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Proportions of circulating transitional B cells associate with MRI activity in interferon beta-treated multiple sclerosis patients

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ABSTRACT

B-cells contribute to MS pathogenesis. The association of circulating B-cell phenotypes with combined unique active lesions (CUA) on MRI at 48 weeks follow-up was investigated in 50 interferon beta-treated MS patients. Transitional B-cell proportions were lower in participants with CUA at week 0 and 48 [$p = 0.004$, $p = 0.002$]. A decrease in circulating anti-EBNA-1 IgG levels between week 0 and 48 associated with absence of CUA [$p = 0.047$], but not with B-cell profiles. In a multi-factor model for CUA-risk, transitional B-cell proportions contributed independent from NK/T-cell ratio, change in anti-EBNA-1 IgG, and vitamin D supplementation. Transitional B-cells may predict treatment response in MS.

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS), of which the pathogenesis is driven by a complex interplay of environmental and genetic factors (Dobson and Giovannoni, 2019). Although the exact underlying disease mechanisms of MS are not yet fully elucidated, several lymphocyte populations have been associated with its disease process. These include T helper 1 cells ($CD4^+IFN\text{-}\gamma^+$ T cell, Th1 cell) (Gutcher and Becher, 2007), T helper 17 cells ($IL\text{-}17A^+CD4^+$ T cell, Th17 cell) (Durelli et al., 2009; van Langelaar et al., 2018), natural killer (NK) cells (McKinney et al., 2021; Mimpen et al., 2020a; Mimpen et al., 2020b), and B cells (Arneth, 2019; Wanleenuwat and Iwanowski, 2019). Several environmental factors associated with MS onset are believed to modulate the functional or phenotypic characteristics of these lymphocytes, including vitamin D levels (Pierrot-Deseilligny and Souberbielle, 2017; Smolders et al., 2008; Smolders et al., 2019), infection with Epstein-Barr virus (EBV) (Ascherio and Munger, 2016; Bar-Or et al., 2020; Kvistad et al., 2014), smoking (Arneth, 2020) and obesity (Ascherio and Munger, 2016). Especially EBV, a B cell-tropic virus that remains latent in memory B populations, appears to be a prerequisite for developing MS. (Abrahamyan et al., 2020; Pakpoor et al., 2013; Tselis, 2012) Circulating antibodies against the EBV nuclear antigen type 1 (EBNA-1) are also associated with a higher risk of radiological MS activity (Kvistad et al., 2014).

Of the lymphocyte populations, B cells have recently gained a lot of attention, largely due to the positive effects of B cell depleting CD20-targeted therapies on MS disease activity in both relapsing and progressive MS. (Gelfand et al., 2017; Hauser et al., 2017) Where B cells were traditionally viewed as a relatively passive population in the older MS disease models (Hemmer et al., 2002), more recent models give them a more central role (Wanleenuwat and Iwanowski, 2019; Milo, 2019; Probstel and Hauser, 2018), partially due to the growing appreciation for their antibody-independent functions in (auto)immunity (Li et al., 2018). This includes their role as antigen presenting cell (APC), their production of pro-inflammatory cytokines, as well as their involvement in establishing and maintaining meningeal tertiary follicles, as seen in secondary progressive MS patients (Serafini et al., 2004). These tertiary follicles in the secondary progressive (SP)MS phase are associated with an earlier onset of disease, as well as a more severe disease course (Magliozzi et al., 2007). Since EBV primarily infects (memory) B cells

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(Bar-Or et al., 2020; Laurence and Benito-Leon, 2017) and EBV infected B cells have been found in the tertiary follicles by some (Magliozzi et al., 2013), but not all groups (Peferoen et al., 2010; Willis et al., 2009), it is suggestive that these factors are closely linked in the pathogenesis of MS.

Besides treatment of existing clinical manifestations, predicting subsequent disease activity is a challenge in MS. Accurate prediction would facilitate timely intervention, thereby reducing lesions and limiting permanent damage to the CNS. Recently, we showed that supplementation of vitamin D_3 for 48 weeks in interferon-beta-1a treated relapsing-remitting (RR)MS patients did not increase the proportion reaching no evidence of disease activity (NEDA), but was associated with a reduced proportion of combined unique active (CUA) lesions on week 48 MRI (*i.e.* new T2 or gadolinium-enhancing lesions) (Hupperts et al., 2019). In $N = 50$ participants of a Dutch sub-study of this trial, we found that a low ratio between NK cells and IL-17A producing T helper cells (NK/IL-17A$^+$CD4$^+$ cell ratio) at week 0 predicted the presence of CUA at 48 weeks (Mimpen et al., 2020a). Moreover, this ratio was associated with IL-2 receptor alpha chain expression and shedding (Mimpen et al., 2021).

