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Original Research Paper

Conversion from clinically isolated syndrome to multiple sclerosis: A large multicentre study

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Abstract

Background and objective: We explored which clinical and biochemical variables predict conversion from clinically isolated syndrome (CIS) to clinically definite multiple sclerosis (CDMS) in a large international cohort.

Methods: Thirty-three centres provided serum samples from 1047 CIS cases with at least two years' follow-up. Age, sex, clinical presentation, T2-hyperintense lesions, cerebrospinal fluid (CSF) oligoclonal bands (OCBs), CSF IgG index, CSF cell count, serum 25-hydroxyvitamin D3 (25-OH-D), cotinine and IgG titres against Epstein-Barr nuclear antigen 1 (EBNA-1) and cytomegalovirus were tested for association with risk of CDMS.

Results: At median follow-up of 4.31 years, 623 CIS cases converted to CDMS. Predictors of conversion in multivariable analyses were OCB (HR = 2.18, 95% CI = 1.71–2.77, $p < 0.001$), number of T2 lesions (two to nine lesions vs 0/1 lesions: HR = 1.97, 95% CI = 1.52–2.55, $p < 0.001$; >9 lesions vs 0/1 lesions: HR = 2.74, 95% CI = 2.04–3.68, $p < 0.001$) and age at CIS (HR per year inversely increase = 0.98, 95% CI = 0.98–0.99, $p < 0.001$). Lower 25-OH-D levels were associated with CDMS in univariable analysis, but this was attenuated in the multivariable model. OCB positivity was associated with higher EBNA-1 IgG titres.

Conclusions: We validated MRI lesion load, OCB and age at CIS as the strongest independent predictors of conversion to CDMS in this multicentre setting. A role for vitamin D is suggested but requires further investigation.

Keywords: Clinically definite multiple sclerosis (CDMS), clinically isolated syndrome (CIS), Epstein-Barr nuclear antigen 1 (EBNA-1), oligoclonal bands (OCBs), serum 25-hydroxyvitamin D3 (25-OH-D).

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Introduction

Multiple sclerosis (MS) is a debilitating disorder of the central nervous system (CNS) characterised by immune-mediated demyelination and progressive neuronal degeneration.[1] The cause of MS is unknown, but the disease appears to develop in genetically susceptible populations as a result of environmental exposures. Environmental factors that have been most

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strongly associated with MS risk are vitamin D deficiency, Epstein-Barr virus (EBV) infection and smoking.[2]

In approximately 85% patients the disease starts as a single clinical episode, the clinically isolated syndrome (CIS).[1,3] Prospective studies demonstrate that 60%–70% of CIS patients develop a second clinically evident demyelinating event within 20 years and will, therefore, be diagnosed with clinically definite MS (CDMS).[3,4] The identification of factors influencing the risk of conversion to CDMS is relevant to allow prognostication, thus directing early intervention strategies. This also carries the potential to further our understanding of the biological mechanisms driving MS. Several studies have investigated clinical and laboratory variables that can help predict conversion to CDMS. The presence and number of magnetic resonance imaging (MRI) lesions in the CNS and the presence of oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF) of CIS patients have been independently associated with an increased risk of conversion.[3–5] Other studies have focused on candidate environmental factors in MS and demonstrated that CIS patients with low vitamin D levels and high anti-EBV antibody titres are more susceptible to conversion to CDMS.[6–8]

Given the potential interdependence between risk factors, it is vital to assess all candidate risk factors collectively and test their association with risk of conversion using a large population of CIS cases. To our knowledge, only one study has comprehensively analysed a number of suggested factors in a cohort of patients experiencing a first demyelinating event. However, the cohort was relatively small (n = 302) and only cases with paediatric onset were included.[9] In this multicentre study we aimed to assess which clinical and environmental variables predict the risk of conversion from adult CIS to CDMS using the largest cohort of adult CIS cases studied to date (n = 1047).

Materials and methods

Participants and inclusion criteria

We performed an international collaborative study across 33 centres located in 17 different countries (eTable 1). Each centre was asked to provide baseline clinical data and stored serum samples from CIS patients, for whom a minimum of two years of follow-up data were available. Inclusion criteria were: (1) the presence of a monophasic clinical episode suggestive of MS (CIS), not attributable to other diseases (for example infectious, neoplastic, congenital, metabolic or vascular disease);[10] (2) clinical follow-up of at least two years; (3) available basic demographic and clinical data (age at serum sampling or month and year of birth, gender, dates of CIS onset, serum sampling, CSF examination, MRI, conversion to CDMS (if present) and last follow-up visit); (4) information on presence or absence of OCBs in CSF at time of CIS; (5) available data on T2 hyperintense lesions on cranial MRI at time of CIS.

