Harvard T.H. Chan School of Public Health

Toward a Common Research Agenda in Infection-Associated Chronic Illnesses

Biomarkers for EBV and associations with Multiple Sclerosis (MS)

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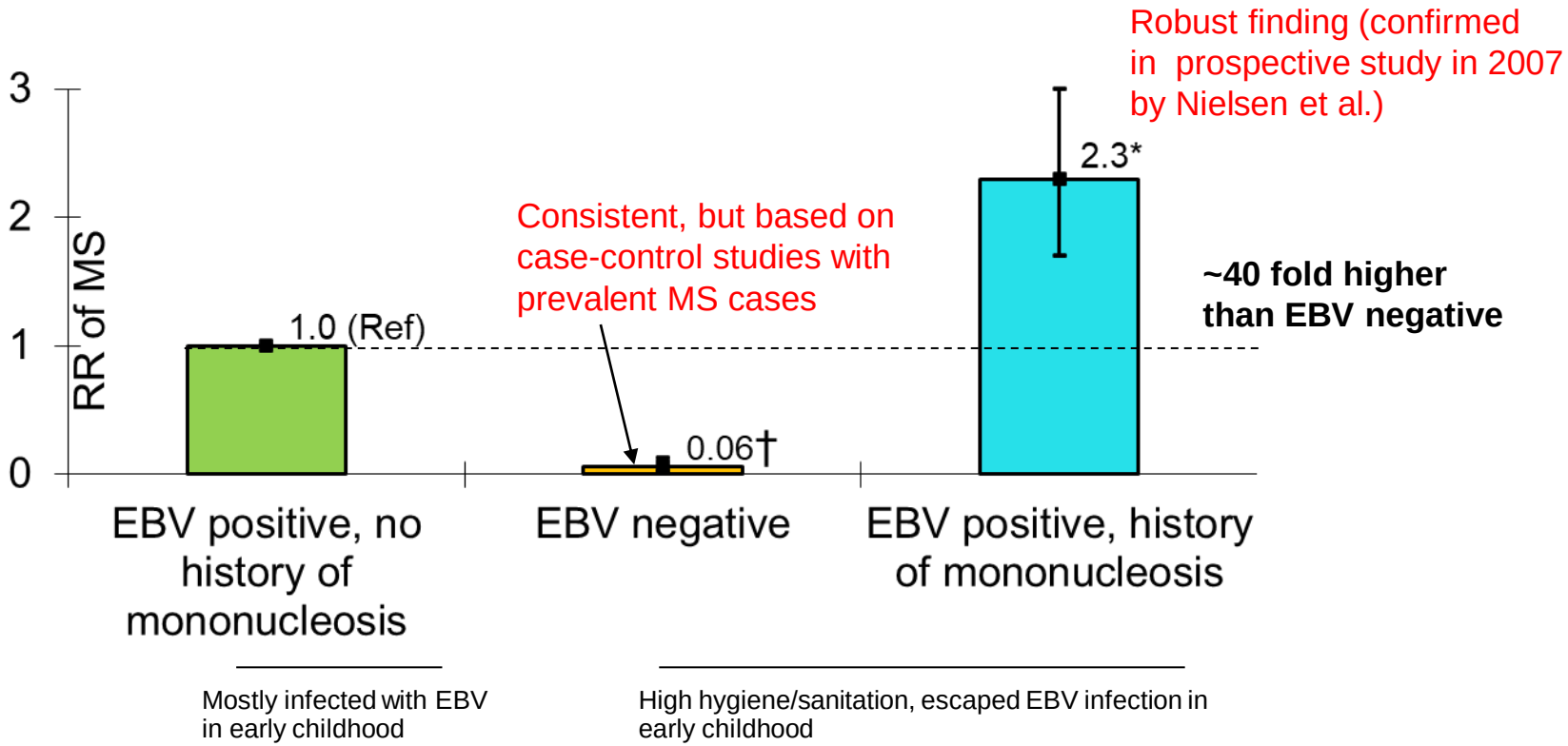
Causality

- This is the key question in examining the relation between infection and chronic diseases
- Requires demonstration that the disease risk in infected > than in uninfected **,***
- The above question can only be answered by rigorous longitudinal epidemiological studies

** When feasible: that prevention of infection results in disease reduction

*** Infection may also aggravate pre-existing chronic disease

MS risk according to EBV infection and history of mononucleosis (= late age at EBV infection)



