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Original article

Virus antibody responses in patients with myelin oligodendrocyte glycoprotein antibody-associated disease and multiple sclerosis

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ABSTRACT

Background: Myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) is an antibody-mediated inflammatory disorder of the central nervous system, which is characterized by demyelination targeting the optic nerve, brain, and spinal cord.
The etiology of MOGAD still remains to be elucidated. Here we performed a retrospective study comparing virus antibody levels of monophasic MOGAD patients to relapsing-remitting multiple sclerosis patients (RRMS) and healthy controls (HCs).
Methods: We enrolled 19 patients with monophasic MOGAD (F:M 13:6, mean 41 years) and 30 patients with RRMS (F:M 21:9, mean 41 years) referred to the Department of Neurology at Rigshospitalet Glostrup, Denmark. Moreover, 15 HCs (F:M 8:7, mean 43 years) were included. Patients and HCs were matched according to age and gender when possible. Antibody levels to Epstein-Barr virus (EBV) Epstein-Barr nuclear antigen (EBNA)1, human herpes virus (HHV) 6A polymerase processivity factor, John Cunningham virus (JCV) major capsid protein 1, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein and cytomegalovirus (CMV) phosphoprotein 52 were measured by enzyme-linked immunosorbent assay.
Results: No differences in antibody levels to HHV6, CMV, and JCV proteins were observed between MOGAD patients and HCs. Similarly, no significant differences in antibody levels to SARS-CoV-2, HHV6, CMV, and JCV were found between MOGAD patients and RRMS patients. However, RRMS patients presented with significantly increased EBV EBNA1 IgG levels in when compared to MOGAD patients and HCs.
Conclusions: Our findings suggest that development of MOGAD is neither associated with SARS-CoV-2, HHV6, CMV nor JCV alone. Furthermore, results presented indicate that EBV serology is different in MOGAD patients compared to RRMS, confirming a role of EBV in the development of MS and suggesting that the etiology of MOGAD is different from MS.

1. Introduction

Autoantibodies targeting myelin oligodendrocyte glycoprotein (MOG) are associated with various demyelinating diseases, which collectively have become recognized as myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) [Marignier et al., 2020].

The disease mechanism still remains to be determined. However, MOGAD has been suggested to begin in the periphery, by activation of T cells and production of antibodies to MOG, which eventually transfer

Abbreviations: AP, alkaline phosphatase; BARF, *Bam*HI-A rightward frame; BBB, blood brain barrier; gB, glycoprotein; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebrospinal fluid; EA, early antigen; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; HC, healthy control; HHV, human herpes virus; HSV, herpes simplex virus; JCV, John Cunningham virus; MeV, measles virus; MuV, Mumps virus; MOG, myelin oligodendrocyte glycoprotein; MOGAD, myelin oligodendrocyte glycoprotein antibody-associated disease; NuP, nucleoprotein; *p*NPP, *p*-nitrophenylphosphate; pp, phosphoprotein; RRMS, relapsing-remitting multiple sclerosis; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SP, spike protein; VP, viral protein; VZV, varicella zoster virus.

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into the central nervous system (CNS) and recognize the transmembrane MOG protein found on the outer surface of nerve fibers [Brunner, 1989; Corbali and Chitnis, 2023; Marignier, 2020, Peschl, 2017].

MOGAD appears to be equally common among men and woman, and recent reports indicate a prevalence of approximately 1.3–2.5/ 100,000, and an annual incidence of approximately 3.4–4.8/million [Hor, 2023].

The disease is found in both children and adults, although the disease may present differently [Cobo-Calvo, 2019, A. 2021; Wendel, 2022]. Common symptoms include vision loss, muscle weakness, stiffness or paralysis, confusion, seizures, and headaches [Cobo-Calvo, 2019, 2021; Wendel, 2022].

Approximately 40–50 % of patients with MOGAD only experience a single attack, also referred to as monophasic MOGAD [Cobo-Calvo, 2019;2021; Corbali and Chitnis, 2023; Jarius, 2012; Peschl, 2017]. MOGAD patients with a monophasic disease typically present with elevated MOG IgG levels, which decrease over time [Cobo-Calvo, 2019, 2021; Satukijchai, 2022]. In contrast, other MOGAD patients experience multiple attacks, also referred to as relapsing MOGAD, which typically is associated with persistently elevated MOG IgG levels [Cobo-Calvo, 2019; Satukijchai, 2022]. Persistently high MOG IgG titers are often associated with increased relapse risk and with a worse disease outcome [Akaishi 2020, 2021].