Patients with neuromyelitis optica (NMO), or a history of a progressive disease course from onset were excluded. CDMS was diagnosed across all centres according to Poser criteria. This implied the exclusion of alternative diagnoses and the presence of a second clinically evident demyelinating attack which had to be separated in time and space from the first episode (i.e. occurring after an interval of at least one month and in a separate CNS location).[11] The study was approved by the corresponding local ethics committees and participants gave written informed consent. United Kingdom (UK) ethical approval was not required as all the samples analysed were collected outside of the UK. The use of the samples was covered by individual material transfer agreements between Barts and The London National Health Service (NHS) Trust and respective participating institutions.

Data collection

MRI, CSF and clinical assessments were performed in each participating centre as part of the diagnostic workup. The number of T2 hyperintense lesions on cranial MRI at time of CIS was used to group patients into three separate categories (0–1 lesions, two to nine lesions and >9 lesions). Grouping of lesion load was performed in order to control for variations in imaging protocol and individual analysis among centres. The presence of immunoglobulin G (IgG) OCBs was determined by isoelectric focusing combined with immunoblotting of matched serum and CSF sample pairs in all centres.[12] Additional clinical and CSF data were provided for a more limited number of patients (topography of CIS, CSF IgG index and CSF cell count).[13]

Serum samples were aliquoted and stored at −80°C according to international consensus guidelines.[14] Liquid chromatography-tandem mass spectrometry (LCMS/MS) was used to measure 25-hydroxyvitamin D3 levels (25-OH-D) (Royal London Hospital, Barts Health NHS Trust, London, UK). Daily internal quality samples are measured by LCMS/MS and the institution follows an external quality assurance scheme (The International Vitamin D Quality

Table 1. Demographic and clinical characteristics of the total CIS cohort, patients who converted and not converted to CDMS during follow-up.

	All CIS patients (n = 1047)	Missing values	Converted to CDMS (n = 623, 59.5%)	Missing values	Not converted to CDMS (n = 424, 40.5%)	Missing values
Age (years)	32.0 (26.0–39.0)	0 (0)	31.0 (25.2–38.1)	0 (0)	33.2 (27.1–39.7)	0 (0)
Females	714 (68.2)	0 (0)	440 (70.6)	0 (0)	274 (64.7)	0 (0)
Follow-up (days)	1574 (1042–2330)	0 (0)	1768 (1110–2558)	0 (0)	1332 (965–1971)	0 (0)
Type of presentation	ON=288 (31.6)	136 (13.0)	ON=160 (30.7)	102 (16.4)	ON=128 (32.8)	34 (8.0)
	BS=188 (20.6)		BS=103 (19.8)		BS=85 (21.8)	
	Spinal=257 (28.2)		Spinal=151 (29.0)		Spinal=106 (27.2)	
	Other=178 (19.5)		Other=107 (20.5)		Other=71 (18.2)	
Days to conversion to CDMS	NA	NA	421 (212 - 853)	0 (0)	NA	NA
Cotinine (> 14 ng/ml)	350 (33.7)	10 (1.0)	205 (33.2)	5 (0.8)	145 (34.6)	5 (1.2)
OCB positive	778 (74.3)	0 (0)	525 (84.3)	0 (0)	253 (59.7)	0 (0)
MRI T2 lesions	0–1=151 (14.4)	0 (0)	0–1=48 (7.7)	0 (0)	0–1=103 (24.3)	0 (0)
	2–9=438 (41.8)		2–9=249 (40.0)		2–9=189 (44.6)	
	>9=458 (43.7)		>9=326 (52.3)		>9=132 (31.1)	
25-OH-D (nmol/l)	49.3 (32.2–72.5)	6 (0.6)	47.9 (31.3–71.9)	4 (0.6)	50.5 (34.7–73.5)	2 (0.5)
EBNA1 IgG	11.3 (6.7–14.7)	3 (0.3)	11.6 (6.7–15.0)	1 (0.2)	10.9 (6.7–14.2)	2 (0.5)
CMV IgG	1.7 (0.2–3.3)	12 (1.1)	1.9 (0.2–3.3)	8 (1.3)	1.2 (0.2–3.4)	4 (0.9)
CSF IgG index	0.7 (0.5–1.1)	351 (33.5)	0.8 (0.6–1.1)	231 (37.1)	0.6 (0.5–0.9)	120 (28.3)
CSF cell count (n/µl)	5.0 (2.8–12.0)	534 (51.0)	5.8 (2.0–12.0)	315 (50.6)	5.0 (3.0–11.0)	219 (51.7)