† * Values based on meta-analyses of case-control studies, both $p<10^{-8}$

Main findings

6.8% EBV -

EBV +

EBV -

EBV -

EBV +

MS

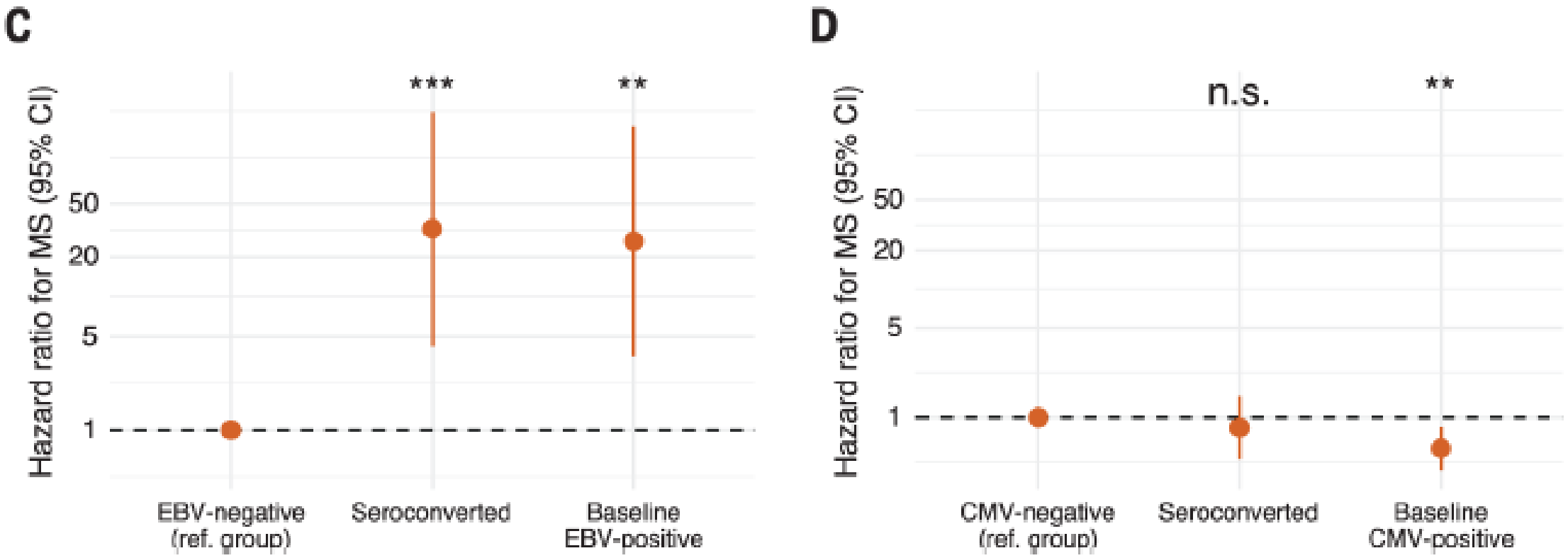
766

1

34*

51% of EBV negative seroconverted during follow-up

***All 34 with onset after EBV infection**

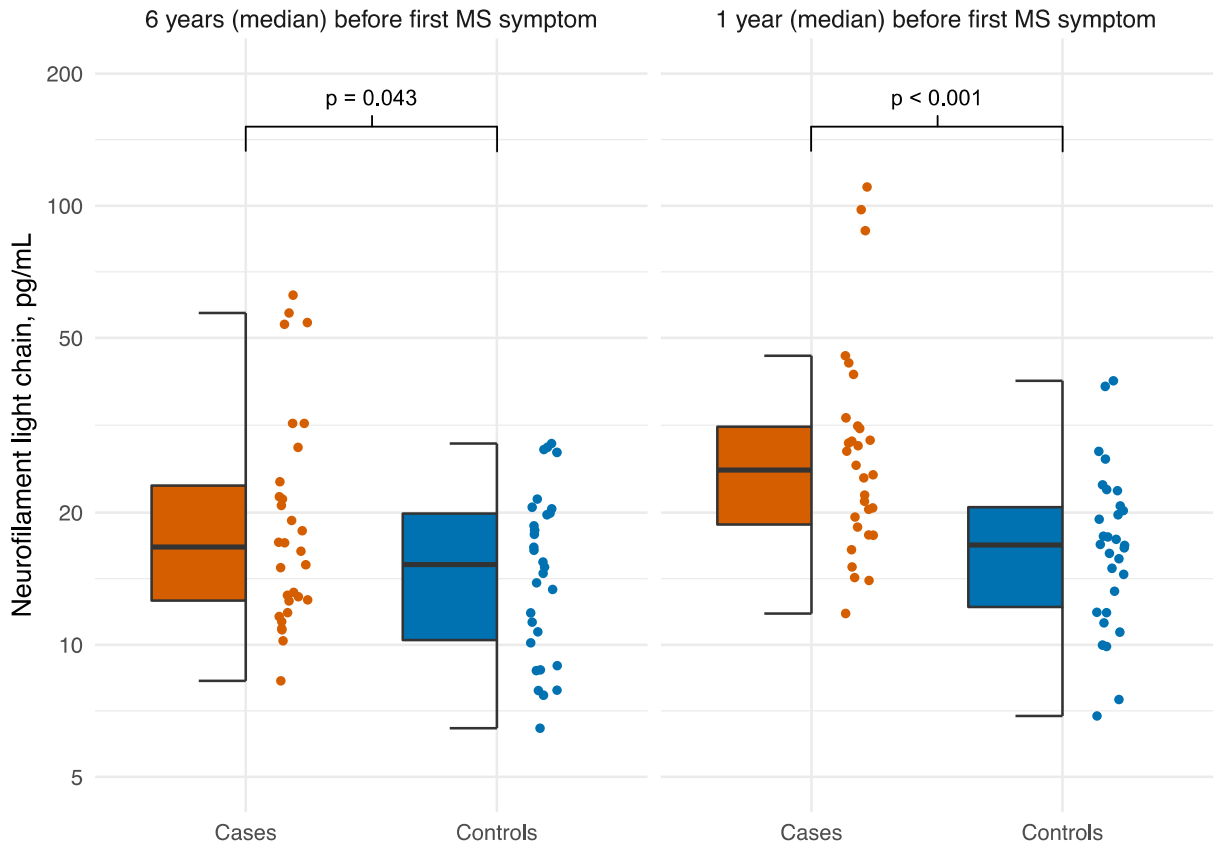


EBV

CMV

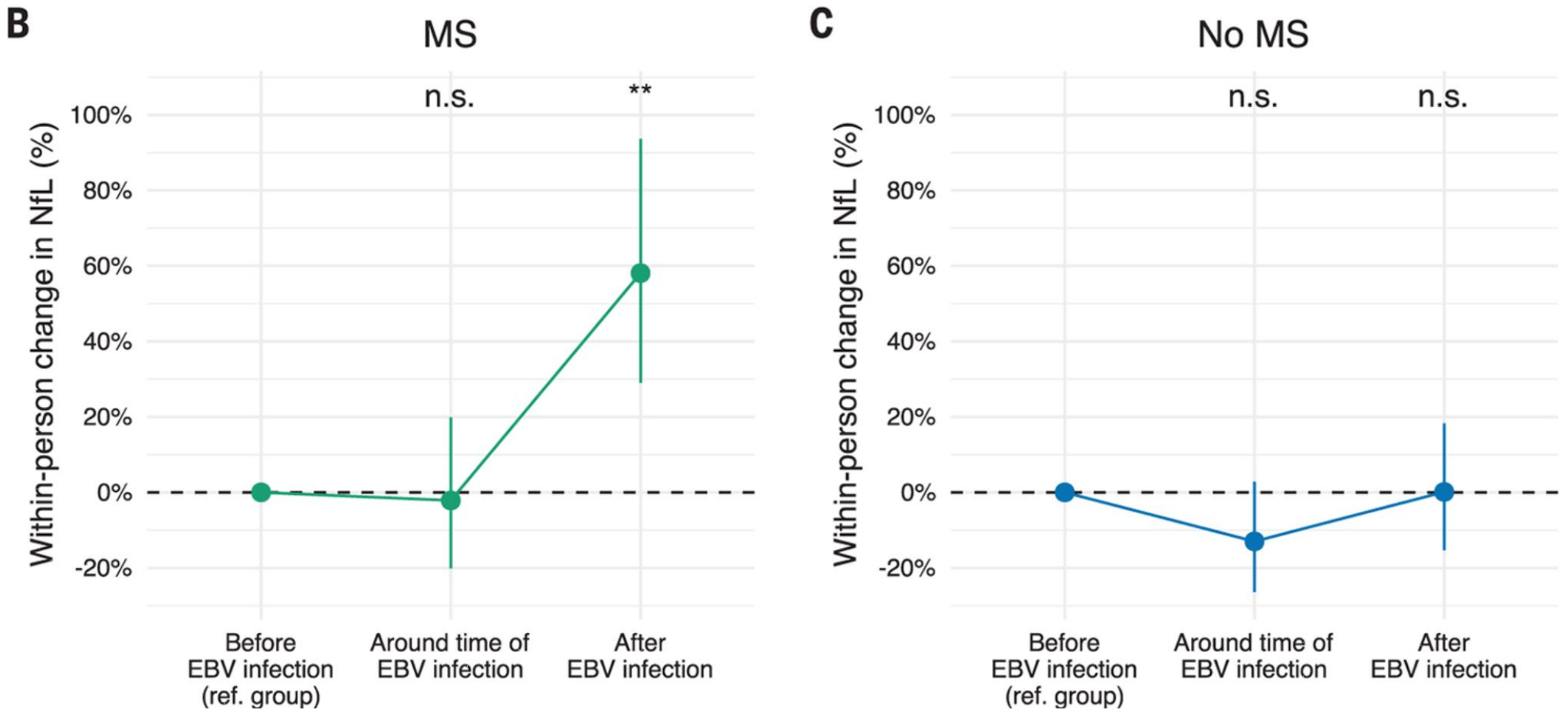
Bjornevik et al. Science 2022

sNfL levels were elevated years before MS onset



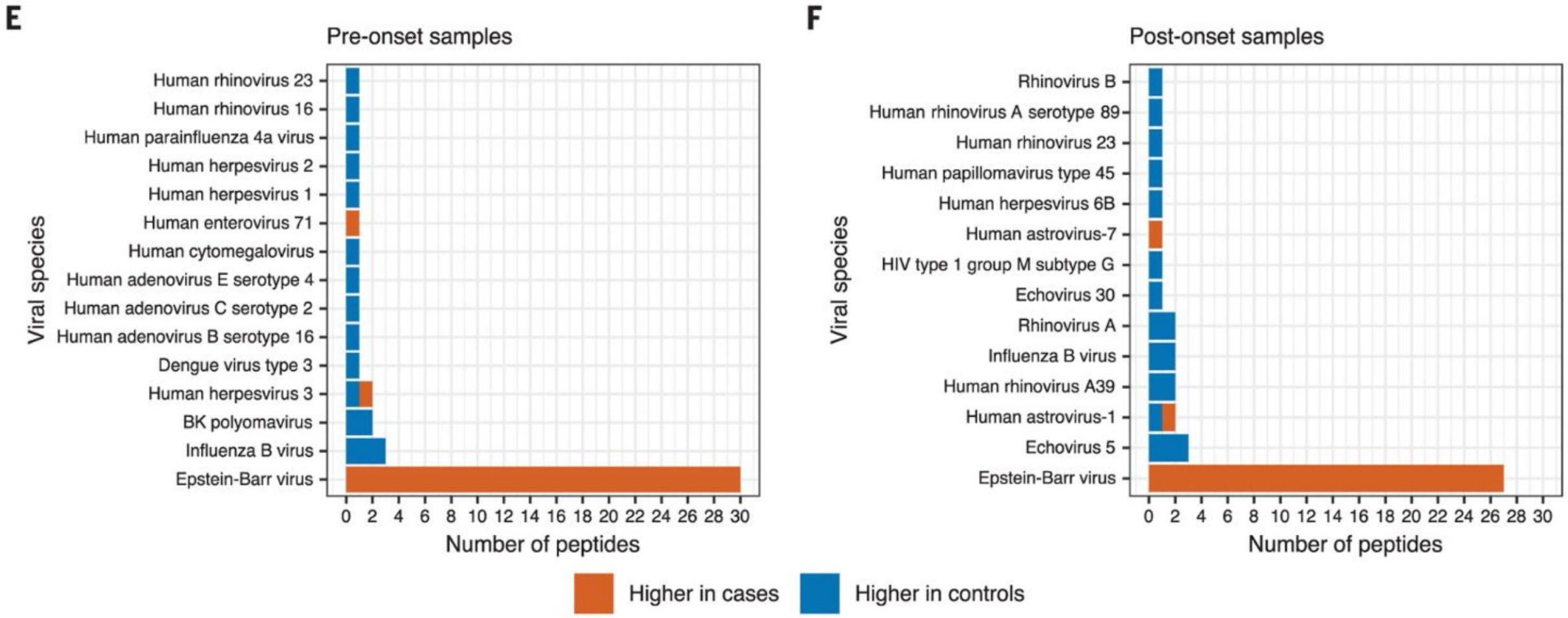
Bjornevik et al. JAMA Neurol. 2020

Does EBV infection precede NfL increase?



Bjornevik et al. Science 2022

MS associated with immune dysregulation?



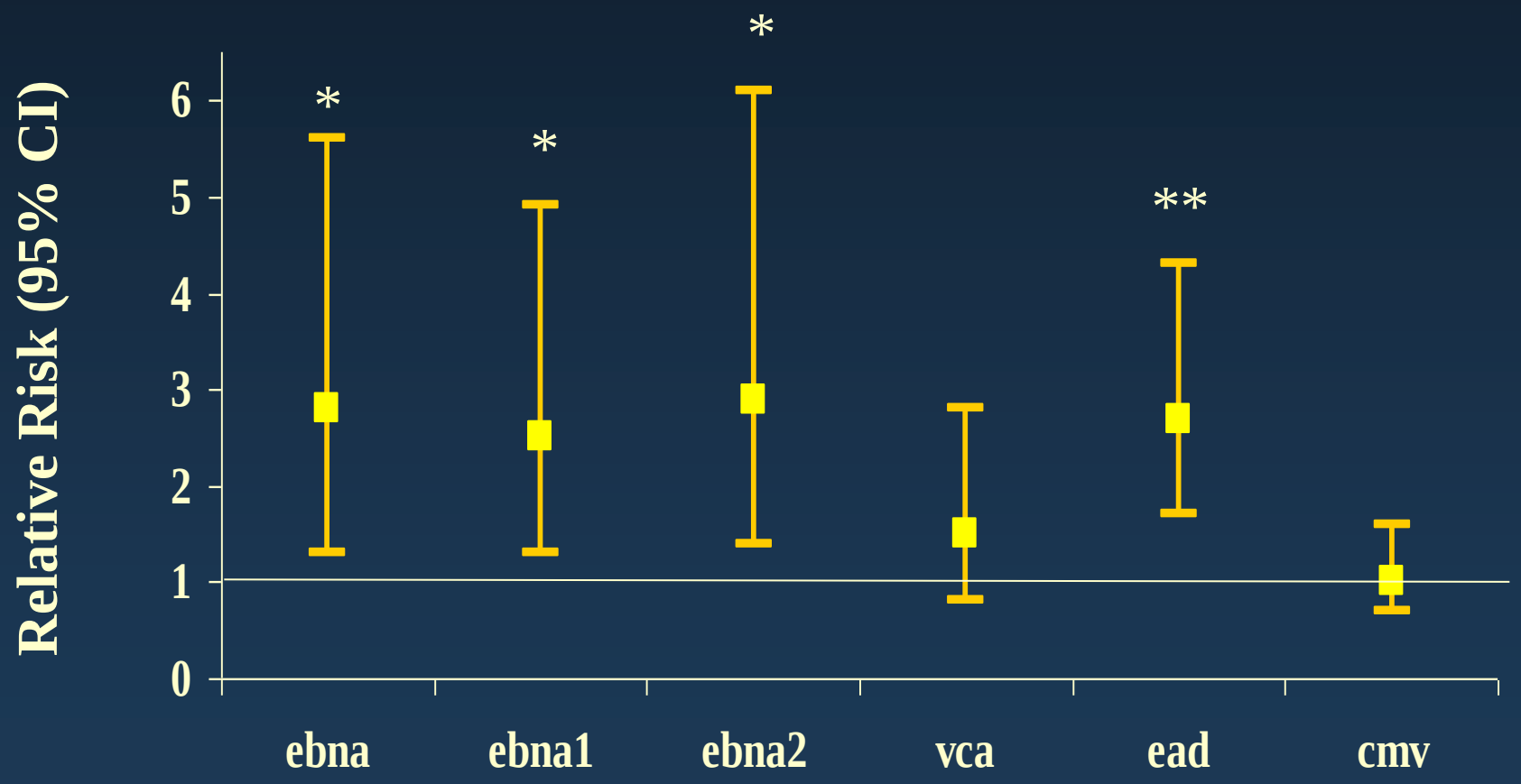
$p<0.05$ (two-sided Fisher Exact test)

- MS is a rare complication of EBV infection
- Factors that increase risk include:

  - Family history of MS / genotype
  - History of IM (age at EBV infection?)
  - Vitamin D deficiency or insufficiency
  - Tobacco smoking
  - Obesity during adolescence
  - Low intake of alpha-linolenic acid

