OPEN ACCESS

ORIGINAL RESEARCH

Complete Epstein-Barr virus seropositivity in a large cohort of patients with early multiple sclerosis

Sargis Abrahamyan,[1,2] Bettina Eberspächer,[3] Muna-Miriam Hoshi,[4] Lilian Aly,[4] Felix Luessi,[5] Sergiu Groppa,[5] Luisa Klotz,[6] Sven G Meuth,[6] Christoph Schroeder,[7] Thomas Grüter ,[7] Björn Tackenberg,[8] Friedemann Paul,[1,9] Florian Then-Bergh,[10] Tania Kümpfel,[11] Frank Weber,[12] Martin Stangel,[13] Antonios Bayas,[14] Brigitte Wildemann,[15] Christoph Heesen ,[16] Uwe Zettl,[17] Clemens Warnke,[18,19] Gisela Antony,[20] Nicole Hessler,[21] Heinz Wiendl,[6] Stefan Bittner,[5] Bernhard Hemmer,[4] Ralf Gold,[7] Anke Salmen ,[22] Klemens Ruprecht ,[1] on behalf of the German Competence Network Multiple Sclerosis (KKNMS)

► Additional material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jnnp-2020-322941).

For numbered affiliations see end of article.

Correspondence to
Dr Klemens Ruprecht, Department of Neurology, Charité - Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany; klemens.ruprecht@charite.de

This paper was presented at the 34th Congress of the European Committee for Treatment and Research in Multiple Sclerosis, 12 October 2018, Berlin, Germany (https://doi.org/10.1177/1352458518799980).

Received 2 February 2020
Revised 9 April 2020
Accepted 16 April 2020
Published Online First 5 May 2020

Check for updates

To cite: Abrahamyan S, Eberspächer B, Hoshi M-M, *et al*. *J Neurol Neurosurg Psychiatry* 2020;**91**:681–686.

ABSTRACT

Objective To determine the prevalence of antibodies to Epstein-Barr virus (EBV) in a large cohort of patients with early multiple sclerosis (MS).

Methods Serum samples were collected from 901 patients with a clinically isolated syndrome (CIS) or early relapsing–remitting multiple sclerosis (RRMS) participating in the German National MS cohort, a prospective cohort of patients with early MS with stringent inclusion criteria. Epstein-Barr nuclear antigen (EBNA)-1 and viral capsid antigen (VCA) antibodies were measured in diluted sera by chemiluminescence immunoassays (CLIAs). Sera of EBNA-1 and VCA antibody-negative patients were retested undiluted by an EBV IgG immunoblot. For comparison, we retrospectively analysed the EBV seroprevalence across different age cohorts, ranging from 0 to >80 years, in a large hospital population (N=16 163) from Berlin/Northern Germany.

Results EBNA-1 antibodies were detected by CLIA in 839 of 901 patients with CIS/RRMS. Of the 62 patients without EBNA-1 antibodies, 45 had antibodies to VCA as detected by CLIA. In all of the remaining 17 patients, antibodies to EBV were detected by immunoblot. Altogether, 901 of 901 (100%) patients with CIS/RRMS were EBV-seropositive. EBV seropositivity increased with age in the hospital population but did not reach 100% in any of the investigated age cohorts.

Conclusion The complete EBV seropositivity in this large cohort of patients with early MS strengthens the evidence for a role of EBV in MS. It also suggests that a negative EBV serology in patients with suspected inflammatory central nervous system disease should alert clinicians to consider diagnoses other than MS.

INTRODUCTION

Strong and consistent evidence indicates an association of multiple sclerosis (MS) and infection with the Epstein-Barr virus (EBV).[1–3] This led to the proposal that, from an epidemiological perspective, MS could be regarded as a late complication of EBV infection.[4] If this was true, one would expect that there should be practically no EBV-seronegative patients with MS.[5] Previous seroepidemiological studies and meta-analyses thereof indeed observed very high EBV seropositivity rates (~98% to 100%) in patients with MS or a clinically isolated syndrome (CIS).[1 6–11] Nevertheless, the detection of few EBV-seronegative persons with a diagnosis of MS in some of those studies suggests that EBV-seronegative MS may occur. However, as inclusion criteria of previous studies on the seroprevalence of EBV in patients with MS were heterogeneous, it cannot be excluded that EBV-seronegative persons with a diagnosis of MS reported in the literature may occasionally have been misclassified and could in fact have diagnoses other than MS.[8] Furthermore, it was shown that the EBV seroprevalence in patients with MS may depend on the sensitivity and specificity of the applied antibody assays and that in the likely most robust studies, that is, those that used two independent methods for detection of EBV antibodies, EBV seropositivity in patients with a diagnosis of MS may reach 100%.[7]

To systematically search for EBV-seronegative patients with MS, we analysed the EBV seroprevalence in 901 patients of the German National MS cohort, a prospective longitudinal observational cohort of patients with early MS with stringent inclusion criteria. For comparison, we retrospectively determined EBV seroprevalence rates across different age cohorts in a large hospital population (N=16 163) from Berlin/Northern Germany.

PATIENTS AND METHODS

Patients with early MS

The German National MS cohort is a multicentre prospective longitudinal observational cohort which recruited a total of 1212 patients between August 2010 and December 2014.[12] Inclusion criteria have previously been reported in detail and comprise female and male patients aged ≥18 years and

- A diagnosis of a CIS (defined as a first clinical event suggestive of inflammatory demyelination) within 6 months before inclusion and fulfilment of three of four Barkhof criteria.