Median and interquartile range (IQR) or n (%). CIS: Clinically isolated syndrome; CDMS: clinically definite multiple sclerosis; ON: optic neuritis; BS: brainstem syndrome; Spinal: spinal cord syndrome; OCB: oligoclonal bands in CSF; 25-OH-D: 25-hydroxyvitamin D3; EBNA1: Epstein-Barr nuclear antigen 1; CMV: cytomegalovirus; CSF: cerebrospinal fluid; Ig: immunoglobulin; NA: not applicable.

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Assessment Scheme, DEQAS). IgG titres against the EBV nuclear antigen 1 (EBNA1) and cytomegalovirus (CMV) were evaluated using commercially available enzyme-linked immunosorbent assays (ELISAs) (ETI-EBNA-G and ETI-CYTOK-G PLUS Diasorin, Saluggia, Italy) following the manufacturers' recommendations. Internally run validation criteria specified by the manufacturer and based on the calibrators were met for all ELISA plates measured. According to the manufacturer's instruction the cut-off for positivity was 20 arbitrary units (AU) for EBNA1 IgG and 0.4 international units (IU) for CMV IgG. Results were calculated by dividing the optical density (OD) of each sample by the OD of the 20 AU/0.4 IU calibrator on each ELISA plate. Serum cotinine levels were assessed using a commercially available ELISA (Calbiotech Inc, Spring Valley, CA, USA) according to the manufacturer's instructions and were used as a marker for smoking behaviour with levels > 14 ng/ml indicating a positive smoking status.[15] Intra- and inter-assay variability for all ELISA measurements was below 15%. All the ELISA assays were performed in a single centre (Blizard Institute London, UK), with the analyst blinded to clinical data.

Statistical analysis

Variables were described by their median and interquartile range (IQR) or by counts and percentages. Serum 25-OH-D varies according to season. In order to correct for this, raw 25-OH-D values were converted into deseasonalised 25-OH-D levels using the methods described by Mowry et al.[16] Briefly, sine and cosine terms were generated to model the influence of the date of blood draw on vitamin D status; these were then included in a linear regression model providing the adjusted 25-OH-D levels.[16]

The impact of each variable on the cumulative risk of conversion to CDMS was assessed in both univariable (Kaplan-Meier survival curves and univariable Cox regression) and multivariable analyses (multivariable Cox regression with backward stepwise selection of variables). The assumption of proportional hazards was tested by including time-dependent covariates (interactions) in the model. Centre information was included in all Cox regression models by using a generalised estimating equation (GEE) sandwich estimate of variance approach. Differences among groups of CIS patients according to their OCB and MRI status were also assessed using logistic and ordinal regression models. All statistical analyses were performed using STATA (http://www.stata.com/) and R (http://www.r-project.org/).

Results

Overall characteristics of CIS patients

A total of 1047 CIS patients were included in the study. These patients had presented to neurology services between November 1986 and December 2011; 1010 (96.5%) after 2000 and 794 (75.8%) after 2005. The median time between onset of neurological symptoms and serum sampling, CSF and MRI examination was 33 (12–81), 28 (10–67) and 19 (4–59) days, respectively. In 729 patients (69.6%) all evaluations were performed within three months since onset of symptoms. Patients were longitudinally followed up for a median time of 1574 days (4.31 years). During this time 623 patients (59.5%) converted to CDMS (median survival time before conversion = 1096 days, 95% confidence interval (CI) = 973–1267).

The demographic and clinical features of CIS patients are shown in Table 1. CSF IgG index was available in 696 patients (66.5%) and CSF cell count in 513 patients (49.0%).[13] CIS patients were more likely to be female than male (female/male ratio = 2.1). The majority of patients had OCBs in their CSF (74.3%) and had more than one CNS T2 lesion on MRI (two to nine T2 lesions = 41.8%; >9 T2 lesions = 43.7%).