We now assess the prognostic value of circulating B cell phenotypes in the same cohort for MRI activity after 48 weeks. Furthermore, we explore how these phenotypes associate with anti-EBV serology, *i.e.* anti-EBNA-1 IgG and anti-VCA IgG, and other lymphocyte subsets.

2. Methods

2.1. Patients

This study is a post-hoc extended analysis of the SOLARIUM study, which was a sub-study of the SOLAR study (NCT01285401). The aim of the SOLAR study was to evaluate disease activity in interferon beta-1a (IFN-β-1a) treated RRMS patients using high dose vitamin D_3 supplements *versus* placebo. Patients in the vitamin D_3 group received cholecalciferol drops (Vigantol Oil, Merck) 7000 IU/day in the first 4 weeks, followed by 14,000 IU/day up to week 48. The SOLARIUM sub-study investigated the effect of high dose vitamin D_3 supplementations on immune system composition. In- and exclusion criteria for the SOLAR and SOLARIUM studies are described elsewhere (Hupperts et al., 2019; Muris et al., 2016). In short, the SOLAR study recruited patients aged 18–55 years, diagnosed with RRMS (according to the McDonald criteria 2005) confirmed by typical MS findings on magnetic resonance imaging (MRI). The first clinical event had to be described within 5 years prior to study screening and signs of active disease must have been present in the last 18 months, but no relapse in 30 days before inclusion. Patients could not participate if they already consumed more than 1000 IU (25 μg) of vitamin D_3 supplements. All patients received IFN-β-1a 44 μg s.c. three times weekly. Eligible participants had used IFN-β-1a at least 90 days, but no longer than 18 months. After randomisation, the patients received either IFN-β-1a and a placebo or IFN-β-1a and vitamin D_3 supplements.

Regarding the MRI outcome, procedures and findings as reported in the SOLAR trial were used (Hupperts et al., 2019). In short, MRI assessments were performed at baseline and week 48. Scans included T2- and T1-weighted images (3 mm slice thickness and 1 mm in-plane resolution) before and after administration of IV gadolinium. MRI was used to find presence of combined unique active (CUA) lesions (new gadolinium-enhancing or new/enlarging T2 lesions) at week 48. Since only $N = 3$ patients had more than 1 CUA after 48 weeks, MRI activity was measured as a dichotomous yes/no outcome.

The SOLARIUM sub-study recruited patients from four of the five participating centers in the Netherlands without adding additional in- or exclusion criteria, being eligible when they consented to participation in the sub-study. Written informed consent was acquired and the SOLARIUM study was approved by the Ethical Committee METC-Z (11-T-03; Heerlen, the Netherlands). Peripheral blood samples were collected at baseline (w0) and after 48 weeks (w48) and analysed using flow cytometry.

For the current study, $N = 50$ participants of whom data regarding B cells and EBV antibody parameters were available could be included.

2.2. Peripheral blood mononuclear cells isolation

The acquirement and analysis of the peripheral blood mononuclear cells (PBMCs) is described elsewhere (Muris et al., 2016). In summary, peripheral blood samples were collected from patients at baseline and week 48 of treatment. Blood was collected in a 10 mL sodium heparin blood sampling tube (BD Biosciences, Breda, The Netherlands) and transported to Maastricht University Medical Center, the Netherlands, at room temperature. Within 24 h PBMCs were isolated by gradient centrifugation as described in previous publications (Hupperts et al., 2019; Muris et al., 2016).

2.3. Flow cytometry

Immediately after isolation, PBMCs were stained with a cocktail of monoclonal antibodies in order to define B cells (CD19$^+$ lymphocytes) and subsequently transitional B cells (IgD$^+$CD27$^-$CD38^{++}), naïve B cells (IgD$^+$CD27$^-$CD38$^+$), non-isotype switched B cells (IgD$^+$CD27$^+$CD38$^{+/-}$), isotype switched B cells (IgD$^-$CD27$^+$CD38$^{+/-}$), plasmablasts (IgD$^-$CD38^{++}) and senescent B cells (IgD$^-$CD27$^-$). The following fluorochrome-conjugated antibodies were used: IgD-FITC (BD Biosciences, Breda, The Netherlands); CD27-PE (BD Biosciences); CD19-PerCP-Cy5–5 (BD Biosciences); CD38-APC (BD Biosciences). Additionally, regulatory B cells (Bregs, CD19$^+$IL-10$^+$) were defined by IL-10 production upon stimulation with CpG, as described in an earlier publication (Muris et al., 2016). For FACS analysis (FACS Canto II flow cytometer (BD Biosciences)), B cells were analysed for 100,000 events in the lymphocyte gate. FACS DIVA software (BD Biosciences) was used to analyse the flow cytometry data. Gating strategies, as well as phenotype definitions, are shown in Fig. 1. The definition of transitional B cells and plasmablasts was validated in a subset of participants at week 48 using an alternative gating strategy (CD19$^+$CD24^{++}CD38^{++} and CD19$^+$CD27^{++}CD38^{++}, respectively; Supplementary Fig. 1).