RR of MS corresponding to a 4-fold increase in anti-EBV and anti-CMV serum antibody titers. Cases (n=18) with blood collected before onset (median: 1.9 yrs before) NHS/NHS2:

* P<0.01; ** P<0.001



Ascherio et al. *JAMA* **286**, 3083-3088 (2001)

Table 2. Geometric Mean Titers of Antibodies in Baseline Serum Samples*

Antibodies	All Subjects			Cases With Blood Collected ≥5 Years Before MS Onset		
	Cases (n = 80)	Matched Controls (n = 153)†	*P* Value	Cases (n = 26)	Matched Controls (n = 50)	*P* Value
IgG to EBV VCA	859	700	.04	792	605	.08
IgA to EBV VCA	3.0	2.7	.25	3.3	2.7	.34
EBNA complex	469	282	<.001	465	229	<.001
EBNA-1	326	230	.05	376	192	<.001
EBNA-2	22	16	.09	21	17	.25
Diffuse early antigen	5.3	3.6	.02	6.0	4.4	.18
Restricted early antigen	3.3	3.1	.46	3.4	3.0	.15
Cytomegalovirus	13	14	.98	11	14	.95

Abbreviations: EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; MS, multiple sclerosis,VCA, viral capsid antigen.
*EBV-negative cases and controls (VCA IgG<1:20) were excluded.
†Baseline antibody titers were missing for 1 control.