Clinical CIS information was available for 911 patients (87%). Of these patients, 288 (31.6%) presented with optic neuritis (ON), 188 (20.6%) with a brainstem attack (BS), 257 (28.2%) with a spinal cord syndrome and the remaining 178 (19.5%) with other symptoms.

Predictors of conversion to CDMS

Each variable was tested as a predictor of conversion from CIS to CDMS using a univariable Cox regression model (Table 2). OCB-positive CIS patients were more than twice as likely to convert to CDMS as OCB-negative individuals (hazard ratio (HR) = 2.49, 95% CI = 1.91–3.23, $p < 0.001$; Figure 1(a)). Similarly, a higher IgG index was associated with conversion to CDMS (HR = 1.22, 95% CI = 1.10–1.36, $p < 0.001$), whilst CSF cell count was not. A gradient of risk of conversion was observed with increasing numbers of T2 lesions (two to nine vs 0/1 lesions: HR = 2.28, 95% CI = 1.70–3.06, $p < 0.001$; >9 vs 0/1 lesions: HR = 3.26, 95% CI = 2.28–4.65, $p < 0.001$; >9 vs two to nine lesions: HR = 1.43, 95% CI = 1.21–1.68, $p < 0.001$; Figure 1(b)). Females were at slightly higher risk of CDMS but this did not reach statistical significance (HR = 1.17, 95% CI = 0.94–1.46, $p = 0.160$; e Figure 1(a)). Age at CIS onset was inversely associated with risk of conversion

Table 2. Univariable and multivariable stepwise Cox regression results for all variables investigated as predictors of conversion to CDMS.

Variable	Category	Univariable			Multivariable		
		HR	95% CI	*p*	HR	95% CI	*p*
Age	–	0.98	0.976–0.99	**<0.001**	0.98	0.98–0.99	**<0.001**
Sex	F vs M	1.17	0.94–1.46	0.160	–	–	–
OCB	Positive vs Negative	2.49	1.91–3.23	**<0.001**	2.18	1.71–2.77	**<0.001**
CSF IgG index	–	1.22	1.10–1.36	**<0.001**	–	–	–
MRI T2 lesions	2-9 vs 0/1	2.28	1.70–3.06	**<0.001**	1.97	1.52–2.55	**<0.001**
	> 9 vs 0/1	3.26	2.28–4.65	**<0.001**	2.74	2.04–3.68	**<0.001**
25-OH-D (quartiles)	2nd vs 1st	0.76	0.60–0.95	**0.016**	0.82	0.70–0.97	**0.019**
	3rd vs 1st	0.69	0.58–0.83	**<0.001**	0.76	0.63–0.91	**0.003**
	4th vs 1st	0.76	0.62–0.94	**0.010**	0.85	0.68–1.07	0.167
EBNA1 IgG (quartiles)	2nd vs 1st	0.85	0.69–1.05	0.127	0.75	0.60–0.94	**0.014**
	3rd vs 1st	0.92	0.70–1.23	0.586	0.81	0.59–1.13	0.220
	4th vs 1st	1.11	0.83–1.49	0.464	1.00	0.75–1.34	0.986
CMV IgG (quartiles)	2nd vs 1st	1.13	0.92–1.38	0.238	1.22	0.98–1.52	0.075
	3rd vs 1st	1.24	0.94–1.62	0.126	1.36	1.10–1.67	**0.004**
	4th vs 1st	1.12	0.89–1.41	0.346	1.25	0.97–1.60	0.07
Cotinine	>14 vs <14 ng/ml	0.95	0.79–1.14	0.549	–	–	–
CSF cell count	–	1.00	0.99–1.01	0.988	–	–	–
Type of CIS	BS vs ON	1.04	0.74–1.46	0.831	–	–	–
	Spinal vs ON	1.12	0.89–1.41	0.318	–	–	–
	Other vs ON	1.22	0.93–1.59	0.155	–	–	–

CDMS: clinically definite multiple sclerosis; HR: hazard ratio; CI: confidence interval; F: female; M: male; vs: versus; CSF: cerebrospinal fluid; MRI: magnetic resonance imaging; Ig: immunoglobulin; OCB: oligoclonal bands in CSF; 25-OH-D: 25-hydroxyvitamin D3; EBNA1: Epstein-Barr nuclear antigen 1; CMV: cytomegalovirus; CIS: clinically isolated syndrome; BS: brainstem syndrome; ON: optic neuritis; Spinal: spinal cord syndrome.