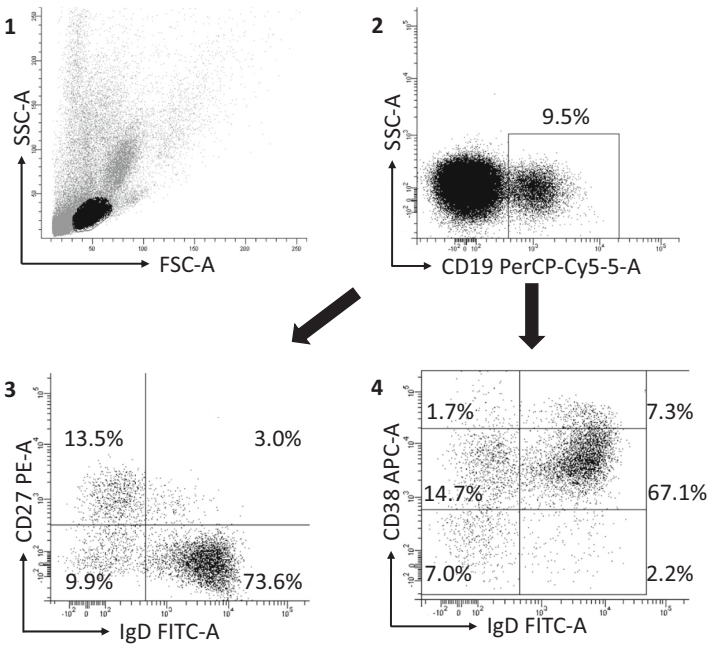
2.4. Antibody measurements

From SOLARIUM participants, blood was drawn at baseline and after a 48-week study period for measurements of several markers. Levels of IgG against the EBV antigens EBNA-1 and viral capsid antigen (VCA) were measured in plasma samples, which were stored at −20 °C until analyses. Tests were performed using the quantitative LIAISON® EBNA or VCA IgG assays (DiaSorin, Saluggia, Italy), which use chemiluminescence immunoassay technology. Results >22 U/mL were considered positive.

2.5. Statistics

SPSS software (IBM SPSS, version 25.0. Chicago, IL) was used to assess associations with disease activity. Normality of data was assessed by visual inspection of histograms with normal curves, skewness and kurtosis. To assess the association between B cell phenotypes and MRI activity after 48 weeks, independent *t*-tests or Mann-Whitney *U* tests were performed based on distribution of data. When assessing the role of multiple parameters for MRI activity after 48 weeks, a binary logistic model was used. If any parameters were not normally distributed according to our earlier mentioned criteria, they were log-transformed in order to normalise the data.

To determine cut-off points for an exploratory multi-factor model, ROC curves were plotted. The cut-off with the highest combined sensitivity and specificity was used. A p-value of <0.05 was considered statistically significant.



Population	Definition
Transitional	IgD^+CD38^{++} 7.3%
Mature naive	IgD^+CD27^- - IgD^+CD38^{++} 73.6% - 7.3% = 66.3%
Non-isotype switched	IgD^+CD27^+ 3.0%
Isotype switched	IgD^-CD27^+ - IgD^-CD38^{++} 13.5% - 1.7% = 11.8%
Plasmablast	IgD^-CD38^{++} 1.7%
Senescent	IgD^-CD27^- 9.9%

Fig. 1. Gating strategy used to analyse and define B cells and subsets. Step 1 shows the gating of lymphocytes from the PBMC sample. Step 2 shows the gating of B cells, defined as $CD19^+$ lymphocytes. Step 3 and 4 show distinctions made based on the IgD, CD27 and CD38 marker. Based on this strategy, six B cell phenotypes can be defined: transitional (IgD^+CD38^{++}), naïve ($IgD^+CD27^-CD38^+$), non-isotype switched ($IgD^+CD27^+CD38^{+/-}$), isotype switched ($IgD^-CD27^+CD38^{+/-}$), plasmablasts (IgD^-CD38^{++}) and senescent (IgD^-CD27^-). APC: allophycocyanin; FITC: fluorescein isothiocyanate; FSC: forward scatter; PE: phycoerythrin SSC: side scatter; PerCP: peridinin chlorophyll protein complex.

3. Results

3.1. Baseline characteristics

The SOLARIUM cohort consisted of 53 RRMS patients, of which $N = 3$ patients were ineligible due to incomplete immunostainings. Additionally, $N = 3$ patients did not undergo an MRI examination after 48 weeks, leaving $N = 47$ patients for analyses regarding MRI outcome. $N = 11$ patients were positive for presence of CUA after 48 weeks follow-up, whereas $N = 36$ were negative. Baseline characteristics did not differ between patients with and without MRI activity, except for a larger proportion placebo-randomized patients in the group with MRI activity (Supplementary Table 1) (Mimpen et al., 2020a; Hupperts et al., 2019). As reported earlier, all participants were EBV-seropositive: 92% of patients were anti-EBNA-1 positive while 96% were positive for anti-VCA, and none were negative for both markers (Rolf et al., 2018).

