Summary

Anti-CD20 monoclonal antibodies are commonly used to manage neuroinflammatory diseases. The rate of B-cell re-emergence after dosing of ocrelizumab or rituximab varies considerably between individuals, but most people remain completely B-cell depleted at 6-months. Tailoring the dosing according to B-cell re-emergence may improve the safety profile of anti-CD20s but poses logistical challenges such as the need for regular attendances for whole-blood sampling. Here we combined a quantitative dried blood spot sampling technique with a DNA methylation test, to provide a reliable means of remotely monitoring B-cell counts, with 100% sensitivity and specificity for reaching > 10 $x10^6$ cells/L.

Key words:

- Multiple sclerosis
- Disease modifying therapies
- Immunology
- Neuromyelitis Optica (NMO)
- Treatment Response

Background

Anti-CD20 monoclonal antibodies are widely used to manage neuroinflammatory diseases including multiple sclerosis (MS) and neuromyelitis optica (NMO). Tailoring re-dosing of anti-CD20s such as rituximab and ocrelizumab according to the re-emergence of B-cells is gaining traction as a means to mitigate the risk of hypogammaglobulinaemia and/or infection in those on long-term therapy.[1] One approach is to re-dose when $CD3^-/CD19^+$ cells (B-cells) reach 10 $x10^6$ cells/L.[2] However, this poses logistical challenges such as the need for in-person attendances for whole-blood sampling or time-sensitive transport of liquid blood samples to a centre offering fluorescence Activated Cell Sorting (FACS). Dried blood spot (DBS) sampling is convenient, and samples are stable at ambient temperatures, but cannot be used for FACS. Here we provide pilot data combining a quantitative DBS sampling device, which can be posted by routine mail from home, with a B-cell specific DNA methylation test, to provide reliable B-cell counts.

Methods

We collected paired whole-blood and quantitative DBS samples from 24 people with MS receiving B-cell depleting therapies or natalizumab and 27 people with immune deficiency. Those with MS were people attending for infusions, enriched for people in an immune-reconstitution phase following ocrelizumab, to provide validation of test performance at low B-cell counts (Supplementary Table 1). People with Immune Deficiency were those requiring regular lymphocyte monitoring, who were willing to try a home-based sampling approach. DBS samples were collected using Capitainer®B50 quantitative micro-sampling devices (https://capitainer.com/capitainerb50/). Whole-blood was collected using EDTA vacutainers. People with MS performed DBS sampling in clinic supervised by a nurse. People with Immune Deficiency performed DBS sampling at home, having been shown once in clinic and with the aid of written and video training resources (https://capitainer.com/wp-content/uploads/2024/01/DLB22-052-02D_CAPITAINER-B50_IFU_EN.pdf and https://capitainer.com/sampling-videos/). Forty-three people with Immune Deficiency, including the

27 reported here who had paired whole-blood samples, were surveyed for satisfaction with remote testing.

Whole-blood was analysed for total and differential lymphocyte counts using FACS analysis according to standard protocol in a UKAS Accredited (ISO 15189) NHS laboratory. Leucocytes were classified according to cell surface expression allowing quantification of $CD3^-/CD19^+$ B cells.

DBS samples were processed as previously described.[3] The number of different immune cell types can be calculated as % of leukocytes and in cells/µl when an internal control of known copy numbers is co-amplified.

Absolute (FACS) and DBS-derived cell counts were compared for total lymphocytes and lymphocyte subsets to measure Pearson correlation coefficients. We used correlation to derive an appropriate correction factor for conversion of counts from DBS to equivalent absolute counts. We calculated the sensitivity / specificity (and 95% confidence intervals (95%CIs)) of our new approach for detecting key B-cell thresholds (CD19+ >/ $\leq$ 5, 10, 20 and 50 $x10^6$ cells/L; see Supplemental Table 2); analysis performed in Graphpad Prism (version 9.5.1).

Results

Pearson's linear regression analysis of paired samples (with removal of a single outlier with lymphocytosis) demonstrated a high degree of correlation between B-cell counts derived from whole-blood and DBS, (CD19 r=0.96 [95% confidence interval (CI) 0.92 – 0.98], p<0.0001, Figure 1 and Supplemental Figure 1 showing correlation with outlier included). Mean B-cell counts were 159 $x10^6$ cells/L in whole-blood and 146 $x10^6$ cells/L in DBS [normal range 50 – 500 $x10^6$ cells/L]. For B-cells, we calculated the correction factor equation [(estimated count – 25.48)/ 1.99; Figure 1] to convert DBS-derived B-cell counts to whole-blood B-cell counts. After applying the correction factor, 100% sensitivity and specificity was achieved for B-cell counts ≤ vs > 10 $x10^6$ cells/L (Table 1).