- A diagnosis of a CIS within 6 months before inclusion and fulfilment of two of four Barkhof criteria and intrathecal IgG production in cerebrospinal fluid (CSF) or abnormal visually evoked potentials.
- A diagnosis of a CIS within 6 months before inclusion and fulfilment of the McDonald 2010 criteria for relapsing–remitting multiple sclerosis (RRMS).
- A diagnosis of RRMS based on the McDonald 2005 criteria within 2 years before inclusion.[12]

Exclusion criteria comprise previous use of any disease-modifying therapy for MS (except for short-term relapse treatment), primary or secondary progressive MS, concurrent progressive neurological diseases and conditions interfering with the assessment plan, for example, contraindications to MRI. The assessment plan includes standardised collection of demographic and clinical data, assessment of the Expanded Disability Status Scale (EDSS) score and standardised sampling of biospecimens. All patients were recruited at specialised MS centres and study data were monitored with a query system.

Sera from therapy-naïve patients were collected, centrifuged and aliquoted during the baseline visit at the participating centres according to standard operating procedures and were shipped overnight to the Department of Neurology, Technische Universität München, where they were stored at −80°C. Baseline serum samples of 901 patients were available for EBV antibody testing and were sent on dry ice to the Department of Neurology, Charité – Universitätsmedizin Berlin.

Detection of EBV antibodies

Serum IgG antibodies to Epstein-Barr nuclear antigen (EBNA)-1 and to the EBV viral capsid antigen (VCA) were measured by Liaison (DiaSorin, Saluggia, Italy) automated quantitative chemiluminescence immunoassay (CLIA) at Labor Berlin GmbH, Berlin, Germany. As only limited amounts of serum (~50 µL) from participants of the German National MS cohort were available, all sera had to be measured in dilution. EBNA-1 IgG antibodies were measured in serum samples diluted either 1:20 (n=40) or 1:10 (n=861) in assay dilution buffer. With these dilutions, EBNA-1 IgG levels <3 U/mL were considered negative, and EBNA-1 IgG levels ≥3 U/mL were considered positive. All EBNA-1 IgG-negative serum samples were tested at a dilution of 1:10 in assay dilution buffer for antibodies to VCA. With this dilution, VCA IgG levels <10 U/mL were considered negative and VCA IgG levels ≥10 U/mL were considered positive. As per the manufacturer's instructions, the assay range of the EBNA-1 IgG CLIA is 3–600 U/mL and that of the VCA IgG CLIA is 10–750 U/mL.

Since we had to determine EBNA-1 and VCA IgG by CLIA in diluted sera of participants of the German National MS cohort, we retested EBNA-1 and VCA IgG-negative sera without dilution by an EBV IgG immunoblot (recomLine EBV IgG, Mikrogen, Germany). As only a small volume of serum (20 µL) is needed for the EBV IgG immunoblot, we were able to use undiluted sera for these measurements. Serum samples that were positive in at least one of the three assays (EBNA-1 IgG CLIA, VCA IgG CLIA or EBV IgG immunoblot) were considered EBV seropositive.

Retrospective analysis of EBV seroprevalence in a large hospital population

We retrospectively analysed results of EBV serologies, which were performed for routine diagnostic purposes in 16 163 persons at Labor Berlin GmbH, Berlin, Germany, between January 2014 and December 2016. Sera were sent for EBV serological testing from persons treated as inpatients or outpatients at university hospitals (Charité – Universitätsmedizin Berlin) located in Berlin or community hospitals located in Berlin and Northern Germany. The 16 163 persons were included irrespective of diagnoses or the reason for ordering EBV serologies. For individuals tested more than once during the study period, only the results of the first EBV serology were considered. From all persons included in the analysis, results of serological tests for EBNA-1 IgG and VCA IgG and VCA IgM had to be available. Notably, EBNA-1 IgG is a marker of past EBV infection, VCA IgG can be found in primary and past EBV infections, and VCA IgM is a marker of primary EBV infection.[13] Testing for EBNA-1 IgG, VCA IgG and VCA IgM was performed by Liaison automated quantitative CLIA using undiluted sera. Thus, the same method (Liaison CLIA) was used for testing of patients with CIS/RRMS and patients of the hospital population, with sera of patients with CIS/RRMS being measured in dilution and sera of patients from the hospital population being measured without dilution. According to the manufacturer's recommendations, in undiluted sera, EBNA-1 IgG levels <5 U/mL were considered negative, levels between 5 and 20 U/mL were considered equivocal and levels ≥20 U/mL were considered positive; VCA IgG levels <20 U/mL were considered negative and VCA IgG levels ≥20 U/mL were considered positive; and VCA IgM levels <20 U/mL were considered negative, levels between 20 and 40 U/mL were considered equivocal and VCA IgM levels≥ 40 U/mL were considered positive. Persons with EBNA-1 IgG and VCA IgG and VCA IgM below the respective cut-offs were considered EBV-seronegative. Persons in whom at least one of the three antibodies, EBNA-1 IgG, VCA IgG or VCA IgM, was above the respective cut-offs were considered EBV-seropositive. From the age of 5 years onwards, persons were grouped in 5-year age cohorts. To analyse the EBV seroprevalence in early life in more detail, newborns and infants below 5 years of age were grouped in smaller age cohorts.