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(HR per year increase = 0.98, 95% CI = 0.976–0.99, $p < 0.001$; e Figure 1(b)).

Individuals in the second, third and fourth quartiles of 25-OH-D levels were at significantly reduced risk of conversion compared to those in the first (i.e. lowest) quartile (second vs first: HR = 0.76, 95% CI = 0.60–0.95, $p = 0.016$; third vs first: HR = 0.69, 95% CI = 0.58–0.83, $p < 0.001$; fourth vs first: HR = 0.76, 95% CI = 0.62–0.94, $p = 0.010$; Figure 1(c)). There was no significant difference between those in the second, third and fourth quartiles (Figure 1(c)). No association between risk of conversion and IgG levels against either EBNA1 or CMV, smoking status as defined by cotinine levels (>14 ng/ml) and type of clinical presentation was found.

Stepwise selection was then used to determine a multivariable Cox regression model. The effect of most variables was consistent with that observed in univariable analyses; age, presence of OCBs and number of T2 MRI lesions were independent predictors of conversion. The significance of the association between 25-OH-D levels and risk of CDMS was attenuated, but the HR of each quartile remained consistent with those of the univariable analyses. Inconsistent significance was present for some quartiles of EBNA1 and CMV IgG (Table 2). There was no evidence that the proportional hazards assumption of the Cox model was violated.

We attempted to make these statistical measures of risk more clinically relevant by estimating the risk of conversion to CDMS in different categories of CIS patients at two and five years of follow-up. The risk of conversion varied widely across different exposure categories (Table 3) and was high among individuals with evidence of OCBs and more than nine T2 lesions on MRI (57.0% and 85.5% at two and five years of follow-up, respectively). Including 25-OH-D information did not considerably change these estimates. Notably, the risk of conversion among OCB-negative CIS cases with either 0 or 1 T2 lesions and higher vitamin D status was low (6.8% and 21.4% at two and five years, respectively). These estimates should, however, be interpreted with caution given the small number of patients lacking all risk factors for conversion and the correspondingly wide CIs.

Predictors of OCBs and MRI lesions

The clinical and biological parameters associated with the presence of OCBs and T2 hyperintense lesions at disease onset were assessed. The median age, sex ratio, vitamin D status, EBNA1 IgG and CMV IgG levels of OCB-positive and OCB-negative CIS patients are described in Table 4. OCB-positive individuals were younger, more likely to be female, and had more T2 lesions and lower 25-OH-D levels than those who were OCB negative. OCB-positive patients also had higher EBNA1 but not CMV IgG levels compared to OCB-negative patients. In a multivariable logistic regression model predicting OCB status, the number of T2 lesions (>9 vs 0/1 lesions: odds ratio (OR) = 5.03, 95% CI = 3.35–7.58, $p < 0.001$), lower age at CIS onset (OR per year increase = 0.98, 95% CI = 0.97–0.997, $p = 0.019$) and higher EBNA1 IgG levels (OR = 1.08, 95% CI = 1.04–1.11, $p < 0.001$) were significantly associated with OCBs (Table 4).

The general features of CIS patients in the three different categories based on number of T2 lesions are shown in eTable 2. We used a multivariable ordinal regression model to investigate which variables were independently associated with an increased number of T2 lesions. Only presence of OCBs was significantly associated with an increased lesion load (OR = 2.73, 95% CI = 2.08–3.58, $p < 0.001$) (eTable 2).

Discussion

We report the largest study ever performed on CIS patients. We confirmed a strong association between an increasing number of T2 hyperintense lesions on baseline MRI and risk of conversion in keeping with several previous studies.[3,4,17] Similarly, CSF markers of B cell activity (OCBs and a higher IgG index) were strongly and independently associated with conversion to CDMS.[18–20] Notably, the risk of conversion appeared particularly high in those patients carrying both OCBs and a high number of T2 lesions (57% and 86% risk of conversion after two and five years of follow-up, respectively). As previously suggested, age at disease onset was also inversely associated with risk of conversion.[21]