Patient survey return rate was 29 out of 43 (67%), showing high levels of satisfaction with the home sampling approach when compared to previous experiences of in-person whole-blood monitoring. A mean satisfaction score of 1.89 for Capitainer compared favourably with 4.37 for conventional blood sampling (range 1-10, with a lower score indicating greater satisfaction). Several positive themes were identified, including ease of use, flexibility of test timing, increased compliance with testing regimes and gain of control for the patient. There were some concerns raised about physical ability to use the Capitainer microsampling device unaided in those with poor manual dexterity and these will be studied further in a wider cohort.

Discussion

Anti-CD20 monoclonal antibody therapies provide an efficacious and convenient disease modifying therapy approach for people with MS and NMO. The rate of B-cell re-emergence after 6-monthly dosing varies considerably between individuals,[4] and appears to be relevant to efficacy outcomes.[5] The majority of people treated with ocrelizumab and rituximab remain completely B-cell depleted at 6-months,[4,6] allowing an opportunity to tailor (usually extend) the dosing interval by monitoring for B-cell re-emergence in the peripheral blood.[1] There is evidence that this approach maintains efficacy but improves the safety profile of rituximab and ocrelizumab for MS/ NMO.[7,8] However, monitoring lymphocyte counts requires attendance in person for venepuncture to provide whole-blood samples, or the sending of time-sensitive liquid-blood samples, which are analysed using flow cytometry (only performed in some centres). This novel approach could reduce treatment burden and reduce cost by up to 60%,[9] as DBS samples are stable within the micro-sampling device sent via the postal system.

We have shown that by combining a quantitative DBS sampling technique with the B-cell targeted measurement of DNA methylation, we can reliably identify the threshold at which to re-dose anti-CD20 therapy. This adds to existing data that measurement of immunoglobulins is already possible

remotely.[10] Patient-satisfaction for a home-based DBS test was high in the 67% of people with Immune Deficiency who responded, although this needs to be more widely tested including in people with MS. A significant advantage of DBS is greater sample stability. These pilot data need to be expanded in larger cohorts since the small sample size cannot fully account for the effect of variations in sampling technique, storage conditions and transport time of liquid and DBS samples. Inclusion of one sample with lymphocytosis slightly weakened the correlation between whole-blood versus DBS B-cell counts, suggesting that further work is needed to explore test performance at high B-cell numbers. Our results hold promise for a remote, patient-empowered approach to the monitoring of B-cell therapies.

Disclosures/ Conflicts of Interest

In the last 5 years ET has received honorarium for consulting work from Biogen, Janssen, Merck, Novartis, and Roche. She has received travel grants to attend or speak at educational meetings from Biogen, Merck, Neuroax, Roche, and Novartis. SJ has received support for conferences, speaker, advisory boards, trials, data and safety monitoring boards, studies and projects with CSL Behring, Takeda, Octapharma, Grifols, BPL, LFB, Kedrion, Pharming, Biocryst, Capitainer, Swedish Orphan Biovitrum, Biotest, Binding Site, GSK, Sanofi, UCB Pharma and HCRW. EMC has received support for conferences, speaker, and advisory boards with CSL Behring, Takeda, Octapharma, Grifols, BPL, LFB, Biocryst, Kalvista and Novartis. JS receives salary from Epimmune diagnostics, who performed detection of cells in DBS samples. SJM, EJ and KB reports no conflicts of interest.

Funding: SJM is supported by the UK Medical Research Council - Rare Disease Research Platform grant number MR/Y008057/1. We would like to acknowledge generous support for this project in the form of the LFB Immunology Grant (awarded to Emily Carne) and consumables from Capitainer.

Data availability statement:

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1 Ellrichmann G, Bolz J, Peschke M, Duscha A, Hellwig K, Lee D-H *et al.* Peripheral CD19+ B-cell counts and infusion intervals as a surrogate for long-term B-cell depleting therapy in multiple sclerosis and neuromyelitis optica/neuromyelitis optica spectrum disorders. *J Neurol* 2019; **266**: 57–67.

2 van Lierop ZYGJ, Toorop AA, van Ballegoij WJC, Olde Dubbelink TBG, Strijbis EMM, de Jong BA *et al.* Personalized B-cell tailored dosing of ocrelizumab in patients with multiple sclerosis during the COVID-19 pandemic. *Multiple Sclerosis Journal* 2021; **28**: 1121–1125.