Statistical analyses

Continuous data were summarised using medians and IQRs. Categorical data are reported as absolute and relative frequencies (%). The significance of different EBV seropositivity rates between patients with early MS and persons in the hospital population was assessed by two-tailed Fisher exact tests (https://www.graphpad.com/quickcalcs/contingency2/). A p value of <0.05 was considered significant.

RESULTS

EBV seroprevalence in early MS

The median (IQR) age of the 901 patients included in this study was 33 (27–41) years, and 630/901 (69.9%) were women. At the time of blood sampling, 380 (42.2 %) had a diagnosis of a CIS and 521 (57.8%) had a diagnosis of RRMS according to the inclusion criteria of the German National MS cohort. The median (IQR) EDSS score of patients with CIS and RRMS was 1.5 (1.0–2.0, data available from n=899 patients).

IgG antibodies to EBNA-1, as measured in diluted sera by CLIA, were positive in 839 of 901 (93.1%) patients with CIS/RRMS. Of the 62 patients without antibodies to EBNA-1, 45 (72.6%) had positive IgG antibodies to VCA, as measured in diluted sera by CLIA. Of the 17 remaining patients, 17 (100%) had IgG antibodies to EBV as detected in undiluted sera by EBV IgG immunoblot. Detailed results of EBV IgG immunoblots

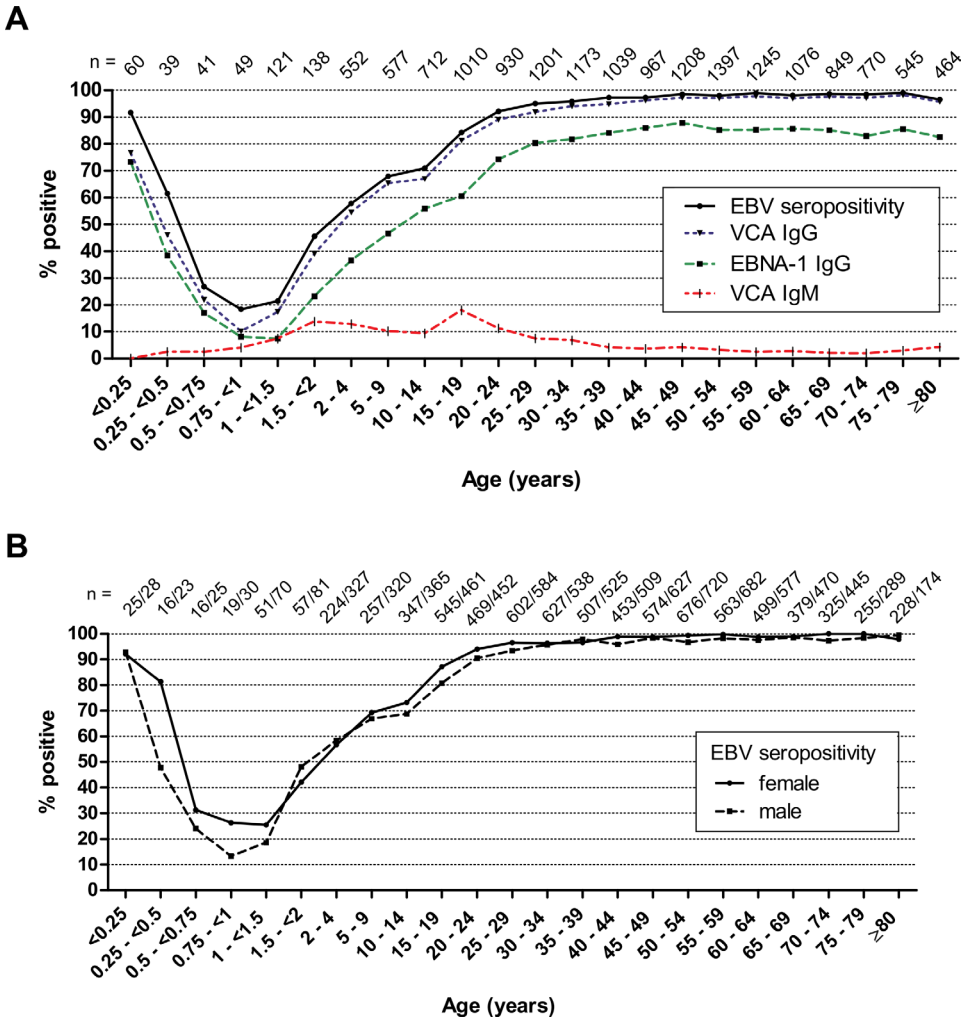

Figure 1 (A) EBV seropositivity rates by age cohorts in a large hospital population (N=16 163). The percentage positivity of IgG antibodies to EBNA-1 and of IgG and IgM antibodies to VCA in different age cohorts is shown. Note that the x-axis is not proportional. EBV seropositivity was defined as seropositivity to at least one of the three antibodies, EBNA-1 IgG, VCA IgG or VCA IgM. The number of persons analysed in each age cohort is indicated above the graph. (B) EBV seropositivity rates by age cohorts in a large hospital population are shown separately for female (n=7714) and male (n=8322) persons. EBV seropositivity was defined as seropositivity to at least one of the three antibodies, EBNA-1 IgG, VCA IgG or VCA IgM. The number of female/male persons analysed in each age cohort is indicated above the graph. EBNA-1, Epstein-Barr nuclear antigen-1; EBV, Epstein-Barr virus; VCA, viral capsid antigen.

are provided in online supplementary table 1. In sum, 12 of 17 sera reacted with the EBNA-1 p72 antigen and 17 of 17 sera reacted with at least one of two VCA antigens (p18 and p23) included in the EBV IgG immunoblot. Altogether, 901 of 901 (100%) patients with CIS/RRMS investigated in this work were EBV seropositive.