3.2. B cell compartment composition associates with MRI activity after 48 weeks

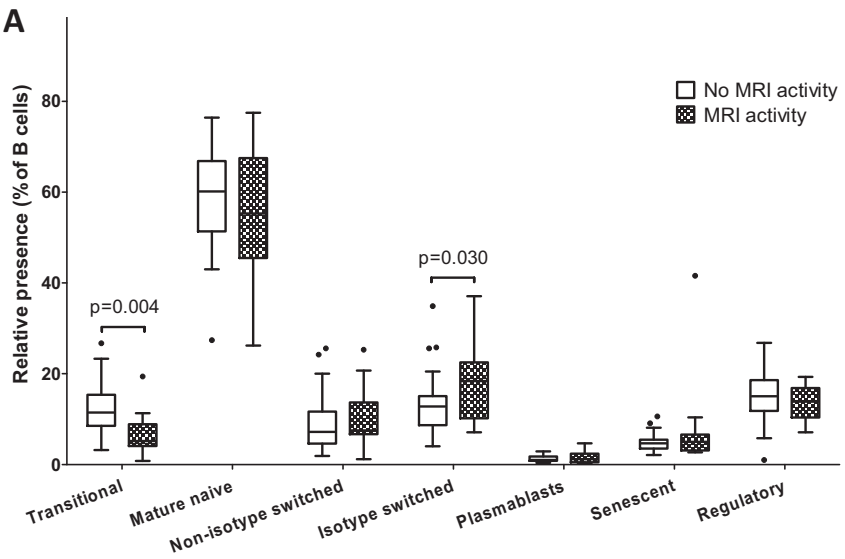
First, we assessed the association between circulating B cell subsets and the presence of CUA on week 48 MRI. At week 0, a lower proportion of transitional B cells [$p = 0.004$] and, to a lesser extent, a higher proportion of isotype switched B cells [$p = 0.030$] were found in patients with CUA on the week 48 MRI (Fig. 2A). At week 48, similar associations were observed for transitional and isotype switched B cells [$p = 0.002$ and $p = 0.015$, respectively] (Fig. 2B) with the addition of a higher percentage of non-isotype switched B-cells in participants with CUA on the week 48 MRI [$p = 0.035$]. These data suggest higher proportions of transitional B cells, and to a lesser extent also lower proportions of isotype-switched B cells, to positively influence the risk for CUA in interferon beta-treated RRMS. Although our manuscript is focused on MRI activity of MS, as being the most sensitive marker for inflammatory disease activity for MS (Barkhof et al., 2011), we also explored an association of B cells subsets with the absence of the clinical and MRI activity as expressed in the composite NEDA-3 endpoint. Patients with a NEDA-3 status at 48 week follow-up showed higher proportions of transitional B cells at baseline ($p = 0.008$, Supplementary Fig. 2). To exclude an effect of vitamin D supplementation on circulating B cell subsets, we explored effects of vitamin D supplementation on these phenotypes. None of the B cell subsets showed a significant change due to vitamin D_3 supplements (Fig. 2C).

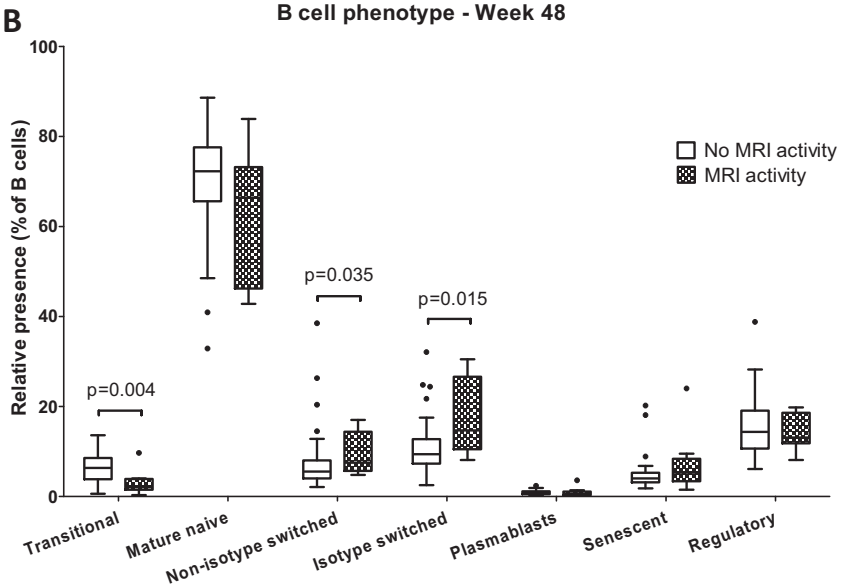
3.3. B cell subsets do not associate with anti-EBNA-1 IgG levels in serum