Levin et al. JAMA 2005

FMC, N=1069 cases
1,867 controls
all EBV positive

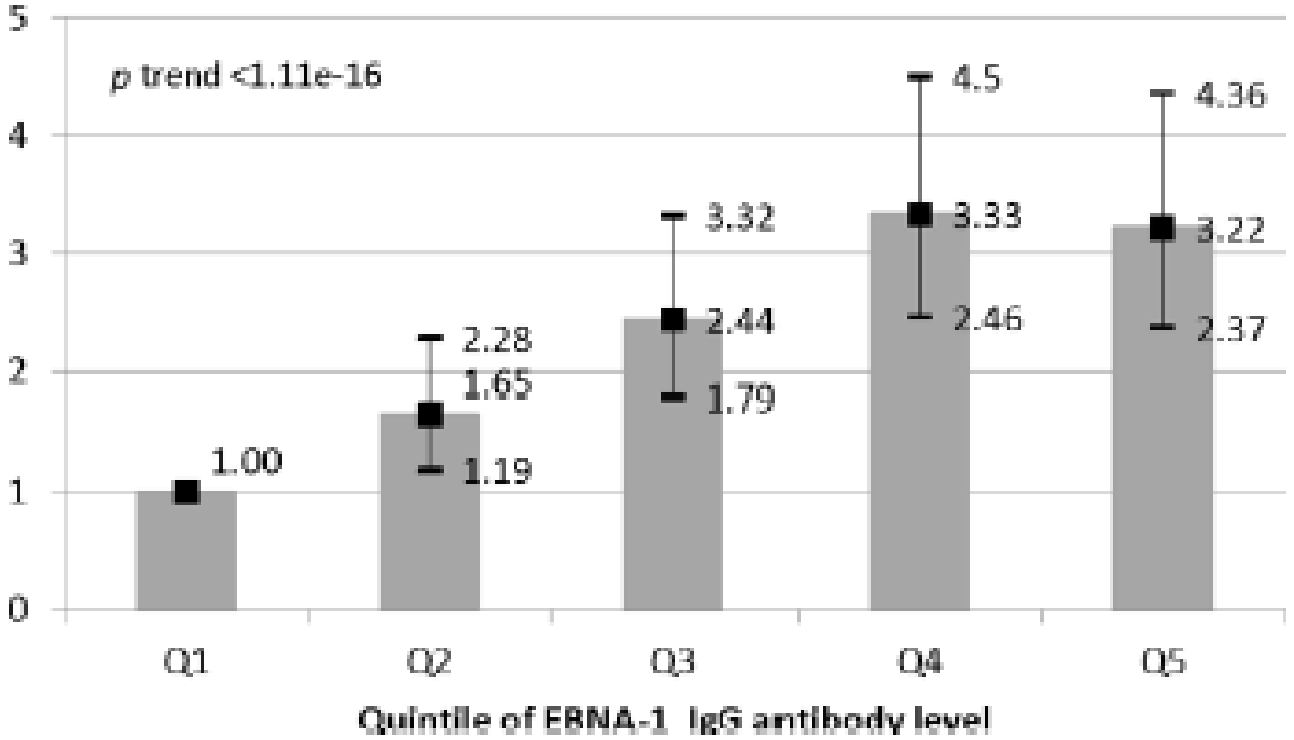
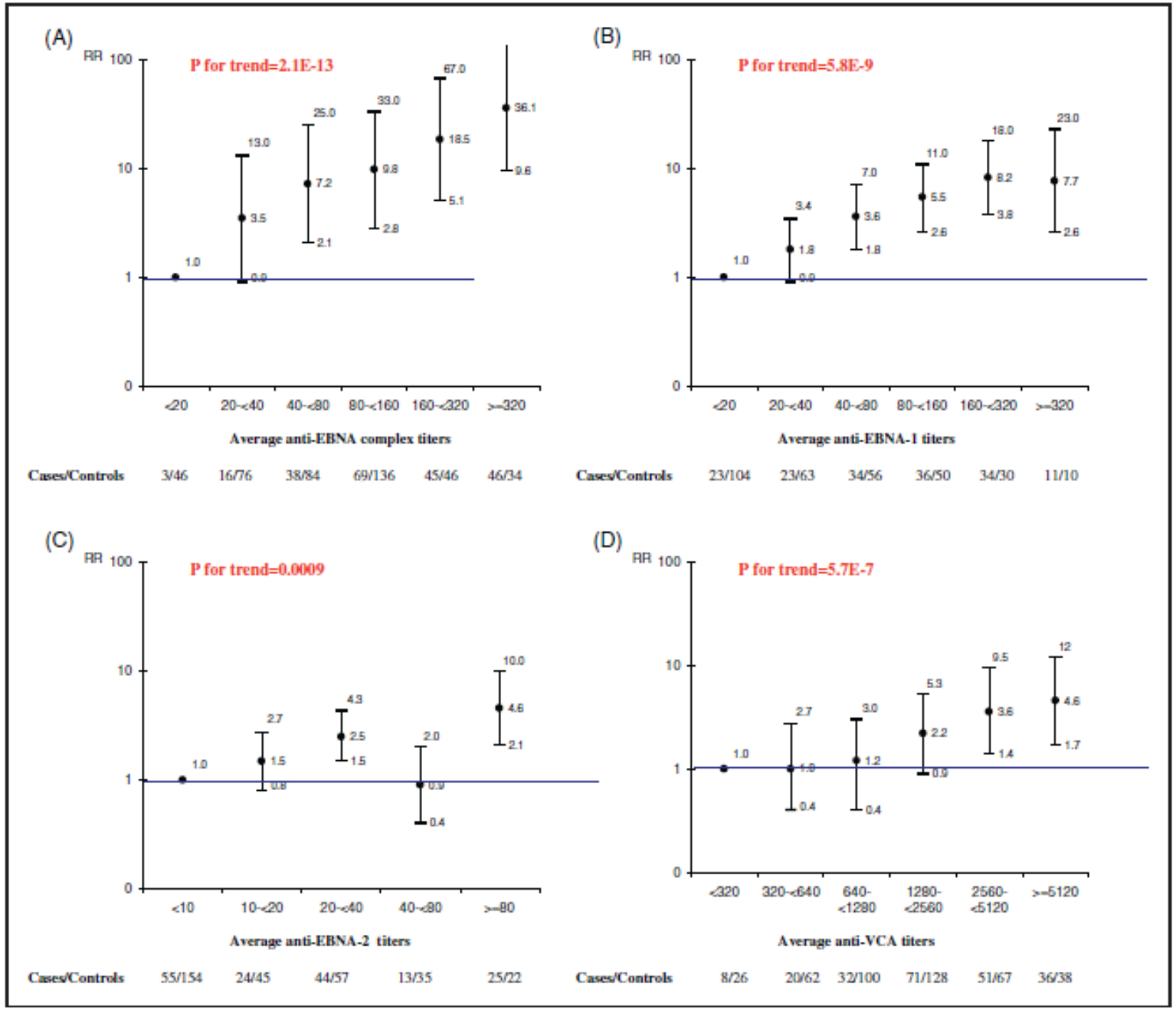


FIGURE 2: Relative risks and 95% confidence intervals of multiple sclerosis in women by quintile of Epstein-Barr nuclear antigen-1 (EBNA-1) IgG index. Adjusted for gravidity, parity, and 25-hydroxyvitamin (<30nmol/l, 30 to <50nmol/l, ≥50nmol/l) and cotinine levels.

