficit in viral immunity caused by chemotherapy. Our results have clinical implications, including the value of a 3rd dose (and probably subsequent doses) of vaccine. The discrepancy between S-antibody results versus T-cell response may also inform clinician choice of SARS-COV-2 treatments for patients with AML/MDS: for example, the use of directly-acting antivirals (e.g. nirmatrelvir-ritonavir) rather than monoclonal antibody therapy (e.g sotrovimab), may be more rational considering the adequate S-antibody titre in patients with AML/MDS.

Acknowledgements

This component of the PACE study was funded by Blood Cancer UK. Cure Leukaemia funded the Trials Acceleration Program (TAP) through which some patients were recruited), and Bristol Myers Squibb provided support and funding for the prospective PACE study. The study was approved by North of Scotland Research Ethics Service (20/NS/0059).

Authorship Contributions and conflicts of interest

JL, DL, SS, CC conceived the study. PM, HP, DL and VU performed experimental work. CG, RB, VH performed statistical analysis. SF, RF, CT, LH, AKu, TT, EY coordinated the trial. AT, YLTC, KR, TR, JO, VD, AP, MD, DM, AKh, FW, SM, PK, YH, SK, JB, RZ recruited patients. All authors had an opportunity to review the manuscript. No authors declared any relevant conflicts of interest

Table 1

Baseline characteristics of patients entered into the study with at least one available sample at the time of analysis.

		Total (n=93)	
	Age (years)	67.8	(24.4 -91.1)
Gender	**Female**	40	(43%)
	Male	53	(57%)
Ethnicity	**African**	1	(1%)
	Pakistani	1	(1%)
	White	79	(85%)
	White and Asian	1	(1%)
	Not Available	11	(12%)
Disease	**AML**	81	(87%)
	MDS-EB2	12	(13%)
AML Type	**Primary AML**	67	(83%)
	Secondary AML	14	(17%)
Cytogenetics (AML patients only (n=81))	**Adverse risk**	9	(11%)
	Favourable risk	24	(30%)
	Intermediate risk	31	(38%)
	Data Pending/Newly diagnosed	17	(15%)
Disease Status	**First diagnosis**	86	(92%)
	Relapsed	6	(6%)
	Not Available	1	(1%)
Disease Response	**CR/CRi**	30	(32%)
	Not CR/CR i*	44	(47%)
	Not Available	19	(20%)
Time From Diagnosis (months)		2.1	(0.0 -199.5)
Time From Relapse (months)		3.3	(0.5 – 7.6)

* Not CR/CRi here contains patients that were in active disease, PR, RD, Relapsed Disease or Not Reassessed at entry to the study

Figure 1 legends

A) SARS-CoV2 spike antibody results (Elecsys® Anti-SARS-CoV-2 S, Roche) within 6 months of vaccination two (n=34), post 6 months of vaccination two (n=25) and post vaccination three (n=56). Diluted assay results with a maximum cut-off of 25000 AU/ml.

B) Changes in spike antibody results for patients who had more than one sample available post vaccination two (green) or three (blue). Red line indicates threshold for positivity on this assay.

C) Comparison of spike antibody and spike antigen T.SPOT COVID results between healthy individuals, patients with CLL and those with AML post vaccine two. Threshold of response for Spike antibody assay was 0.8 AU/ml and T.SPOT assay was $\geq$20 SFU/10^6 cells.

D) Results from pseudovirus neutralization assay for wild type, delta and omicron (BA.1) variants. Samples either within 6 months of vaccination 2, post 6 months of vaccination 2 and post vaccination 3.

E) Correlation plots presenting neutralisation results against spike antibody responses (Elecsys® Anti-SARS-CoV-2 S, Roche) post vaccination 2. Samples for Elecsys® antibody assay diluted with a maximum cut-off for at 25000 AU/ml.

F) Spike antibody results post vaccination two and vaccination three, split by N assay response.

G) T.SPOT COVID T cell response to S1 spike antigen, divided by sample time point.

H) Changes in T cell response for patients who had more than one sample available post vaccination two (green) or three (blue). Red line indicates threshold for positivity on this assay.