Since circulating antibodies against EBV antigens have been reported to be associated with MS MRI activity (Kvistad et al., 2014), associations between B cell phenotypes and IgG responses against EBNA-1 and VCA were explored. In our cohort, there was no difference in anti-EBNA-1 and anti-VCA antibodies between patients with or without CUA on week 48 MRI (Fig. 3A and B). Patients without MRI-activity at 48 weeks showed a more pronounced reduction in circulating anti-EBNA-1 but not anti-VCA IgG antibodies during 48 weeks of follow-up compared to patients with CUA (Fig. 3C). Accordingly, vitamin D_3 supplementation was already shown to be associated with both a lower proportion of CUA at week 48 (Hupperts et al., 2019), and a reduction of circulating anti-EBNA-1 IgG antibodies (Rolf et al., 2018). In our cohort, circulating anti-EBNA-1 IgG antibodies did not correlate with B cell phenotype percentages at any time point (Fig. 3D). This finding suggests that changes in B cell phenotypes and anti-EBNA-1 IgG in serum are independent contributors to CUA-risk in IFN-β-1a treated MS patients.

3.4. Patients with MRI activity show distinct clustering for multiple prognostic parameters

Since B cell phenotypes appeared a correlate of neither vitamin D supplementation, nor anti-EBNA-1 IgG levels, we further explored how individual parameters contribute to CUA lesions at week 48. Our previous work showed that the week 0 ratio between NK cells and $IL\text{-}17A^+CD4^+$ T cells predicted the presence of CUA on week 48 MRI (Mimpen et al., 2020a) and as such, this parameter was also included in our model. When introducing these 4 predictors of CUA on week 48 MRI





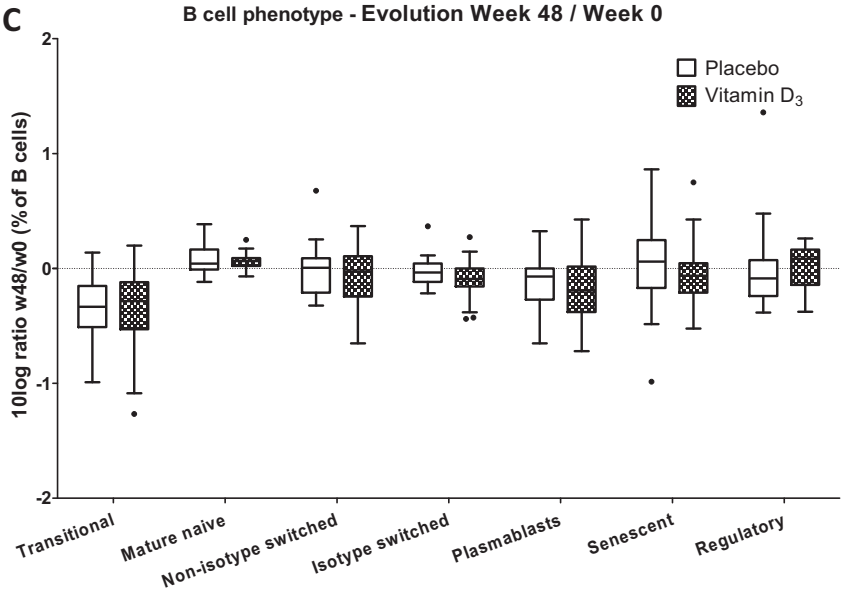


Fig. 2. A: Differences in B cell phenotypes (as defined in Fig. 1, with the addition of regulatory B cells) between patients with and without MRI activity, measured at baseline. Shown *p*-value is calculated using a Mann-Whitney *U* test. B: Differences in B cell phenotypes (as defined in Fig. 1, with the addition of regulatory B cells) between patients with and without MRI activity, measured at week 48. Shown p-value is calculated using a Mann-Whitney U test. C: Differences in the evolution of B cells over 48 weeks between patients with and without vitamin D supplementation. Evolution is calculated as the log ratio of week 48/w0, where negative numbers represent a relative decrease of B cell phenotypes, while positive numbers indicate a relative increase in B cell phenotypes. Shown p-value is calculated using a Mann-Whitney U test. Dotted lines represent the 0 value.