Munger et al. Annals of Neurology 2019

N=217 MS cases,
422 controls
all EBV positive



Munger at al. Mult Scler 2011;18(8):1185-87

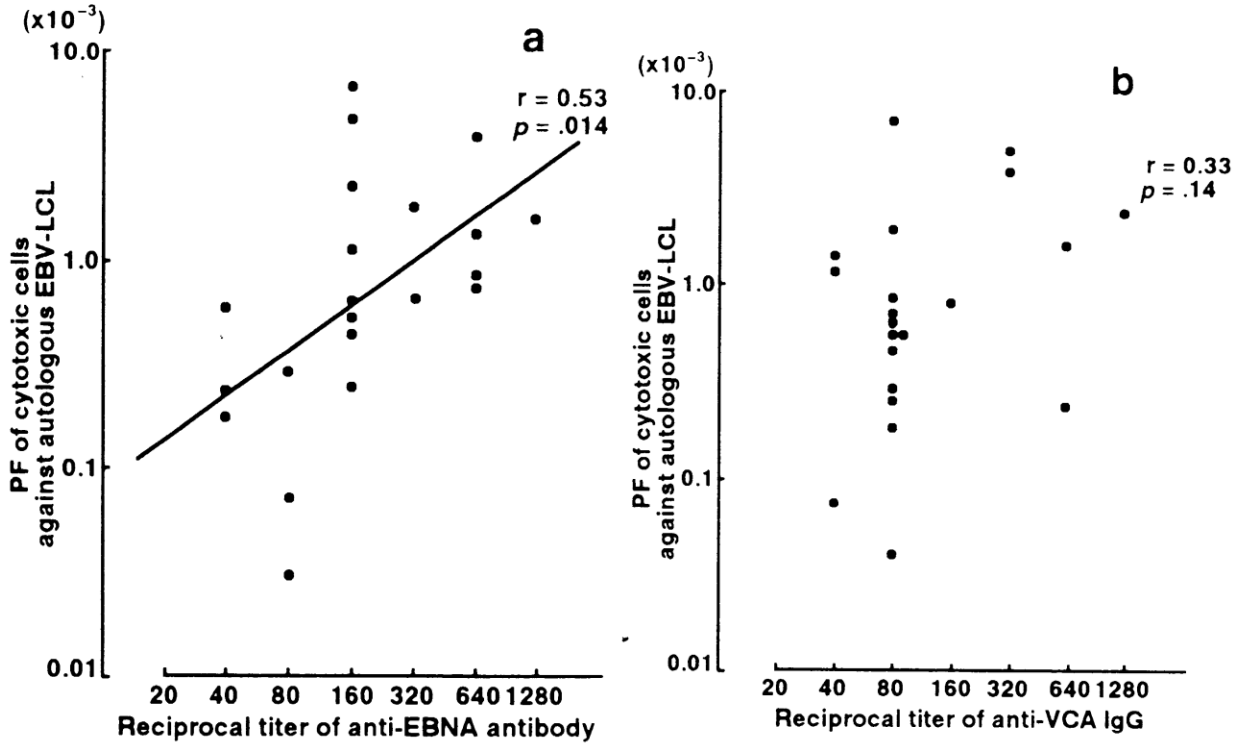


Fig. 3. Relationships between reciprocal titer (inverse of the titer) and precursor frequency (PF) of cytotoxic cells against autologous Epstein-Barr virus transformed lymphoblastoid cell line (EBV-LCL) for 21 seropositive adults. a: reciprocal titer of anti-Epstein-Barr virus-associated nuclear antigen (EBNA) antibody; b: reciprocal titer of anti-viral capsid antigen (VCA) IgG antibody. The correlation is statistically significant for the titer of anti-EBNA antibody but not for that of anti-VCA IgG antibody.

Kusunoki et al. 1993

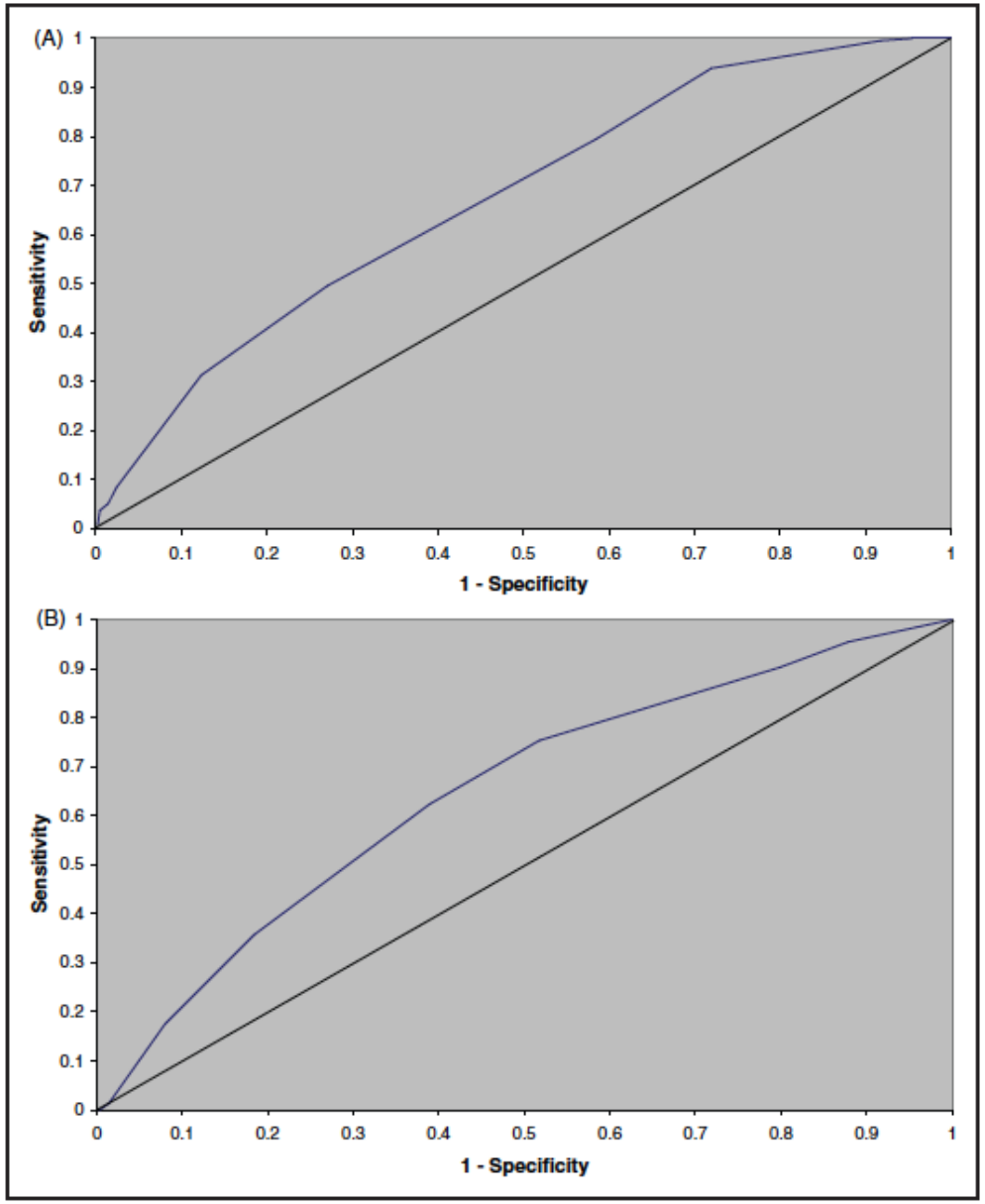
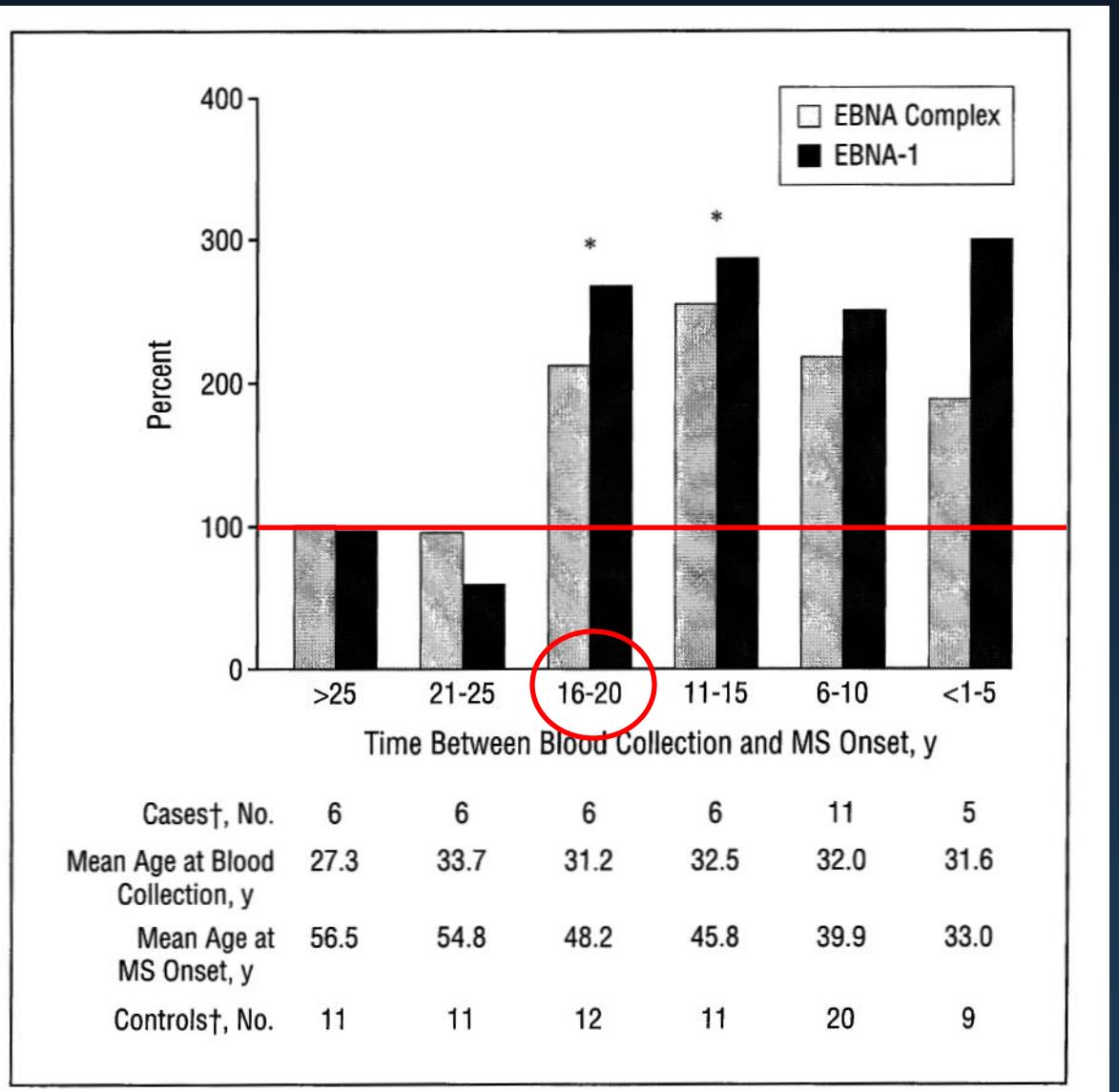