Despite clinical overlaps between MOGAD and multiple sclerosis (MS), disease progression in MOGAD is predominantly relapse-dependent, whereas the presence of oligoclonal bands and progression independent of relapse activity commonly are associated with MS [Akaishi, 2020; Akaishi et al., 2021; Corbali and Chitnis, 2023].

The etiology of MOGAD still remains inconclusive. Genetic risk factors associated with MOGAD remain to be determined, although specific DRB1 and DQB1 alleles may be more frequent in pediatric-onset MOGAD patients [Bruijstens, 2020; Grant-Peters, 2021; Sun, 2020]. Furthermore, no specific environmental factors have been identified, although case studies have reported that COVID-19 infection may trigger disease onset [Johnsson, 2022; Lotan, 2022]. Recently, the possible role of virus infection in disease onset has been elaborated, but without identifying specific viruses to be involved in the development of the disease (Fadda et al., 2024; Maniscalco et al., 2025; Molenaar et al., 2025).

To our knowledge, associations between viruses and MOGAD onset have not been studied in detail yet. Therefore, we performed a retrospective study, comparing the virus antibody status of MOGAD patients to relapsing-remitting (RR)MS patients and healthy controls (HCs).

2. Methods

2.1. Reagents

NaCl was purchased from Unikem (Copenhagen, Denmark). Diethanolamine, Tween-20, Na_2CO_3, $NaHCO_3$, phenol red and $MgCl_2$ were from Merck (Darmstadt, Germany). Alkaline phosphatase (AP)-labelled goat anti-human IgG, Tris HCL and *p*-nitrophenylphosphate (*p*NPP) substrate tablets were from Sigma Aldrich (St. Louis, MO, USA). Polysorp microtiter plates were from Thermo Fisher Scientific (Roskilde, Denmark). Full-length EBV Epstein-Barr nuclear antigen (EBNA)1 was obtained from Abcam (Cambridge, UK). Recombinant CMV phosphoprotein (pp)52 was obtained from ProSpec-Tany TechnologyGene Ltd. (Rehovot, Israel). HHV 6A polymerase processivity factor (p41), EBV glycoprotein B (gB), and EBV *Bam*-HI rightward frame (BARF)1, EBV early antigen D (EAD) were purchased from MyBioSource (San Diego, SD, USA). Measles virus (MeV) nucleoprotein (NuP) and mumps virus (MuV) NuP were from Baltymas (Vilnius, Lithuania). The ectodomain of spike protein (SP) from SARS-CoV-2 was from Baltymas (Vilnius, Lithuania) and was produced in-house at the institute of Biotechnology, Vilnius University, Lithuania. John Cunningham virus (JCV) major capsid viral protein (VP)1 was produced at the Institute of Biotechnology, Vilnius, Lithuania, as previously described [Norkiene, 2015; Sasnauskas, 2022]. Recombinant varicella zoster virus (VZV) glycoprotein E (gE) was from Virogen (Watertown, MA, USA). Herpes simplex virus (HSV) 1 gB was from Biorad (Hercules, CA, USA).

2.2. Patients

We enrolled 19 patients with monophasic MOGAD and 30 patients with RRMS and 15 HCs. RRMS and MOGAD patients were referred to the Department of Neurology, Rigshospitalet Glostrup, Denmark (Table 1). HCs were truly healthy volunteers, which were negative for any known neurological diseases. Individuals enrolled were of Caucasian origin and were age- and gender-matched as far as possible. None of the patients received B-cell-depleting treatment at the time of enrollment. RRMS patients were diagnosed according to the revised McDonald criteria [Thompson, 2018], whereas MOGAD patients were diagnosed based on the presence of MOG antibodies combined with neurological symptoms [Banwell, 2023]. RRMS and MOGAD patients were recruited between 2018 and 2025, whereas HCs were recruited before 2021.