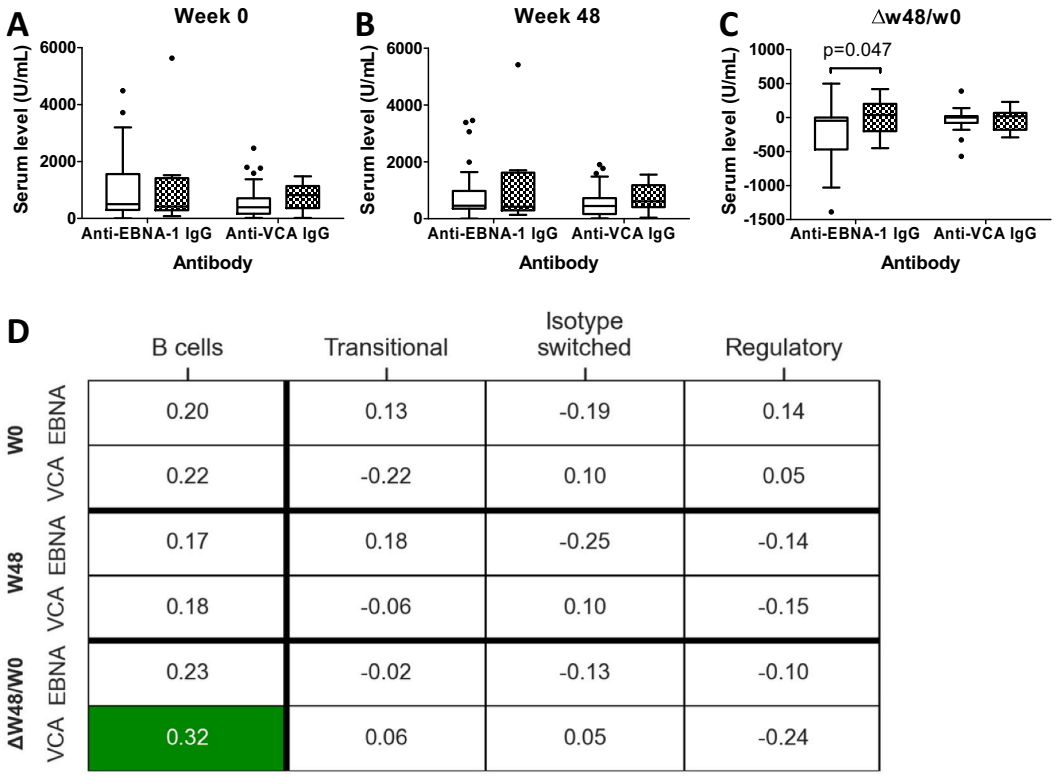


Fig. 3. A: Differences in anti-EBNA-1 IgG and anti-VCA IgG between patients with and without MRI activity, measured at baseline. Shown p-value was calculated using a Mann-Whitney U test. B: Differences in anti-EBNA-1 IgG and anti-VCA IgG between patients with and without MRI activity, measured at week 48C: Differences in anti-EBNA-1 IgG and anti-VCA IgG between patients with and without MRI activity, measured as the difference between week 48 and baseline. D: Heatmap showing correlations between anti-EBV serology and B cell subsets. Only statistically significant correlations are coloured. A green colour indicates a positive correlation. Correlation coefficient shown is a Spearman's rho. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in a 3D plot, patients with MRI activity tended to cluster together (Fig. 4A). After log-transforming data that were not normally distributed, we introduced our parameters in an explorative binary logistic regression model where they explained 57.9% of the variance in CUA-activity in our cohort (Nagelkerke $R^2 = 0.579$), and all factors except Δ anti-EBNA-1 IgG (Δw48/w0) trended to contribute to this model (Supplementary Table 2). To visualize this interplay for all individual participants, we dichotomized all variables based on individual ROC-curves in a high- and low-risk profile for CUA (Fig. 4B). Indeed, participants with CUA on week 48 MRI-scan tended to show a high-risk profile for multiple predictors.

4. Discussion

We investigated the prognostic value of B cell subsets, and explored associations with other predictors of MS MRI activity in a homogenous cohort of interferon-β-1a treated RRMS patients. We show that low circulating proportions of transitional B cells associate with a lower risk of CUA on week 48 MRI, and that this is no correlate of proportions of IL-10^+ Breg cells. Additionally, we show that this association of high transitional B cell proportions with a lower risk of CUA is not dependent on vitamin D supplementation, circulating anti-EBNA-1 IgG levels, or the ratio between NK and IL-$17A^+CD4^+$ T cells. Altogether, we conclude that the risk of MS MRI activity during interferon beta therapy is likely the result of many interacting phenotypes and effector characteristics of individual lymphocyte populations. Herewith, our data are a call for a systems biology approach to further understand the role of lymphocytes in the disease process of RRMS.