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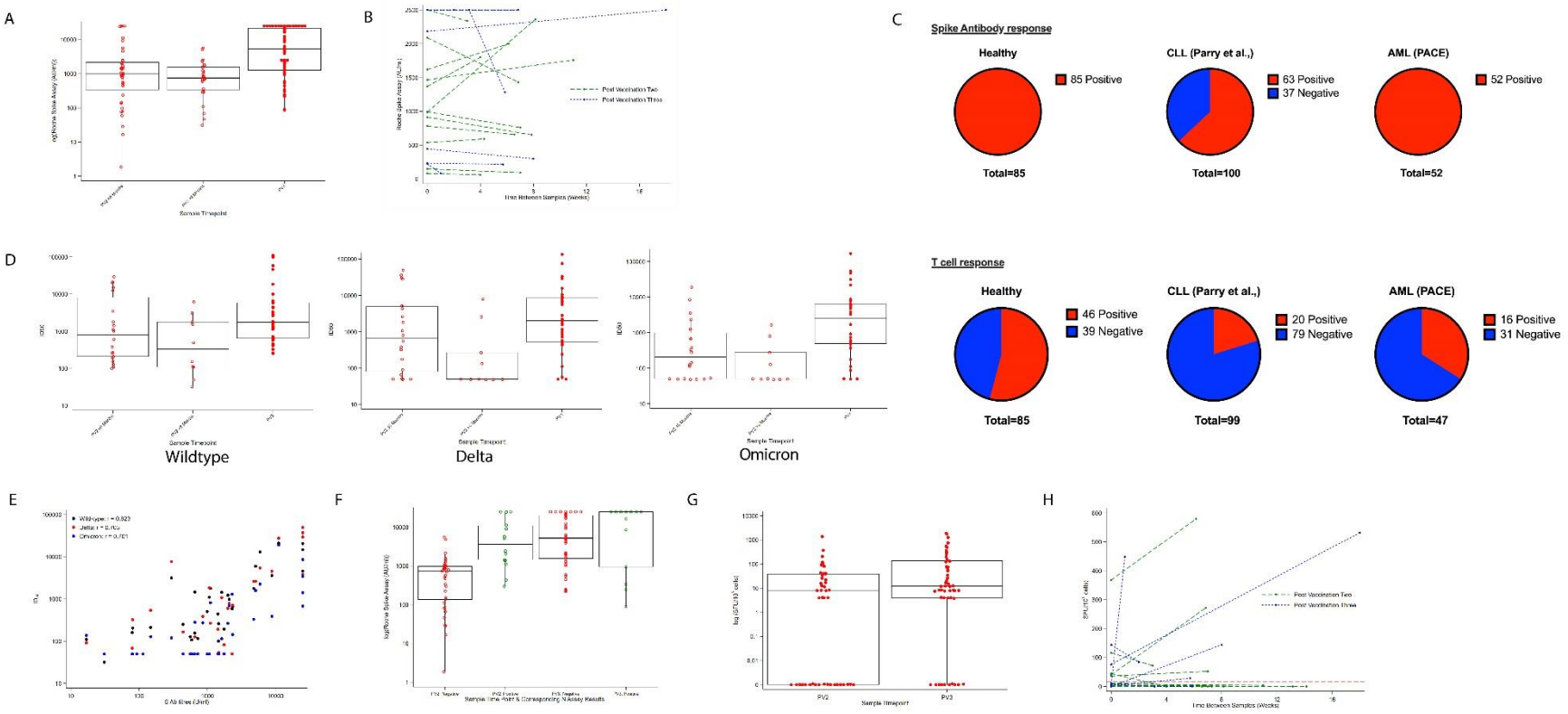
Figure 1


A
B
C
Spike Antibody response
Healthy
85 Positive
Total=85
CLL (Parry et al.,)
63 Positive
37 Negative
Total=100
AML (PACE)
52 Positive
Total=52
D
Wildtype
Delta
Omicron
T cell response
Healthy
46 Positive
39 Negative
Total=85
CLL (Parry et al.,)
20 Positive
79 Negative
Total=99
AML (PACE)
16 Positive
31 Negative
Total=47
E
F
G
H

Supplementary methods

Modeling of factors which influence T cell response (T.SPOT COVID Panel 1 assay)

As there were a low proportion of patients who had more than one sample, repeated measures modelling was deemed not possible. Instead, modelling was focused on the response post each vaccination using frequentist regression models.
An intercept was included to estimate the mean population-level response common to all patients. If this model did not fit the observed data satisfactorily, alternative models and correlation structures were considered.
Once the optimal model had been ascertained, we used stepwise regression (in both directions) to ascertain further population-level terms (also called covariates or fixed effects) influential in the results of the modelling of T.SPOT COVID results. The following covariates were explored:

- Most recent disease response (grouped as complete response (CR) or complete response with incomplete count recovery (CRi) or not CR/CRi),
- Age (centered around the median value, years),
- Sex, and
- time from vaccination to sample collection.