EBV seroprevalence in a large hospital population

To compare the EBV seroprevalence in patients with early MS with the general EBV seroprevalence in a similar geographical region, we analysed the EBV seroprevalence across different age groups in a large hospital population (n=16 163) from Berlin/Northern Germany. Data on sex were available from 16 036 of these persons, of which 7714 (48%) were female and 8322 (52%) were male. Figure 1A summarises the EBV seroprevalence in the entire hospital population; the respective source data are provided in online supplementary table 2. EBV seropositivity was high in newborns (<0.25 years) and lowest in 0.75–<1 year olds. Subsequently, EBV seropositivity increased until adulthood, with steepest increases in the age cohorts of 1.5–2.0 and 15–19 years. The increases of EBV seropositivity in these age cohorts were paralleled by an increased detection of VCA IgM antibodies, indicating primary EBV infections. While EBV seropositivity further increased with increasing age and was ≥98% in all 5-year age cohorts from 45 to 79 years, it did not reach 100% in any of these age cohorts. EBV seropositivity tended to be overall slightly higher in women than in men, but the course of EBV seropositivity across the life span was similar in both sexes (figure 1B).

When comparing the EBV seropositivity rates in patients with early MS and in participants of the hospital population in the age range of 20–40 years, that is, the typical age of clinical onset of MS,[14] EBV seropositivity among patients with MS (610/610, 100%) was higher than among participants of the hospital population (4134/4343, 95.2%; p<0.0001). In a comparison of EBNA-1 IgG seropositivity by age between patients with early MS and patients from the hospital population, the difference in EBNA-1 IgG seropositivity was strongest in the youngest analysed age cohort (20–24 years) and subsequently declined with increasing age (online supplementary figure 1).

DISCUSSION

The key result of this study is a complete EBV seropositivity in a large cohort (n=901) of patients with early MS. While this finding is consistent with the known high EBV seroprevalence in MS,[1 6–11] the absence of any EBV-seronegative patients with early MS in our cohort appears remarkable and further strengthens the evidence for an association of EBV infection and MS. The already 100% EBV seropositivity in the 380 patients with a CIS, that is, the earliest clinically detectable manifestation of MS, complies with the concept that EBV infection precedes the clinical onset of MS and suggests that EBV exerts its role early in the development of MS.[15]

The German National MS cohort is a prospective longitudinal cohort with stringent inclusion criteria, requiring MRI, CSF or electrophysiological evidence supportive of MS in patients with a CIS.[12] Thus, the likelihood of inclusion of patients not having a true CIS, that is, a CIS as a first clinical manifestation of MS, or not having true MS into this well-characterised cohort of patients with CIS/early RRMS appears very low. Assuming that there is a genuine association of EBV and MS, the high degree of diagnostic certainty in patients participating in the German National MS cohort may therefore explain the 100% EBV seropositivity observed in this cohort. This conclusion is supported by findings of a previous meta-analysis, which in a post hoc analysis found higher ORs for EBNA-1 and VCA IgG seropositivity in serological studies of EBV prevalence in MS that included confirmed cases of MS as compared with studies that included confirmed and probable cases of MS.[8] The most plausible explanation for this observation is that probable cases of MS are more likely to comprise misclassified patients who actually do not have MS. The EBV seroprevalence of those misclassified patients would be expected to correspond to that of the general population and to thus be lower than the EBV seroprevalence of patients with true MS, which could explain the occasional detection of EBV-seronegative persons in some former studies on EBV seroprevalence in MS.[8]

Our study corroborates results obtained in 1047 retrospectively collected patients with a CIS, only one of whom was found to be EBV seronegative.[10 16] Altogether, the present evidence suggests that EBV-seronegative patients with MS, if they should exist at all, occur extremely rarely. An implication of these findings of relevance for clinical practise is that a negative EBV serology in a patient with suspected inflammatory central nervous system disease should alert clinicians to consider diagnoses other than MS.[2 5 7] Given that the difference in the EBV seropositivity rates between patients with MS and controls declines with age (see also online supplementary figure 1), the younger the age of the patient, the more informative testing for EBV should be. Future studies on this issue may therefore focus in particular on children with suspected inflammatory central nervous system disease, as the difference between the likewise high EBV seroprevalence in children with MS and the EBV seroprevalence in paediatric controls is rather pronounced.[17]

The high EBV seropositivity in newborns and the rapid decline until the age of 1 year observed in the hospital population is explained by placental transmission and subsequent disappearance of maternal EBV antibodies. The subsequent steep increases of EBV seropositivity in early infancy and in the age cohort of 15–19 years correspond to previous data on the natural course of EBV infection in industrialised countries of the northern hemisphere.[18–21] The somewhat higher seroprevalence of VCA IgG as compared with EBNA-1 IgG in patients of the hospital population is consistent with findings of previous large seroepidemiological studies and likely related to the known phenomenon that a certain proportion of persons infected with EBV does not develop antibodies to EBNA-1.[8 13 22] Importantly, we found that about 30% of 10–14 year olds in our hospital population from Northern Germany were EBV seronegative. This is of relevance as these individuals are particularly prone to develop symptomatic primary EBV infection in the form of infectious mononucleosis, which is associated with an about twofold increased risk of MS.[23 24]

The higher EBV seropositivity rate in patients with early MS (100%) as compared with persons in the hospital population (95.2%) in the age range of 20–40 years is consistent with previous data[8] and appears compatible with the concept that EBV may be a necessary but not sufficient factor for the development of MS.