3 Baron U, Werner J, Schildknecht K, Schulze JJ, Mulu A, Liebert U-G *et al.* Epigenetic immune cell counting in human blood samples for immunodiagnostics. *Sci Transl Med* 2018; **10**: eaan3508.

4 Baker D, MacDougall A, Kang AS, Schmierer K, Giovannoni G, Dobson R. CD19 B cell repopulation after ocrelizumab, alemtuzumab and cladribine: Implications for SARS-CoV-2 vaccinations in multiple sclerosis. *Mult Scler Relat Disord* 2022; **57**. doi:10.1016/j.msard.2021.103448.

5 Bar-Or A, Grove RA, Austin DJ, Tolson JM, VanMeter SA, Lewis EW *et al.* Subcutaneous ofatumumab in patients with relapsing-remitting multiple sclerosis. *Neurology* 2018; **90**: e1805–e1814.

6 Claverie R, Perriguey M, Rico A, Boutiere C, Demortiere S, Durozard P *et al.* Efficacy of Rituximab Outlasts B-Cell Repopulation in Multiple Sclerosis. *Neurology Neuroimmunology & Neuroinflammation* 2024; **10**: e200152.

7 Schuckmann A, Steffen F, Zipp F, Bittner S, Pape K. Impact of extended interval dosing of ocrelizumab on immunoglobulin levels in multiple sclerosis. *Med* 2023; **4**: 361-372.e3.

8 Rempe T, Elfasi A, Rodriguez E, Vasquez M, Graves J, Kinkel R. Ocrelizumab B-cell repopulation-guided extended interval dosing versus standard dosing – similar clinical efficacy with decreased immunoglobulin M deficiency rates. *Mult Scler Relat Disord* 2023; **79**: 105028.

9 Martial LC, Aarnoutse RE, Schreuder MF, Henriet SS, Brüggemann RJM, Joor MA. Cost evaluation of dried blood spot home sampling as compared to conventional sampling for therapeutic drug monitoring in children. *PLoS One* 2016; **11**. doi:10.1371/journal.pone.0167433.

10 Haberstroh H, Hirsch A, Goldacker S, Zessack N, Warnatz K, Grimbacher B *et al.* A Toolkit for Monitoring Immunoglobulin G Levels from Dried Blood Spots of Patients with Primary Immunodeficiencies. *J Clin Immunol* 2023; **43**: 1185–1192.

Figure 1 CD19+ B Cell Counts Quantified from Whole Blood and *Capitainer*®B50 Dried Blood Spots. Comparisons were made against CD19+ B cell counts obtained via whole blood EDTA vacutainers using fluorescence activated cell sorting (FACS) analysis with *Capitainer*®B50 quantitative microsampling devices using CD19 specific DNA methylation quantitative polymerase chain reaction (PCR) analysis. Sample size n= 50. Statistical comparisons were made using Pearson's linear regression ($r=0.96$, $p<0.0001$) with correction factor [(estimated count - 25.48)/1.99] applied. The panel inset shows a magnified version of the correlation at low cell counts.

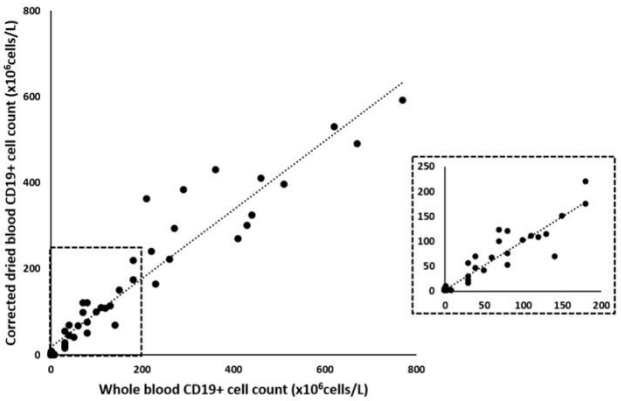

Table 1 Sensitivity and specificity for CD19+ B cell counts in Whole Blood and *Capitainer®*B50 Dried Blood Spots

		Dried blood spot CD19 ≤10				
		Yes	**No**	**Total**		
Whole blood CD19 ≤10	**Yes**	14	0	**14**		**Sensitivity = 100%**
	No	0	36	**36**		**Specificity = 100%**
	Total	**14**	**36**	**50**		