Our finding that transitional B cells may contribute to a protective effect in RRMS is in line with earlier research. Transitional B cells are shown to have regulatory properties (Zhou et al., 2020). As such, they have been implied in the pathogenesis of MS. Indeed, reduced transitional B cell numbers (Lee-Chang et al., 2011; Miyazaki et al., 2014) and regulatory function (Cencioni et al., 2020) and increased migratory capacity (Lee-Chang et al., 2011) have been reported in clinically isolated syndrome and MS. Additionally, some MS treatments associated with reduced disease activity in MS have been associated with increased transitional B cell proportions, including fingolimod (Miyazaki et al., 2014; Miyazaki et al., 2018; Blumenfeld et al., 2016) and interferon-β (Dooley et al., 2016). As such, one should exercise caution to extrapolate our findings to MS patients treated with other disease modifying therapies. Both fingolimod and IFN-β have been reported to increase transitional B cells through the increased circulating levels of B-cell activating factor of the TNF family (BAFF) (Miyazaki et al., 2018; Hedegaard et al., 2011). However, since BAFF was not significantly affected by high-dose vitamin D_3 supplementation in a preceding pilot-study (Knippenberg et al., 2011), we did not include BAFF measurements in the current SOLARIUM study-design.

In several studies, the protective effect of transitional B cells has been attributed to their capability of producing IL-10 (Miyazaki et al., 2014; Cencioni et al., 2020; Blumenfeld et al., 2016). In our study, an association with MRI-activity after 48 weeks follow-up was found only for transitional B cells, but not for IL-10^+ B cells. This interesting contrast may be explained by the method to analyse IL-10^+ B cells. In our study, B cells were stimulated with CpG for 24 h, after which IL-10^+ B cells were gated. One study shows that a combination of CpG and CD40L stimulation mainly expands regulatory B cells with a memory phenotype (Banko et al., 2017). As such, it may be that transitional B cells exert their protective effect through a Breg phenotype, but that the transitional Breg population remains undetected by our induction method.