Of note, due to only limited amounts of serum available, sera of patients from the MS cohort had to be measured by CLIA in dilution. All EBNA-1 and VCA antibody-negative patients with CIS/early RRMS, as determined in diluted sera by CLIA, were thus retested by an EBV IgG immunoblot, which requires only a small volume of serum, enabling us to analyse undiluted sera. Detection of EBV antibodies by immunoblot in patients, who were EBV and VCA IgG negative by CLIA, is therefore explained by the fact that those sera were measured undiluted, that is, at a 10-fold higher concentration than in the CLIA. The majority of the 17 sera tested by the EBV immunoblot contained antibodies to EBNA-1, and all 17 sera contained antibodies to VCA (see online supplementary table 1), further supporting the conclusion that antibody responses in those 17 patients differed only quantitatively but not qualitatitvely from that of the other 884 patients.

Of further note, unlike patients with CIS/RRMS, EBV-seronegative participants of the hospital population, as determined by CLIA using undiluted sera, were not retested by an EBV immunoblot. We consider it very unlikely that this could have resulted in a higher rate of EBV seronegativity in the hospital population than in patients with MS for the following reasons: first, in routine diagnostic serology, absence of EBNA-1 IgG, VCA IgG and VCA IgM, as determined by CLIA in undiluted sera, is generally accepted to reliably indicate EBV seronegativity with no further confirmatory tests being required.[13 21 25] Second, while we are not aware of published studies that directly compared the recomLine EBV IgG immunoblot with the liaison CLIA applied in our work, in a previous comparative study, the sensitivity of the recomLine EBV IgG immunoblot was not higher than that of another CLIA method (Architect; Abbott, Wiesbaden, Germany),[25] which is similar to the CLIA Method used in our work.[26] Third, when we re-tested 28 sera, which were EBNA-1 IgG and VCA IgG negative, as determined in undiluted sera by the Liaison CLIA, by EBV immunoblots, all of these sera were likewise EBV seronegative in the EBV immunoblot (unpublished observation). Altogether, the available evidence therefore clearly argues against a higher sensitivity of the EBV IgG immunoblot as compared with determination of EBNA-1 IgG and VCA IgG in undiluted sera by the liaison CLIA.

A limitation of this study is that we did not determine EBV seroprevalence in the general population, but, similar to a previous large investigation,[21] used a hospital population as a surrogate instead. The hospital population may have included patients in whom EBV serologies were ordered for a suspected primary EBV infection, which could potentially result in higher EBV seropositivity rates than in the general population. Conversely, our hospital population may also have included immunosuppressed patients in whom EBV serologies could potentially result false

negative. However, given the very high number of patients analysed, it seems conceivable that the data obtained in the hospital population are overall representative of EBV seropositivity rates across different age ranges in the general population. Finally, as is the case with every serological test, we cannot exclude the occurrence of rare false-positive results. Nevertheless, such rare false-positive results would be highly unlikely to explain the 100% EBV seropositivity observed in patients with CIS/RRMS in our study.

CONCLUSION

The complete EBV seropositivity in this large cohort of patients with CIS/RRMS strengthens the evidence for a role of EBV in MS. It also suggests that a negative EBV serology in patients with suspected inflammatory central nervous system disease should alert clinicians to consider diagnoses other than MS. The results of this study are compatible with the concept that MS could be a rare late complication of EBV infection. Future studies should focus on the clarification of the mechanisms underlying the role of EBV in MS.

Author affiliations
[1]Department of Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany
[2]Yerevan State University, Yerevan, Armenia
[3]Labor Berlin Charité-Vivantes GmbH, Berlin, Germany
[4]Department of Neurology, Klinikum rechts der Isar der Technischen Universität München, Munich, Germany
[5]Department of Neurology, University Medicine Mainz, Johannes Gutenberg University Mainz, Mainz, Germany
[6]Department of Neurology with Institute of Translational Neurology, University of Münster, Münster, Germany
[7]Department of Neurology, St. Josef-Hospital, Ruhr-University Bochum, Bochum, Germany
[8]Department of Neurology, Philipps-Universität Marburg, Marburg, Germany
[9]NeuroCure Clinical Research Center, Charité – Universitätsmedizin Berlin, Berlin, Germany
[10]Department of Neurology, University of Leipzig, Leipzig, Germany
[11]Institute of Clinical Neuroimmunology, Ludwig-Maximilians-Universität, Munich, Germany
[12]Neurological Clinic, Sana Kliniken des Landkreises Cham, Cham, Germany
[13]Clinical Neuroimmunology and Neurochemistry, Department of Neurology, Hannover Medical School, Hannover, Germany
[14]Department of Neurology, Universitätsklinikum Augsburg, Augsburg, Germany
[15]Department of Neurology, University of Heidelberg, Heidelberg, Germany
[16]Department of Neurology, University Hospital Hamburg-Eppendorf, Hamburg, Germany
[17]Department of Neurology, University of Rostock, Rostock, Germany
[18]Department of Neurology, University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany
[19]Department of Neurology, University of Düsseldorf, Düsseldorf, Germany
[20]Central Information Office (CIO), Philipps-Universität Marburg, Marburg, Germany
[21]Institute of Medical Biometry and Statistics, University Hospital Schleswig-Holstein, Campus Lübeck, Lübeck, Germany
[22]Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

Acknowledgements The authors and representatives of the Kompetenznetz Multiple Sklerose express their deep gratitude to all patients participating in the German National MS cohort, as well as to the study nurses, for their motivated collaboration and recruitment efforts, and to the data monitoring and administrative personnel of the study.