Figure 2. Receiver operating characteristic curves for Epstein–Barr virus (EBV) IgG antibodies in the sample collected most recently before multiple sclerosis symptom onset: (A) anti-EBNAc IgG antibodies ($C = 0.67$); (B) anti-EBNA-1 IgG antibodies ($C = 0.65$).

Munger at al. Mult Scler 2011;18(8):1185-87

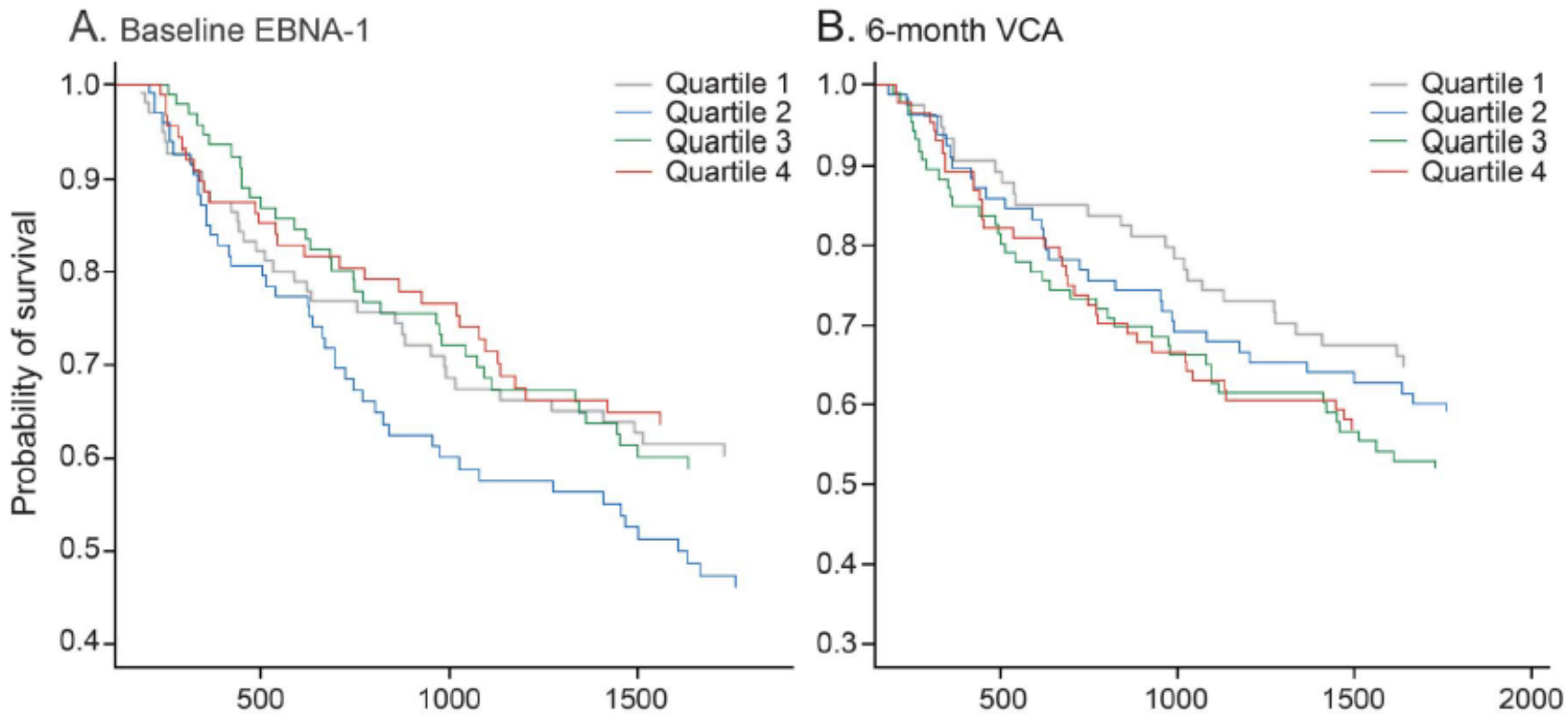
EBNA titers in MS cases as % of matched controls – Kaiser Multiphasic Health Study



	>25	21-25	16-20	11-15	6-10	<1-5
Cases†, No.	6	6	6	6	11	5
Mean Age at Blood Collection, y	27.3	33.7	31.2	32.5	32.0	31.6
Mean Age at MS Onset, y	56.5	54.8	48.2	45.8	39.9	33.0
Controls†, No.	11	11	12	11	20	9

Delorenze et al., *Arch Neurol*, 2006 Jun;63(6):839-44.