2.3. Detection of virus antibodies in serum by enzyme-linked immunosorbent assay

The presence of virus IgG in serum samples from MOGAD and RRMS patients and HCs was assessed by enzyme-linked immunosorbent assay (ELISA). Briefly, Polysorp microtiter plates were coated with virus protein (1 μg/mL) in carbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, 0.5 % Phenol red, pH 9.6) overnight at 4 °C. Following coating, plates were blocked for 30 min in TTN (0.3 M NaCl, 20 mM Tris, 1 % Tween-20, pH 7.5). Next, patient pools or individual sera diluted in TTN (1:100) were added to each well and incubated for 1 hour, whereafter wells were washed 3 times in TTN and incubated for another hour with AP-labelled goat-anti-human IgG (1:5000). Bound antibodies were quantified by addition of *p*NPP (1 mg/mL) diluted in AP-substrate buffer (1 M diethanolamine, 0.5 mM $MgCl_2$, pH 9.8), whereafter absorbances were measured at 405 nm with background subtractions measured at 650 nm using a microtiter plate reader (Versamax, Molecular Devices, Sunnyvale, Ca, USA). Plates were washed between each step in TTN buffer (200 μL, 3 × 1 min).

For each microtiter plate, a standard curve was included, which was composed of a two-fold serial dilution of a MOGAD serum pool ($n = 19$) starting from a dilution of 1:100. A MOGAD pool ($n = 19$) and a donor pool ($n = 100$) were used as high positive and low positive controls, respectively. Samples were tested in duplicates. See supplementary A for further information.

2.4. Determination of oligoclonal bands

Samples were analysed for oligoclonal bands by isoelectric focusing, which was conducted using a semi-automatic Hydrasys instrument (Sebia, Blackwater, Surrey, UK) according to the manufacturer's instructions. Briefly, a hydrogel 9 CSF isofocusing kit was used, where the CSF and serum samples (10 μL/well) were loaded onto the gel using an applicator. After separation, the gel was immuno-fixated with peroxidase-labelled anti-IgG, and the gel was washed before enzymatic

Table 1
Patient characteristics of included individuals. MOGAD, myelin oligodendrocyte glycoprotein antibody-associated disease, RRMS, relapsing-remitting multiple sclerosis, HC, healthy controls.

	MOGAD	RRMS	HCs
n	19	30	15
Sex F:M	13:6	21:9	8:7
Average age (years)	41	41	43
Age range (years)	23–71	25–62	34–63
Oligoclonal bands (%)	16	92	0

development as described elsewhere [Shan and Yeh, 1998].

2.5. Determination of total IGG and albumin concentrations

Total IgG levels and albumin concentrations were measured in serum and CSF samples by nephelometry (BN ProSpec System, Siemens, Erlangen, Germany) according to the manufacturer's instructions.

The IgG index was calculated using the formula [Hottenrott, 2017, 2018]:

IgG index = CSF_{IgG} * $serum_{albumin}$ / $CSF_{albumin}$ * $serum_{IgG}$ (Cit-off = 0.7).

AI values were calculated using the formula:

$AI = Q_{IgG\ [spec]}/Q_{IgG\ [total]}$ (cut-off = 1.5)

$Q_{IgG\ [spec]} = CSF_{IgG\ [spec]}/serum_{IgG\ [spec]}$

$Q_{IgG\ [total]} = CSF_{IgG\ [total]}/serum_{IgG\ [total]}$

If $Q_{IgG\ [total]} > Q_{lim}$ AI was calculated as

$AI = Q_{IgG\ [spec]}/Q_{lim}$ where

$Q_{lim} = 0.93 * (Q_{alb}^2 + 6 \times 10^{-6})^{0.5} - 1.7 \times 10^{-3}$

2.6. Ethics

The study was conducted according to current guidelines and was approved by the Regional Scientific Committee of Copenhagen (No H-20012823, no. H-19036891). All patients and HCs were notified and provided written informed consent.

2.7. Data analysis

MyAssays.com was used to create a 4-parameter logistic curve fit, for calculation of IgG concentrations in serum and CSF (U/mL). Antibody levels being lower than the quantification limit of the standard curve were re-tested in a lower concentration or set to a background value, before being included in the statistical analysis. Antibody levels above the upper point of the standard curve were extrapolated using the 4-parameter polynomic function. See supplementary A for further information.