Two recent studies have demonstrated a significant inverse relation between vitamin D status and risk of conversion.[6,8] Our study appears to confirm this finding, and indeed extends this in a heterogeneous sample of CIS patients from a variety of latitudes. It is noteworthy that our study included both a considerably larger number of patients[8] and also incorporated information on OCBs.[6] Even if the effect of 25-OH-D levels on risk of conversion appears partially attenuated in multivariable analyses, our overall results confirm that CIS patients with lower 25-OH-D levels tend to convert to CDMS more rapidly. Studies outside the field of MS have reported that vitamin D levels may fall in the presence of systemic inflammation

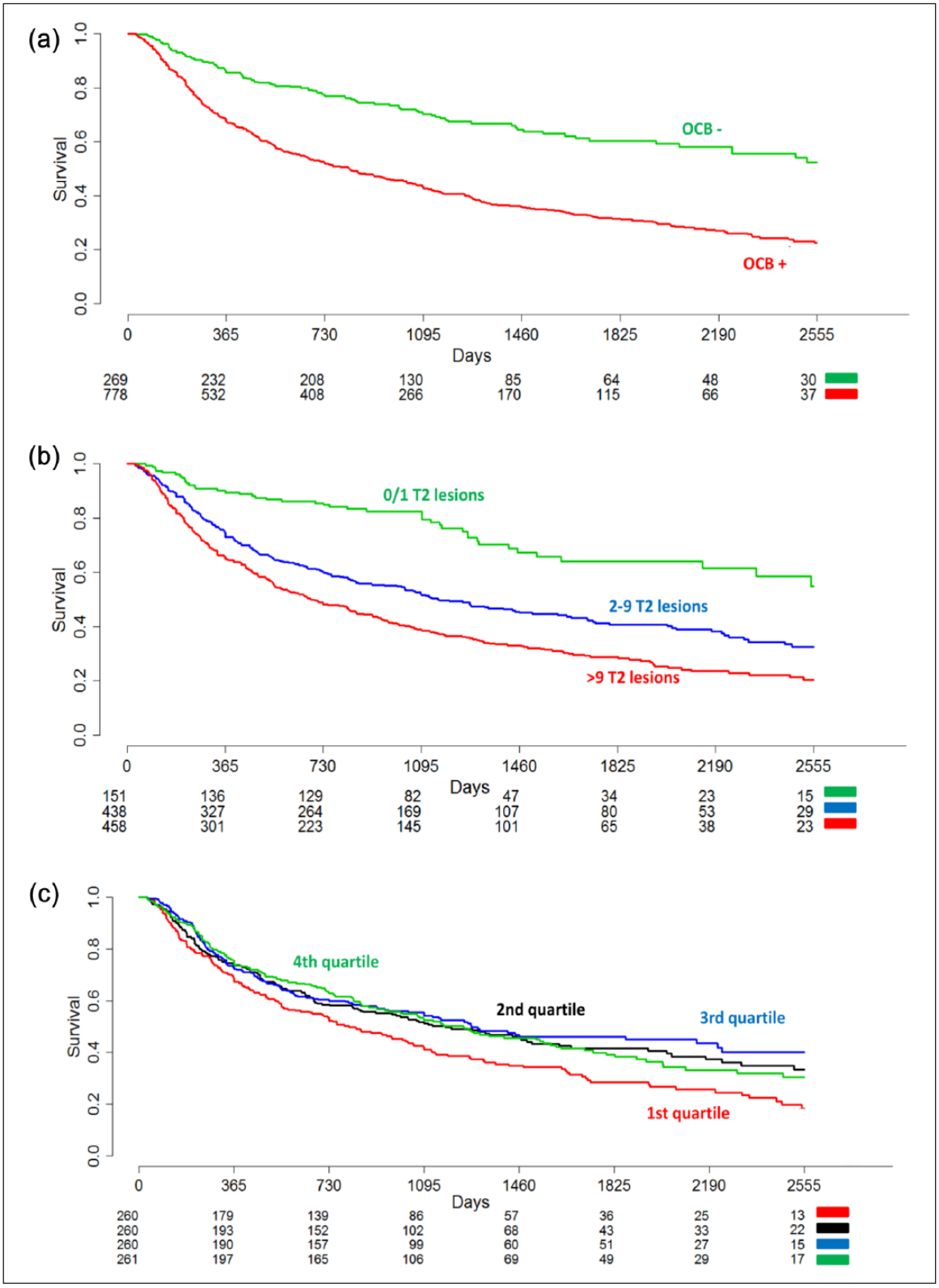

Figure 1. Survival curve of conversion to CDMS and numbers of individuals at risk of conversion (below the x axis) stratified according to presence (+) or absence (–) of oligoclonal bands (OCBs) in CSF (a), number of T2 hyperintense lesions on cranial MRI (b) and serum 25-hydroxyvitamin D3 by quartiles (patients with lowest concentration in first quartile) (c). CDMS: clinically definite multiple sclerosis; CSF: cerebrospinal fluid; MRI: magnetic resonance imaging.