Figure Risk of conversion to CDMS by quintiles of EBV/CMV IgG antibody and tobacco use



Munger et al. Neurology 2015

Top EBV peptides and potential mimics

Table S1. Viral peptides in the *pre-onset* samples with significantly different antibody binding between cases and controls[1]

Viral species	Strain	Protein	UniProt ID	Protein length	Peptide start	Peptide end	Proportion in Cases	Proportion in Controls	P-value
Epstein-Barr virus	B95-8	EBNA-1	P03211	641	365	420	0.73	0.2	0.000073
Epstein-Barr virus	B95-8	Capsid protein VP26	P14348	176	113	168	0.87	0.47	0.0022
Epstein-Barr virus	B95-8	Envelope glycoprotein M	P03215	405	365	405	0.37	0.03	0.0025
Epstein-Barr virus	GD1	EBNA-1 (Fragment)	Q5MJ03	237	29	84	0.47	0.1	0.0034
Epstein-Barr virus	B95-8	EBNA-3	P12977	944	701	756	0.63	0.23	0.0038
Epstein-Barr virus	B95-8	EBNA-1	P03211	641	421	476	0.83	0.47	0.0061
Human enterovirus 71	BrCr	Genome polyprotein	Q66478	2193	589	644	0.43	0.1	0.0074
Epstein-Barr virus	AG876	EBNA-3	Q69138	925	729	784	0.6	0.23	0.0082
Epstein-Barr virus	GD1	Nuclear antigen 1 (Fragment)	Q19NX3	89	29	84	0.63	0.27	0.0089
Epstein-Barr virus	GD1	EBNA-2	Q3KSV2	451	197	252	0.63	0.27	0.0089
Epstein-Barr virus	B95-8	EBNA-3	P12977	944	813	868	0.47	0.13	0.01

Bjornevik K, Cortese M et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science. 2022

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	Epstein-Barr virus	GD1	Nuclear antigen 1 (Fragment)	Q19NX3	89	29	84	0.63	0.27	0.0089
	Eps							63	0.27	0.0089
	Eps							47	0.13	0.01

Mimic	Peptide	Aminoacid sequence	Sequence in EBV	Position in EBV antigen	Peptide rank
GlialCAM	GlialCAM 370-389	ATGRTHSSPPRAPSSPGRSR	SQSSSSGSPPRRPPPGRRPF	EBNA1 386-405	1
Alpha-b crystallin	CRYAB 43-57	SLSPFYLRPPSFLRA	RRPFF	EBNA1 402-406	1
	CRYAB 1-15	MDIAIHHPWIRRPFF	RRPFF	EBNA1 402-406	1
Anoctamin	ANO2 140-149	PGDIELGPLD	PGAIEQGPAD	EBNA1 431-440	6
MBP	MBP 85-99	ENPVVHFFKNIVTPR	TGGVYHFVKKHVHES	BALF5 627-641	Outside top 30 EBV pep

JEM Journal of Experimental Medicine

BRIEF DEFINITIVE REPORT

Broader Epstein–Barr virus–specific T cell receptor repertoire in patients with multiple sclerosis

Tilman Schneider-Hohendorf[1*], Lisa Ann Gerdes[2,3,4*], Béatrice Pignolet[5*], Rachel Gittelman[6], Patrick Ostkamp[1], Florian Rubelt[7], Catarina Raposo[8], Björn Tackenberg[8,9], Marianne Riepenhausen[1], Claudia Janoschka[1], Christian Wünsch[1], Florence Bucciarelli[5], Andrea Flierl-Hecht[2,3,4], Eduardo Beltrán[2,3,4], Tania Kümpfel[2,3,4], Katja Anslinger[10], Catharina C. Gross[1], Heidi Chapman[6], Ian Kaplan[6], David Brassat[8], Hartmut Wekerle[2,11], Martin Kerschensteiner[2,3,4], Luisa Klotz[1], Jan D. Lünemann[1], Reinhard Hohlfeld[2,3], Roland Liblau[5*], Heinz Wiendl[1*], and Nicholas Schwab[1*]

Epstein–Barr virus (EBV) infection precedes multiple sclerosis (MS) pathology and cross-reactive antibodies might link EBV infection to CNS autoimmunity. As an altered anti-EBV T cell reaction was suggested in MS, we queried peripheral blood T cell receptor β chain (TCRβ) repertoires of 1,395 MS patients, 887 controls, and 35 monozygotic, MS-discordant twin pairs for multimer-confirmed, viral antigen–specific TCRβ sequences. We detected more MHC-I–restricted EBV-specific TCRβ sequences in MS patients. Differences in genetics or upbringing could be excluded by validation in monozygotic twin pairs discordant for MS. Anti–VLA-4 treatment amplified this observation, while interferon β– or anti-CD20 treatment did not modulate EBV-specific T cell occurrence. In healthy individuals, EBV-specific CD8⁺ T cells were of an effector-memory phenotype in peripheral blood and cerebrospinal fluid. In MS patients, cerebrospinal fluid also contained EBV-specific central-memory CD8⁺ T cells, suggesting recent priming. Therefore, MS is not only preceded by EBV infection, but also associated with broader EBV-specific TCR repertoires, consistent with an ongoing anti-EBV immune reaction in MS.