Correlations between clinical parameters were determined using Pearson r correlations, where the correlation (r) was defined as follows: 0–0.25 = no correlation, 0.25–0.5 = weak positive correlation, 0.5–0.75 = moderate positive correlation, and 0.75–1.0 = strong positive correlation.

Statistical analysis using GraphPad Prism (v 5.0) was performed by a nonparametric approach, where Mann Whitney u tests were used to determine statistically different significance. A value of $p < 0.05$ was accepted as statistically significant with * = $p < 0.05$, ** = $p < 0.01$, ***$p < 0.001$.

3. Results

3.1. Screening of serum pools

Initially, to screen for general trends among patients, MOGAD, RRMS, and HC pools were tested for specific antibody reactivity to various virus proteins by ELISA (Fig. 1,2). Antibody reactivity to multiple EBV proteins was determined (Fig. 1), as MS patients typically present with elevated antibodies to selected EBV proteins [Ali, 2024; Bjornevik 2022; Gåsland, 2023; Munger et al., 2011], although EBNA1 IgG titers have been reported not to be increased in MOGAD patients [Fadda et al., 2024; Maniscalco et al., 2025; Molenaar et al., 2025].

As found, no significant differences in EBV antibody levels were

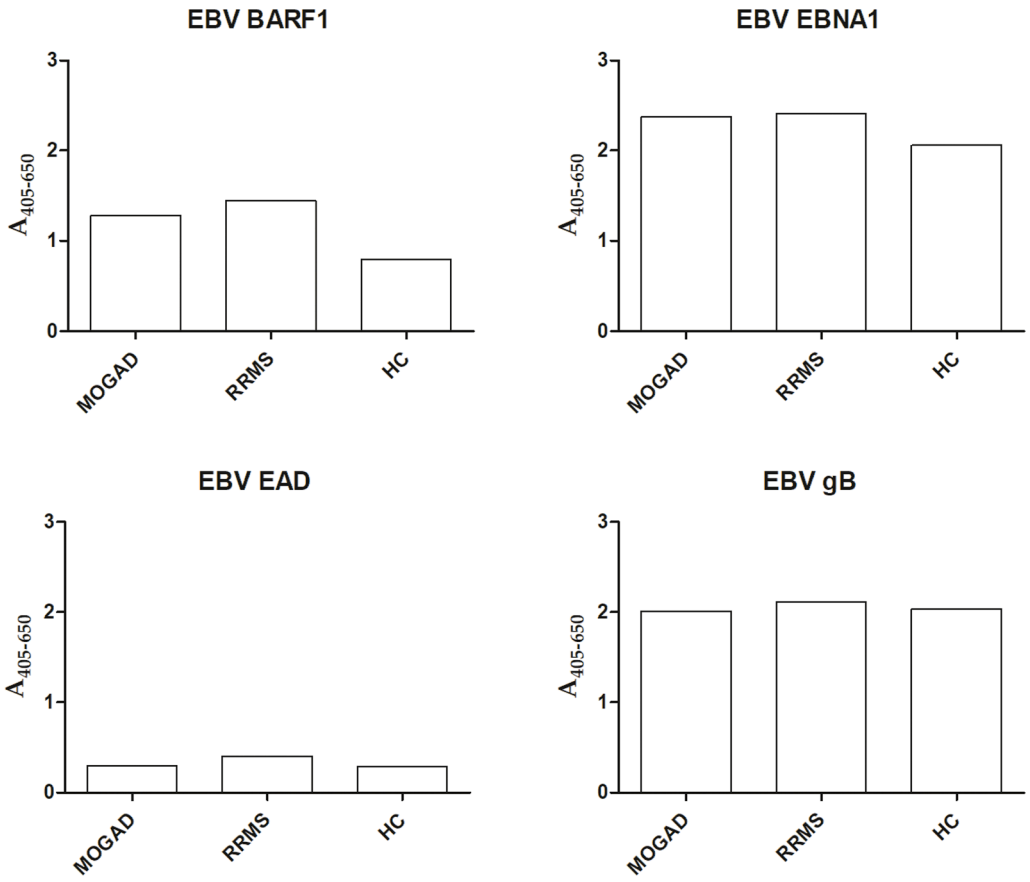

Fig. 1. Epstein-Barr virus (EBV) IgG in serum pools of patients with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), relapsing-remitting multiple sclerosis (RRMS) and healthy controls (HCs) analyzed by enzyme-linked immunosorbent assay. BARF, ***Bam*HI-A rightward frame; EAD, early antigen D; EBNA, Epstein-Barr nuclear antigen; gB, glycoprotein B.**