Collaborators Katrin Pape, Department of Neurology, University Medicine Mainz, Johannes Gutenberg University Mainz, Germany; Gerd Meyer zu Hörste, Department of Neurology with Institute of Translational Neurology, University of Münster, Germany; Maria Seipelt, Department of Neurology, Philipps-Universität Marburg, Germany; Sandra Nischwitz and Matthias Knop, Department of Neurology, Max-Planck-Institute of Psychiatry, Munich, Germany; Susanne Rothacher, Department of Neurology, Universitätsklinikum Augsburg, Germany; Hayrettin Tumani, Department of Neurology, University of Ulm, Germany, and Clinic of Neurology Dietenbronn, Schwendi, Germany; Ulf Ziemann, Department of Neurology, Eberhard-Karls-Universität Tübingen, Germany; Ralf A Linker, Department of Neurology, University Hospital Regensburg, Germany.

Contributors SA and BE participated in study design, performed antibody measurements and analysed the data. M-MH, LA, FL, SG, LK, SGM, CS, TG, BT, FP, FT-B, TK, FW, MS, AB, BW, CH, UZ, CW, GA, NH, HW, SB, BH, RG and AS contributed patients and clinical data. GA, RG, BH, AS and HW designed and conceptualised the German National MS cohort. KR conceived the study, participated in the study design, analysed the data and drafted the manuscript. All authors reviewed and revised the manuscript.

Funding The German National MS Cohort and the Kompetenznetz Multiple Sklerose are supported by grants from the German Federal Ministry for Education and Research, grant number 01GI0914 (Bochum), 01GI0916, 01GI1601G (Lübeck) and 01GI1601B (Marburg). This study was supported by the Charité Research Fund and Stiftung Charité (BIH Clinical Fellow Program).

Competing interests SA reports no disclosures. BE reports no disclosures. M-MH received travel expenses from Bayer Health Care and honoraria for an advisory board from Merck Serono GmbH. LA reports no disclosures. FL serves as an advisory board member for Roche Pharma and has received travel grants from Teva Pharma. SG reports no disclosures. LK received compensation for serving on scientific advisory boards (Genzyme, Novartis Pharma); speaker honoraria and travel support (CSL Behring, Merck Serono, Roche, Novartis Pharma); research support (Biogen, Novartis Pharma). SGM receives honoraria for lecturing, and travel expenses for attending meetings from Almirall, Amicus Therapeutics Germany, Bayer Health Care, Biogen, Celgene, Diamed, Genzyme, MedDay Pharmaceuticals, Merck Serono, Novartis, Novo Nordisk, ONO Pharma, Roche, Sanofi-Aventis, Chugai Pharma, QuintilesIMS and Teva. His research is funded by the German Ministry for Education and Research (BMBF), Bundesinstitut für Risikobewertung (BfR), Deutsche Forschungsgemeinschaft (DFG), Else Kröner Fresenius Foundation, Gemeinsamer Bundesausschuss (G-BA), German Academic Exchange Service, Hertie Foundation, Interdisciplinary Center for Clinical Studies (IZKF) Muenster, German Foundation Neurology and Alexion, Almirall, Amicus Therapeutics Germany, Biogen, Diamed, Fresenius Medical Care, Genzyme, HERZ Burgdorf, Merck Serono, Novartis, ONO Pharma, Roche, and Teva.CS reports no disclosures.TG received travel reimbursement from Biogen Idec; not related to this work. BT received personal speaker honoraria and consultancy fees as a speaker and advisor from Bayer Healthcare, Biogen, CSL Behring, GRIFOLS, Merck Serono, Novartis, Octapharma, Roche, Sanofi Genzyme, TEVA und UCB Pharma. His University received unrestricted research grants from Biogen-idec, Novartis, TEVA, Bayer Healthcare, CSL-Behring, GRIFOLS, Octapharma, Sanofi Genzyme und UCB Pharma; none related to this work.FP serves on the scientific advisory board for Novartis; received speaker honoraria and travel funding from Bayer, Novartis, Biogen Idec, Teva, Sanofi-Aventis/Genzyme, Merck Serono, Alexion, Chugai, MedImmune, and Shire; is an academic editor for PLoS ONE; is an associate editor for Neurology® Neuroimmunology & Neuroinflammation; consulted for SanofiGenzyme, Biogen Idec, MedImmune, Shire, and Alexion; and received research support from Bayer, Novartis, Biogen Idec, Teva, Sanofi-Aventis/Genzyme, Alexion, Merck Serono, German Research Council, Werth Stiftung of the City of Cologne, German Ministry of Education and Research, Arthur Arnstein Stiftung Berlin, EU FP7 Framework Program, Guthy Jackson Charitable Foundation, and National Multiple Sclerosis of the USA; none related to this work. FTB received personal compensation for speaking and attending advisory boards from Actelion, Bayer, Biogen, Genzyme, Merck, Novartis, Teva and Roche; financial support, through his institution, to attend scientific meetings or for investigator initiated studies from Actelion, Bayer, Biogen, Genzyme, Merck, Novartis and Teva. TK received travel expenses and personal compensations from Bayer Healthcare, Teva Pharma, Merck-Serono, Novartis, Sanofi-Aventis/Genzyme, Roche and Biogen, as well as grant support from Bayer-Schering AG, Novartis and Chugai Pharma; and none related to this work. FW received honoraria from Genzyme, Novartis, TEVA, Bayer and Biogen for speaking or for serving on a scientific advisory board, a travel grant for the attention of a scientific meeting from Merck-Serono and Novartis and grant support from Merck-Serono, Novartis and from the Federal Ministry of Education and Research (BMBF, Projects Biobanking and Omics in ControlMS as part of the Competence Network Multiple Sclerosis). MS received honoraria for scientific lectures or consultancy from Bayer Healthcare, Biogen, Baxter/Baxalta, CSL Behring, Euroimmune, Grifols, Merck-Serono, Novartis, Roche, Sanofi-Aventis, and Teva. His institution received research support from Bayer Healthcare, Biogen Idec, Genzyme, Merck-Serono, Novartis, and Teva; and none related to this work. AB received personal compensation from Merck Serono, Biogen, Bayer, Novartis, TEVA, Roche, Sanofi/Genzyme, Celgene, Alexion and grants for congress trips and participation from Biogen, TEVA, Novartis, Sanofi/Genzyme, Merck Serono, Celgene; none related to this work. BW reports grants from Deutsche Forschungsgemeinschaft, grants from Bundesministerium für Forschung und Technologie, grants from Dietmar Hopp Stiftung, grants from Klaus Tschira Stiftung, grants and personal fees from Merck Serono, personal fees from Biogen, personal fees from Bayer Healthcare, personal fees from TEVA, grants and personal fees from Novartis, grants and personal fees from Sanofi Genzyme, personal fees from Roche, outside the submitted work. CH received research grants and speaker honoraria from Biogen, Genzyme, Roche, and Merck; none related to this work. UKZ received speaker fees from Aventis, Almirall, Biogen, Bayer, Merck, Novartis, Roche, and Teva. CW has received institutional fees for consultancy, speaking, or research from Novartis, Biogen, Sanofi-Genzyme and Roche. GA reports no disclosures.NH reports