Interactions were considered, as appropriate. Stepwise regression will occur on a model without group-level effects, however where there were patients who contributed more than 1 sample at any given time point, such terms (also called random effects) were included to reflect patient heterogeneity and account for the fact patients contribute multiple results (both over the course of the study and potentially per vaccine time point).
Analysis presented incorporated treatment at the relevant time as a covariate. This only included patients for whom their treatment was able to be ascertained and where there was greater than 1 patient per treatment group.

Serum Antibody Responses to SARS-CoV2 vaccination

Antibody levels were measured using the Roche serum Spike antibody assay (Elecsys® Anti-SARS-CoV-2 S, Roche). The limit of detection was 0.4 U/mL and a level of ≥0.8 U/mL was interpreted as positive. Samples which reached the maximum threshold of the test (>2500 U/mL) were diluted 10-fold with phosphate buffered saline (PBS) and repeated.

T cell responses to SARS-CoV2 vaccination

Heparinised blood samples were couriered immediately after collection to Oxford Immunotec and T cell responses to SARS-CoV2 antigens were assayed using the COVID T.SPOT test. Responses to S1 spike proteins (“Panel 1”) are reported. Results are reported here as spot forming units (SFU) per 10^6 cells but were provided as SFU per 250,000 cells; the raw result was

therefore multiplied by 4. A result of >16 (≥20) was interpreted as 'adequate' (i.e. including borderline results) and a result of >28 (≥32) was interpreted as a clear positive.

CLL data on antibody and T cell responses

Patients with chronic lymphocytic leukemia (CLL) were recruited as part of a study from the University of Birmingham, UK.[6] Antibody titers and T cell responses were measured using the same methodology.

Neutralization assay

A SARS-CoV-2 neutralization assay was performed in accordance with the protocol described previously.[7] Briefly, serially diluted serum samples from patients were incubated with a HIV-pseudovirus expressing the SARS-CoV-2 S protein of wild-type, Delta or Omicron variants and luciferase gene. HeLa cells, engineered to express ACE2, were then added to the respective wells. After 72 hours, plates were read for luminescence after cell lysis and addition of substrate. Neutralization capacity of each patient sample is shown as inhibitory dilution 50 (ID_{50}), i.e., the serum dilution at which 50% of infection is inhibited compared to the virus alone.

Supplementary Table 1

Characteristics of patients providing samples at each timepoint (Post Vaccine, PV).

		Sample Timepoint			
		PV2 (n=56)		PV3 (n=57)	
Age†(years)		65.0	(24.0 -91.0)	70.0	(25.0 -91.0)
Time Since Vaccination (days)		151.0	(19.0 -270.0)	39.0	(1.0 -173.0)
Disease Response*	CR/Cri	28	(50%)	27	(47%)
	Not CR/Cri	22	(39%)	20	(35%)
	Not Available	6	(11%)	10	(18%)
Vaccine Type	Moderna	1	(2%)	7	(12%)
	Oxford/Astrazeneca	23	(41%)	0	(0%)
	Pfizer/BioNtech	30	(54%)	49	(86%)
	Other	1	(2%)	0	(0%)
	Unknown	1	(2%)	1	(2%)
Treatment ‡	Has not started treatment	16	(29%)	18	(32%)
	Intensive	12	(21%)	10	(18%)
	Not intensive	4	(7%)	8	(14%)
	Venetoclax based	9	(16%)	10	(18%)
	Completed treatment	3	(5%)	5	(9%)
	Not available	12	(22%)	6	(11%)

† Age at the date the sample was taken, median (range)

* Not CR/CRi here contains patients that were in active disease, PR, RD, Relapsed Disease or Not Reassessed at entry to the study

‡ Treatment type here taken to be the classification of the treatment taken closest to the sample

Supplementary Table 2

Characteristics of individuals in a healthy cohort providing samples at Post vaccine 2.

Age (Median (Range))	73.1 (39-81) years
Sex	Female n= 56 (66%)
	Male n =29 (34%)
Vaccine dose 2 type	Pfizer/Biontech n= 30 (35%)
	AztraZeneca n=55 (65%)
Median time from vaccination to sample	178 days