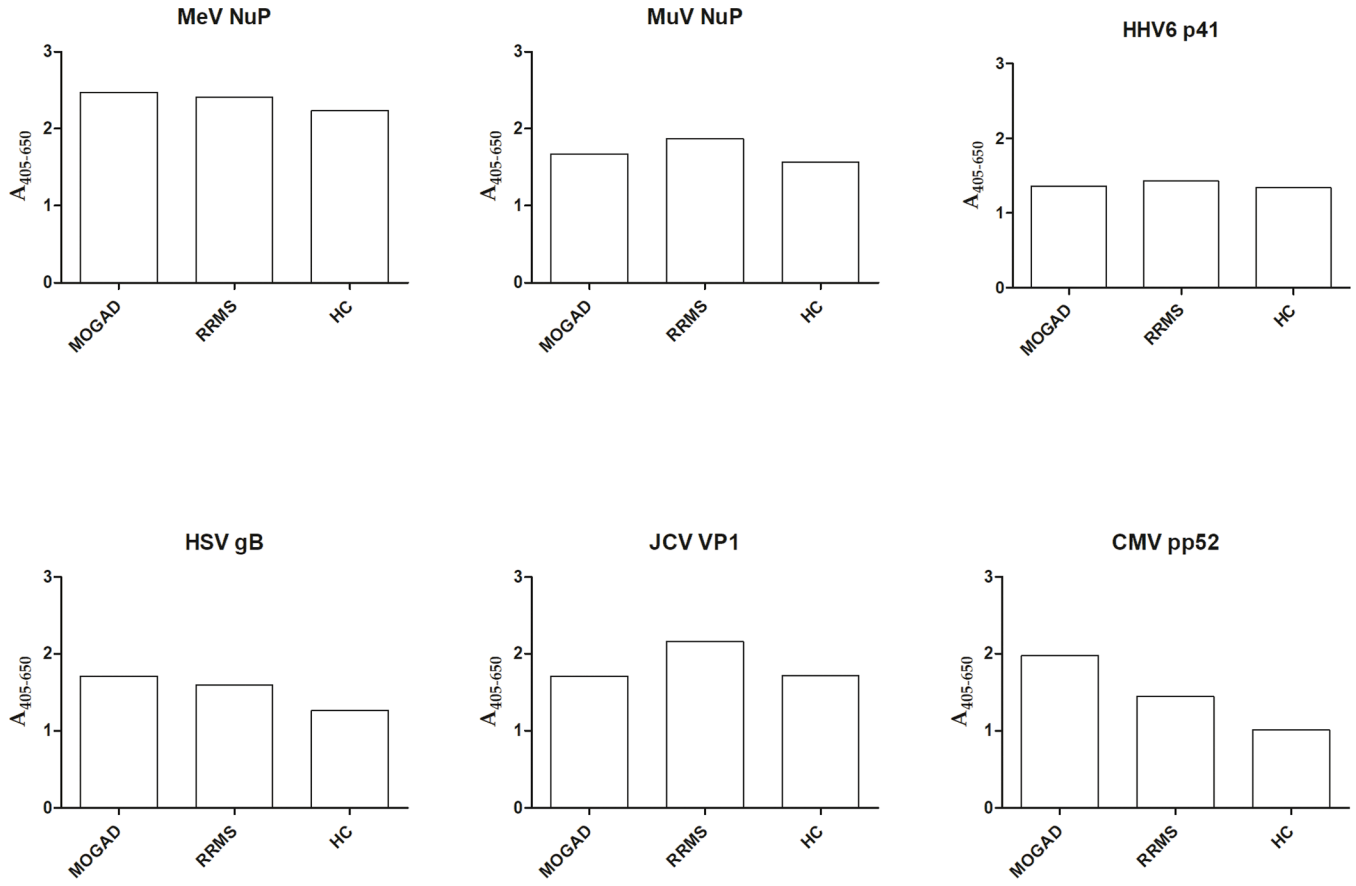

Fig. 2. Virus IgG in serum pools of patients with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), relapsing-remitting multiple sclerosis (RRMS) and healthy controls (HCs) analyzed by enzyme-linked immunosorbent assay. NuP, nucleoprotein; p41, polymerase processivity factor; gB, glycoprotein B; VP, viral protein; pp, phophoprotein. MeV, measles birus; MuV, Mumps virus; HHV6, Human herpes virus; HSV, Herpes simplex virus; JCV, John Cunningham virus; CMV, Cytomegalovirus.

determined between the MOGAD pool and the HC pool, although a trend indicated slightly elevated antibodies to BARF1 in the MOGAD and RRMS patient pools compared to HCs.