no disclosures.HW receives honoraria for acting as a member of scientific advisory boards and as a consultant for Biogen, Evgen, MedDay Pharmaceuticals, Merck Serono, Novartis, Roche Pharma AG, Sanofi-Genzyme, as well as speaker honoraria and travel support from Alexion, Biogen, Cognomed, F. Hoffmann-La Roche Ltd, Gemeinnützige Hertie-Stiftung, Merck Serono, Novartis, Roche Pharma AG, Sanofi-Genzyme, TEVA, and WebMD Global. Professor Wiendl is acting as a paid consultant for Abbvie, Actelion, Biogen, IGES, Novartis, Roche, Sanofi-Genzyme, and the Swiss Multiple Sclerosis Society. His research is funded by the BMBF, DFG, Else Kröner Fresenius Foundation, Fresenius Foundation, Hertie Foundation, NRW Ministry of Education and Research, Interdisciplinary Center for Clinical Studies (IZKF) Muenster and RE Children's Foundation, Biogen GmbH, GlaxoSmithKline GmbH, and Roche Pharma AG, Sanofi-Genzyme. SB has received honoria and compensation for travel from Biogen Idec, Merck Serono, Novartis, Sanofi-Genzyme and Roche. BH served on scientific advisory boards for F. Hoffmann-La Roche Ltd, Novartis, Bayer AG, and Genentech; he has served as DMSC member for Allergy Care and TG Therapeutics; he or his institution have received speaker honoraria from Biogen Idec, Teva Neuroscience, Merck Serono, Medimmune, Novartis, Desitin, and F. Hoffmann-La Roche Ltd; his institution has received research support from Chugai Pharmaceuticals; holds part of two patents; one for the detection of antibodies and T cells against KIR4.1 in a subpopulation of patients with MS and one for genetic determinants of neutralizing antibodies to interferon β during the last 3 years. RG serves on scientific advisory boards for Teva Pharmaceutical Industries Ltd, Biogen Idec, Bayer Schering Pharma, and Novartis; has received speaker honoraria from Biogen Idec, Teva Pharmaceutical Industries Ltd., Bayer Schering Pharma, and Novartis; serves as editor for Therapeutic Advances in Neurological Diseases and on the editorial boards of Experimental Neurology and the Journal of Neuroimmunology; and receives research support from Teva Pharmaceutical Industries Ltd., Biogen Idec, Bayer Schering Pharma, Genzyme, Merck Serono, and Novartis; none related to this work. AS received speaker honoraria and/or travel compensation for activities with Almirall Hermal GmbH, Biogen, Merck, Novartis, Roche, and Sanofi Genzyme; none related to this work. KR received research support from Novartis, Merck Serono, German Ministry of Education and Research, European Union, Stiftung Charité (BIH Clinical Fellow), Arthur Arnstein Stiftung Berlin, as well as speaking fees and travel grants from Bayer Healthcare, Biogen Idec, Merck Serono, Sanofi-Aventis/Genzyme, Teva Pharmaceuticals, Roche, Novartis, and Guthy Jackson Charitable Foundation.

Patient consent for publication Not required.