Table 3. Risk of conversion to CDMS in different categories of CIS patients after two and five years of follow-up.

Category	At two years of follow-up		At five years of follow-up	
	n	% risk of conversion (95% CI)	*n*	% risk of conversion (95% CI)
OCB positive	778	47.9 (44.4–51.5)	607	81.1 (77.6–84.0)
OCB negative	269	22.7 (17.9–28.2)	154	58.4 (50.2–66.2)
MRI > 9 T2 lesions	458	51.7 (47.1–56.4)	372	82.5 (78.2–86.2)
MRI 2–9 T2 lesions	438	39.9 (35.4–44.7)	314	74.5 (69.3–79.2)
MRI 0-1 T2 lesions	151	14.6 (9.5–21.4)	75	54.7 (42.8–66.0)
25-OH-D 1st quartile	260	47.3 (41.1–53.6)	205	82.4 (76.4–87.2)
25-OH-D 2nd quartile	260	41.5 (35.5–47.8)	182	76.4 (69.4–82.2)
25-OH-D 3rd quartile	260	40.0 (34.0–46.3)	179	71.5 (64.2–77.9)
25-OH-D 4th quartile	261	36.8 (31.0–43.0)	191	74.3 (67.4–80.2)
OCB positive/> 9 T2	377	57.0 (51.8–62.1)	310	85.5 (80.9–89.1)
OCB negative/> 9 T2	81	27.2 (18.1–38.4)	62	67.7 (54.5–78.7)
OCB positive/2-9 T2	328	43.9 (38.5–49.5)	251	77.7 (71.9–82.6)
OCB negative/2-9 T2	110	28.2 (20.2–37.7)	63	61.9 (48.8–73.6)
OCB positive/0–1 T2	73	19.2 (11.2–30.4)	46	69.6 (54.1–81.8)
OCB negative/0–1 T2	78	10.3 (4.8–19.7)	29	31.0 (16.0–51.0)
OCB positive/>9 T2 / 25-OH-D < median	192	56.2 (48.9–63.3)	154	87.0 (80.4–91.7)
OCB negative/0–1 T2 / 25-OH-D > median	44	6.8 (1.8–19.7)	14	21.4 (5.7–51.2)

CDMS: clinically definite multiple sclerosis; CIS: clinically isolated syndrome; CI: confidence interval; MRI: magnetic resonance imaging; OCB: oligoclonal bands in CSF; 25-OH-D: 25-hydroxyvitamin D3.

and this may happen to a greater extent in those CIS patients who go on to convert to CDMS.[22] Although reverse causation cannot be excluded, the increasing evidence for regulatory effects of vitamin D on the immune system support a potential causal link between vitamin D deficiency and conversion to CDMS.[23,24]

Previous studies have suggested that individuals with higher antibody levels against EBV are at increased risk of conversion.[7,9] However, we did not see any association between IgG production against EBNA1 and CMV and conversion to CDMS in univariable analyses. Some quartiles appeared significantly associated in the multivariable model, but this was not consistent across quartiles. Given the lack of significance in univariable analyses and the known risk of false-positive associations in stepwise models, we believe these data should be interpreted with caution.[25] The difference in the methods used by previous studies could at least partly contribute to the discrepancy in these results. We tested a single EBV antigen using only an ELISA-based kit, which is less accurate than immunofluorescence-based methods.[26]

We were particularly interested in the relation between OCBs and IgG against EBNA1, as previous studies have suggested a potential link between these markers of B cell activation in MS. A similarly large proportion of patients with MS have OCBs in their CSF and antibodies against EBV in their serum.[12,27] In addition, both the presence of OCBs and high antibody titres against EBV proteins are positively associated with the human leucocyte antigen *(HLA)-DRB1*1501* allele, the main genetic risk factor in MS.[28–31] Our finding that EBNA1 but not CMV IgG levels are significantly associated with the presence of OCBs further strengthens the link between EBV and intrathecal B cell activation. This highlights a potential connection between exposure to this virus and the most consistent immunological finding in MS patients.