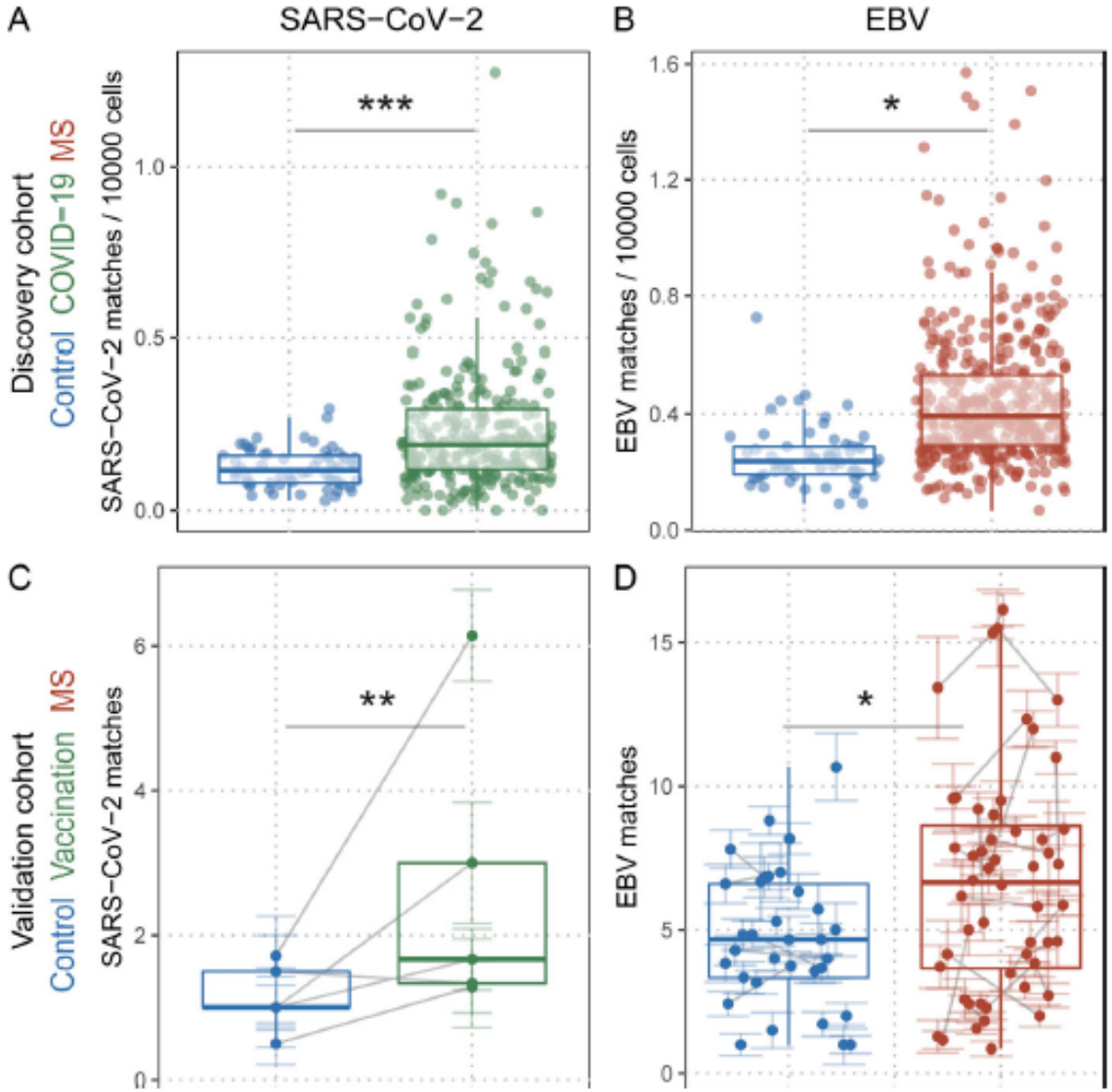

Figure 1. **Quantification of SARS-CoV-2– and EBV-specific T cell rearrangements in TCRβ repertoires of the discovery cohort and the validation cohort. (A and B)** SARS-CoV-2 ($q_{COVID-19} = 4e^{-05}$; $n_{HD} = 62$; $n_{COVID-19} = 278$; A) and EBV ($q_{MS} = 0.01088$; $n_{HD} = 62$; $n_{MS} = 430$; B) TCRβ sequence matches quantified in HD (blue dots), patients with acute COVID-19 (COVID-19, green dots), and MS patients (red dots); *q* values indicate adjusted significance of disease state (COVID-19 or MS) in linear models with the covariates sequencing depth, age, sex, and HLA. **(C)** SARS-CoV-2 TCRβ sequence matches quantified in HD before their first (blue dots) and after their second SARS-CoV-2 vaccination (green dots; $q_{Vaccination} = 0.00196$; $n = 5$). Colored lines indicate standard error of the mean of the biological replicates (sequencing pools) for the respective sample, and gray lines connect samples from the same individual. *q* values indicate adjusted significance of vaccination in linear mixed models with the covariates sequencing depth, vaccination status, and sequencing pools nested within samples within individuals. **(D)** EBV TCRβ sequence matches quantified in control donors (blue dots), and MS patients (red dots; $q_{MS} = 0.0298172$; $n_{Control} = 27$; $n_{MS} = 25$). Colored lines indicate standard error of the mean of the sequencing pools for the respective sample, and gray lines connect samples from the same individual. *q* values indicate adjusted significance of MS in linear mixed models with the covariates sequencing depth, age, sex, treatment, and sequencing pools nested within samples within individuals.

"Therefore, MS is not only preceded by EBV infection, but also associated with broader EBV-specific TCR repertoires, consistent with an ongoing anti-EBV immune reaction in MS."

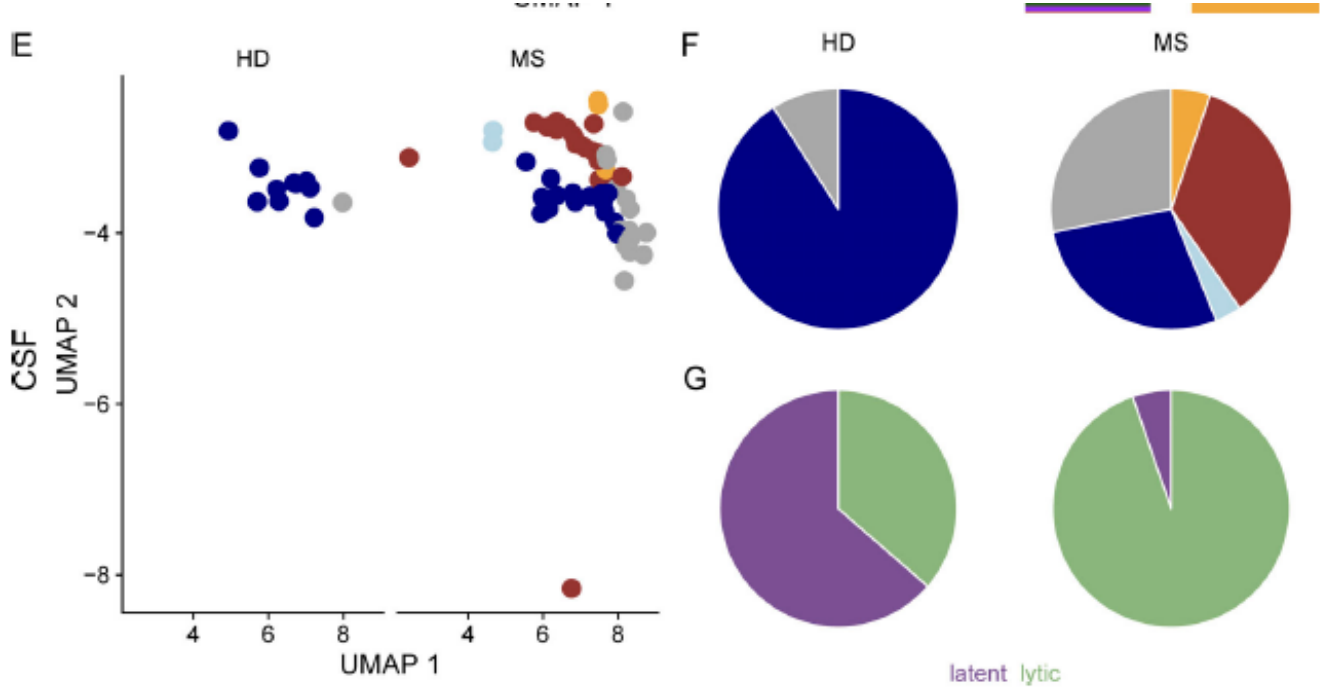

Figure 4. **scRNAseq analysis illustrates phenotype of the EBV-specific CD8⁺ T cells in HD and MS patients. (A)** UMAP plot of the level-3 granularity mapped CD8⁺ T cells of four deeply sequenced healthy controls previously described by Boutet et al. (2019). Color indicates cluster annotation. **(B)** Quantification of the cluster affiliation of EBV-specific CD8⁺ T cells. **(C)** UMAP plot of the level-3 granularity mapped EBV-specific CD8⁺ T cells, split according to their specificity against latent or lytic EBV epitopes. **(D)** Quantification of latent (left) and lytic (right) cluster affiliation. **(E)** UMAP plot of the level-3 granularity mapped CSF cells from 6 HD and 5 MS patients from Pappalardo et al. (2020). Only EBV sequence–matched CD8⁺ T cells are shown. **(F)** Quantification of the cluster affiliation of EBV-specific CSF cells from HD (left) and MS patients (right). **(G)** Quantification of the specificity against either latent (purple) or lytic (green) proteins of EBV-specific CSF cells from HD (left) and MS patients (right).

Acknowledgements


KM


KB


MC

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