Ethics approval The study protocol, including collection of biospecimens for scientific purposes, was approved by the lead ethics committee, Ruhr-University Bochum (registration number 3714-10), and local ethics committees of all participating centres.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iDs
Thomas Grüter http://orcid.org/0000-0001-8927-9818
Christoph Heesen http://orcid.org/0000-0001-8131-9467
Anke Salmen http://orcid.org/0000-0002-4751-299X
Klemens Ruprecht http://orcid.org/0000-0003-1962-6014

REFERENCES

1 Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: the role of infection. *Ann Neurol* 2007;61:288–99.
2 Ascherio A, Munger KL, Lünemann JD. The initiation and prevention of multiple sclerosis. *Nat Rev Neurol* 2012;8:602–12.
3 Belbasis L, Bellou V, Evangelou E, *et al*. Environmental risk factors and multiple sclerosis: an umbrella review of systematic reviews and meta-analyses. *Lancet Neurol* 2015;14:263–73.
4 Ascherio A, Munger KL. Epidemiology of multiple sclerosis: from risk factors to Prevention-An update. *Semin Neurol* 2016;36:103–14.
5 Deuschle K, Hofmann J, Otto C, *et al*. Are there Epstein-Barr virus seronegative patients with multiple sclerosis? *Mult Scler* 2013;19:1242–3.
6 Goodin DS. The causal cascade to multiple sclerosis: a model for MS pathogenesis. *PLoS One* 2009;4:e4565.
7 Pakpoor J, Disanto G, Gerber JE, *et al*. The risk of developing multiple sclerosis in individuals seronegative for Epstein-Barr virus: a meta-analysis. *Mult Scler* 2013;19:162–6.
8 Almohmeed YH, Avenell A, Aucott L, *et al*. Systematic review and meta-analysis of the sero-epidemiological association between Epstein Barr virus and multiple sclerosis. *PLoS One* 2013;8:e61110.
9 Horakova D, Zivadinov R, Weinstock-Guttman B, *et al*. Environmental factors associated with disease progression after the first demyelinating event: results from the multi-center set study. *PLoS One* 2013;8:e53996.
10 Dobson R, Kuhle J, Middeldorp J, *et al*. Epstein-Barr-negative MS: a true phenomenon? *Neurol Neuroimmunol Neuroinflamm* 2017;4:e318.
11 Gieß RM, Pfuhl C, Behrens JR, *et al*. Epstein-Barr virus antibodies in serum and DNA load in saliva are not associated with radiological or clinical disease activity in patients with early multiple sclerosis. *PLoS One* 2017;12:e0175279.
12 von Bismarck O, Dankowski T, Ambrosius B, *et al*. Treatment choices and neuropsychological symptoms of a large cohort of early MS. *Neurol Neuroimmunol Neuroinflamm* 2018;5:e446.
13 Niller H-H, Bauer G. Epstein-Barr virus: clinical diagnostics. *Methods Mol Biol* 2017;1532:33–55.
14 Compston A, Coles A. Multiple sclerosis. *The Lancet* 2008;372:1502–17.
15 Levin LI, Munger KL, O'Reilly EJ, *et al*. Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. *Ann Neurol* 2010;67:NA–30.
16 Kuhle J, Disanto G, Dobson R, *et al*. Conversion from clinically isolated syndrome to multiple sclerosis: a large multicentre study. *Mult Scler* 2015;21:1013–24.
17 Pohl D, Krone B, Rostasy K, *et al*. High seroprevalence of Epstein-Barr virus in children with multiple sclerosis. *Neurology* 2006;67:2063–5.
18 Rickinson AB, Kieff E. Epstein-Barr virus. In: Knipe DM, Howley PM, eds. *Virology*. 4th edn. New York: Lippincott Williams and Wilkins, 2001: 2575–627.
19 Morris MC, Edmunds WJ, Hesketh LM, *et al*. Sero-Epidemiological patterns of Epstein-Barr and herpes simplex (HSV-1 and HSV-2) viruses in England and Wales. *J Med Virol* 2002;67:522–7.
20 Balfour HH, Sifakis F, Sliman JA, *et al*. Age-Specific prevalence of Epstein-Barr virus infection among individuals aged 6-19 years in the United States and factors affecting its acquisition. *J Infect Dis* 2013;208:1286–93.
21 Fourcade G, Germi R, Guerber F, *et al*. Evolution of EBV seroprevalence and primary infection age in a French hospital and a City laboratory network, 2000-2016. *PLoS One* 2017;12:e0175574.
22 De Paschale M, Agrappi C, Manco MT, *et al*. Seroepidemiology of EBV and interpretation of the "isolated VCA IgG" pattern. *J Med Virol* 2009;81:325–31.
23 Balfour HH, Odumade OA, Schmeling DO, *et al*. Behavioral, virologic, and immunologic factors associated with acquisition and severity of primary Epstein-Barr virus infection in university students. *J Infect Dis* 2013;207:80–8.
24 Handel AE, Williamson AJ, Disanto G, *et al*. An updated meta-analysis of risk of multiple sclerosis following infectious mononucleosis. *PLoS One* 2010;5:e12496.
25 Guerrero-Ramos A, Patel M, Kadakia K, *et al*. Performance of the architect EBV antibody panel for determination of Epstein-Barr virus infection stage in immunocompetent adolescents and young adults with clinical suspicion of infectious mononucleosis. *Clin Vaccine Immunol* 2014;21:817–23.
26 François C, Segard C, Bouvier M, *et al*. Comparison of Abbott Architect®, Siemens Immulite®, and Diasorin Liaison® for determination of Epstein-Barr virus serological diagnosis. *Diagn Microbiol Infect Dis* 2018;90:96–101.