OCB status was also positively associated with MRI T2 lesion load and lower age at onset. Notably, these variables were also associated with risk of conversion and this confirms the importance of OCBs and B cell activation within the CNS in the pathology of this disease.

Our multicentre approach enabled the retrospective collection of more than 1000 CIS patients who have been followed up for several years, but this real-life clinical setting also inevitably leads to some limitations. Potential bias can arise from centre-specific

Table 4. Association between demographic and clinical features of CIS patients with presence or absence of OCB.

Variable		OCB positive	OCB negative	Univariable			Multivariable		
				OR	95% CI	*p*	OR	95% CI	*p*
Age	Median (IQR)	31.27 (25.4–39.00)	33.6 (27.7–38.8)	0.98	0.97–0.998	**0.025**	0.98	0.97–0.997	**0.019**
T2 MRI	2–9	328 (42.2%)	110 (40.9%)	3.19	2.17–4.68	**<0.001**	3.16	2.13–4.70	**<0.001**
	> 9	377 (48.5%)	81 (30.1%)	4.97	3.34–7.41	**<0.001**	5.03	3.35–7.58	**<0.001**
EBNA1 IgG	Median (IQR)	11.8 (7.6–14.9)	9.3 (4.2–14.1)	1.08	1.05–1.11	**<0.001**	1.08	1.04–1.11	**<0.001**
25-OH-D	Median (IQR)	49.0 (31.2–71.3)	50.1 (36.1–76.8)	0.995	0.99–0.9996	**0.035**	–	–	–
Sex	Females	544 (69.9%)	170 (63.2%)	1.35	1.01–1.81	**0.042**	–	–	–
CMV IgG	Median (IQR)	1.5 (0.2–3.4)	2.2 (0.2–3.3)	1.03	0.98–1.09	0.225	–	–	–

CIS: clinically isolated syndrome; OCB: oligoclonal bands in cerebrospinal fluid (CSF); OR: odds ratio; CI: confidence interval; IQR: interquartile range; EBNA1: Epstein-Barr nuclear antigen 1; 25-OH-D: 25-hydroxyvitamin D3; CMV: cytomegalovirus; Ig: immunoglobulin.

effects, despite adjusting for them in the regression models. This includes the potential consequences of various different clinical care protocols employed across the participating sites, and possible recall bias depending on the frequency of follow-up visits. Furthermore, we should remember that patients who participate in studies are those who fulfil the inclusion criteria, and differences may be present between these and the overall patient population. We did not include in our analysis additional variables that could potentially act as confounders or independently influence the risk of conversion to CDMS such as the potential effect of disease-modifying treatment, genetic factors, history of infectious mononucleosis, latitude, time spent outdoors, vitamin D intake and previous history of smoking. Vitamin D levels were only measured at a single time-point, which may not appropriately reflect vitamin D status over the long term. Finally, MRI protocols and T2 lesion counting methods could not be pre-specified and hence may not be uniform across the different centres. We therefore chose not to use MRI as evidence of dissemination in time and space (as in the current diagnostic criteria).[32] We instead applied Poser criteria to define CDMS, which allow a more reliable evaluation of disease activity in multicentre studies such as this one. Nonetheless, the occurrence of new neurological symptoms represents an important and unequivocal clinical endpoint which is relevant both for patients and neurologists. Similarly, in order to reduce inter-rater variability in T2 lesion counts, we grouped patients in categories (0–1, two to nine and >9 lesions) and used these rather than the original lesion counts for all analyses.

In conclusion, OCB status, number of T2 lesions and lower age at CIS onset are associated with an increased risk of conversion from CIS to CDMS. A role for lower vitamin D levels is also suggested. We confirm that patients both with OCBs and several T2 lesions are highly likely to convert, with their risk of developing CDMS in five years at almost 90%. Given that MRI activity and OCB status are inextricably linked with immune activation, these data support targeting the immune system early in the disease in order to slow or prevent MS disease activity.

The effect of vitamin D is intriguing and supports the need for large-scale clinical trials of vitamin D supplementation in order to conclusively answer the question as to whether this environmental factor is causal or consequential for disease activity. The future integration of additional parameters including genetic variants associated with the risk of MS will allow a

more accurate assessment of risk of conversion and more targeted intervention strategies.

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Conflicts of interest

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