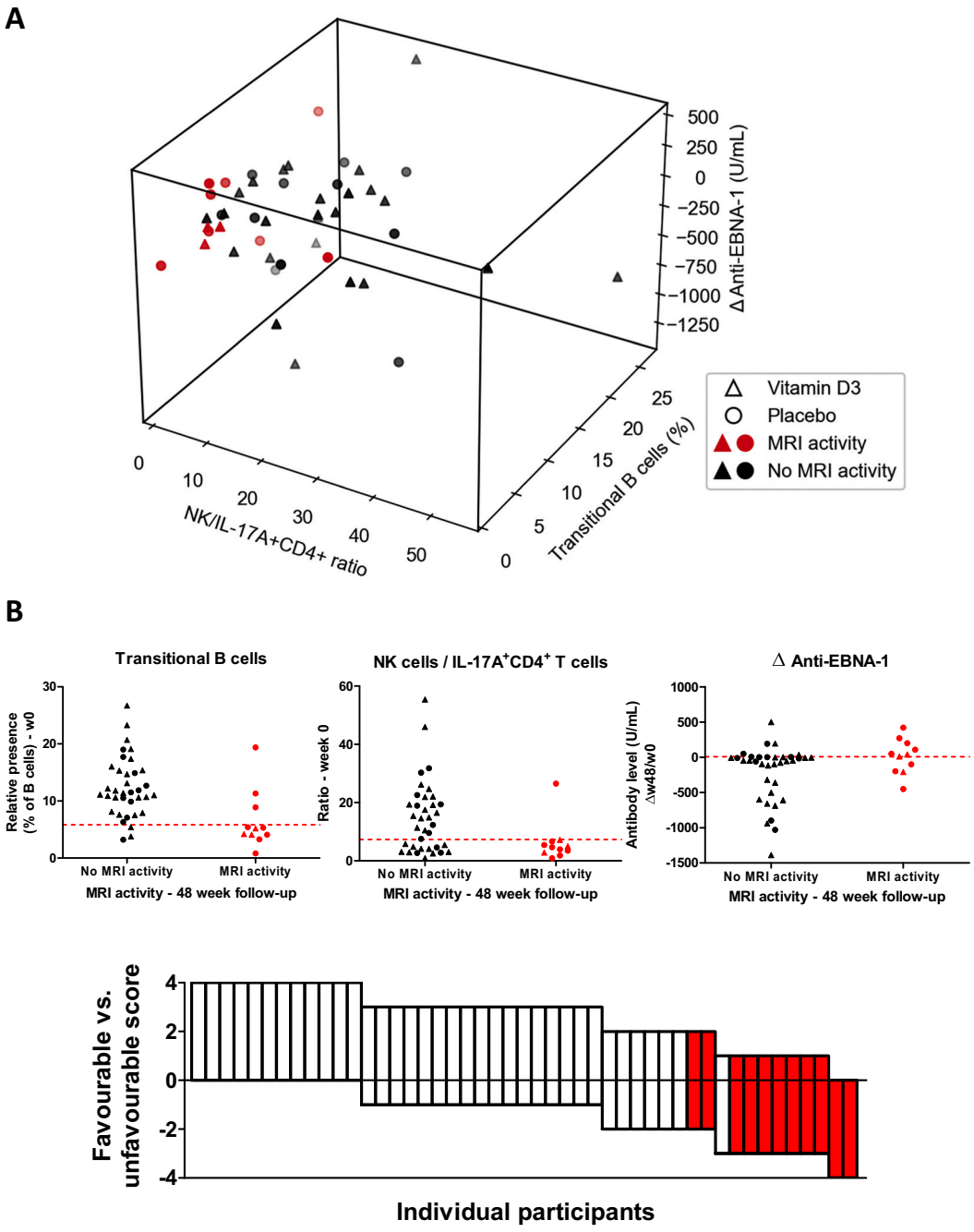


Fig. 4. A: 3D plot using three prognostic markers: relative presence of transitional B cells in the B cell population, NK/IL-17A^{+}CD4^{+} T cell ratio and the difference in anti-EBNA-1 IgG antibodies between week 48 and baseline. Red markers represent patients with MRI activity after 48 weeks, while black markers represent patients without MRI activity. Triangle markers represent patients who received high dose vitamin D_3 supplementation, while round markers represent patients who received placebo. B: Visualisation of multiple-hit model. When grouping patients based on the amount of favourable or unfavourable prognostic markers using cutoffs shown, patients with MRI activity (shown in red) tend to cluster around multiple negative markers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Alternatively, transitional B cells may control CD4^{+} T cell proliferation and MS disease activity by other effector mechanisms than IL-10 (Simon et al., 2016).

Isotype-switched memory B cells are generally being viewed as an unfavourable cell subset in MS. (Dooley et al., 2016) Our current results are in line with these notion, as higher percentages of isotype switched B cells are associated with a higher risk of CUA presence. The lack of correlation between anti-EBNA-1 IgG and isotype switched B cells is interesting, as EBV is known for its latent infection of memory B cells. One possible explanation may lie in recent evidence, suggesting that memory B cells only develop into antigen secreting cells after migrating to the CNS (van Langelaar et al., 2021). Isotype switched B cells as measured in peripheral blood may, therefore, give an incomplete view of anti-EBNA-1 production.

We showed that transitional B cell frequencies associate with MS CUA presence, as also observed for other immune-related predictors identified earlier in this cohort, including anti-EBNA-1 IgG (Rolf et al., 2018), vitamin D supplementation (Hupperts et al., 2019), and NK/T cell ratios (Mimpen et al., 2020a). This observation fits the growing appreciation for the complex interplay between environmental factors

and genetic background that influences cell subsets in (auto)immune diseases (Brodin and Davis, 2017; Davis et al., 2017; Ma'ayan, 2017). As such, the need for integrative models has increased, which led to an increase in popularity for a systems biology approach, where larger interacting systems are taken into account. For MS, a systems biology approach has been used to identify *e.g.* regulatory genetic pathways (International Multiple Sclerosis Genetics, C, 2019) as well as potential biomarkers for disease activity (Chase Huizar et al., 2020). An approach on a cellular level remains poorly investigated. In our exploratory model, the combination of several prognostic factors, mainly the relatively increased or decreased presence of transitional B cells, NK cells and IL-17A producing T helper cells, leads to a model which currently explains over half of the variance in the prognosis of CUA in IFN-β-1a treated RRMS patients. To make more definitive claims on this approach, more biomarkers should be investigated and integrated into a larger model using a larger dataset. Nonetheless, our findings underline the importance of looking at the interaction rather than merely the presence of individual immune components to understand the complex pathogenesis of diseases like MS.

Our study has a some limitations. First, our data is derived from the SOLAR and SOLARIUM studies, which both were designed to answer a different research question. Thus, the exploratory nature of our research brings an increased risk for false negatives, in addition to the absence of a few potentially relevant parameters like the aforementioned BAFF levels. Additionally, our patients exclusively used interferon-β-1a. While this greatly increased homogenisation of the participants, it may influence the extrapolation of our data. Strengths of this study include its double-blinded nature, as well as its broad view of the B cell compartment.

In conclusion, our data underline the protective role for transitional B cells in IFN-β treated RRMS patients, as well as its potential as a prognostic biomarker for MRI activity. As noted earlier, our data suggests an interplay between several prognostic factors and calls for a systems biology approach to further grasp the interactions of lymphocyte subsets in RRMS. More research is necessary to confirm the prognostic value of transitional B cells in treatment-naïve RRMS patients and also to investigate other potential biomarkers in a systems biology approach.

Data availability statement

The data presented in this study are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

MM has nothing to disclose; *JD* has nothing to disclose; *LR* has nothing to disclose; *AHM* has nothing to disclose; *WD* has nothing to disclose; *RH* received institutional research grants and fees for lectures and advisory boards from Biogen, Merck, and Genzyme-Sanofi; *ML* has nothing to disclose; *OG* has nothing to disclose; *JS* received lecture and/or consultancy fees of Biogen, Merck, Sanofi-Genzyme, and Novartis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2021.577664.

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