Similarly, antibody levels to a broad spectrum of virus antigens originating from MeV, MuV, HHV6, HSV, JCV and CMV were determined (Fig. 2). As observed, antibody levels to CMV pp52 were elevated in the MOGAD pool when compared to the HC pool.

No notable difference in IgG levels to MeV NuP, MuV NuP and HHV6 p41 was observed between MOGAD and RRMS patients and HCs. CMV pp52 IgG and HSV gB IgG were slightly elevated in the MOGAD pool compared to the RRMS pool and the HC pool, whereas JCV VP1 IgG levels were slightly reduced in the MOGAD pool compared to the RRMS pool.

3.2. *Screening of individual serum samples*

Following pool screenings, individual serum samples were tested for antibody reactivity to selected antigens by ELISA (Fig. 3). As presented, no notable differences between JCV VP1 IgG levels in RRMS, MOGAD and HCs could be determined ($p > 0.05$). A trend indicated reduced HHV6 p41 IgG levels in MOGAD patients compared to HCs ($p = 0.0532$), whereas HHV6 p41 IgG levels were significantly reduced in RRMS patients when compared to HCs ($p = 0.0484$). No difference in HHV6 p41 IgG levels were found between MOGAD and RRMS patients ($p = 8626$). CMV pp52 IgG levels tended to be slightly elevated in MOGAD patients compared to RRMS patients, although not significant ($p = 0.1264$), whereas similar CMV pp52 IgG levels were found in the MOGAD cohort and the HC group ($p = 0.8673$). No differences in JCV IgG levels were observed between MOGAD, RRMS, and HC samples (MOGAD vs HCs $p = 0.5901$, MOGAD vs RRMS $p = 0.7686$, RRMS vs HCs $= 0.6684$). Furthermore, serum samples from MOGAD and RRMS patients presented with similar SARS-CoV-2 SP IgG levels in serum of MOGAD and RRMS patients, as no difference in SP IgG levels could be determined ($p = 0.6001$). SARS-CoV-2 SP IgG in serum of MOGAD and RRMS patients was significantly elevated when compared to HCs ($p < 0.0001$), as serum samples from HCs were collected before 2021.

Finally, EBV EBNA1 IgG levels were determined in MOGAD patient serum. No difference in EBV EBNA1 IgG levels were observed between MOGAD patients and HCs ($p = 0.8245$). In contrast, EBV EBNA1 IgG levels in patients with RRMS were significantly elevated when compared to MOGAD patients ($p = 0.0305$) and HCs ($p = 0.0406$).

Following screening of patient sera, EBV EBNA1, SARS-CoV-2 SP and HHV6 p 41 IgG levels were analysed in the CSF of MOGAD, RRMS, and HCs (Fig. 4). EBV EBNA1 IgG levels were significantly elevated in RRMS patients compared MOGAD patients ($p = 0.0096$) and HCs ($p < 0.0001$). No statistically significant difference in EBV EBNA1 IgG levels was observed between MOGAD patients and HCs ($p = 0.1450$). Furthermore, no difference in CSF HHV6 p41 IgG was observed between MOGAD, RRMS and HCs groups ($p > 0.0500$) and no difference was observed for SARS-CoV-2 SP IgG levels between MOGAD and RRMS patients ($p = 0.5166$), whereas SARS-CoV-2 SP IgG levels were significantly elevated in MOGAD ($p = 0.006$) and RRMS ($p < 0.0001$) patients when compared to the HC group.

Although no differences in virus IgG levels in serum and CSF of MOGAD samples was determined relative to HCs, a few individuals were observed to have increased virus IgG titers. To determine whether these patients experienced intrathecal antibody synthesis, the AIs of the tested MOGAD patients were determined (Supplementary B). Four out of 19

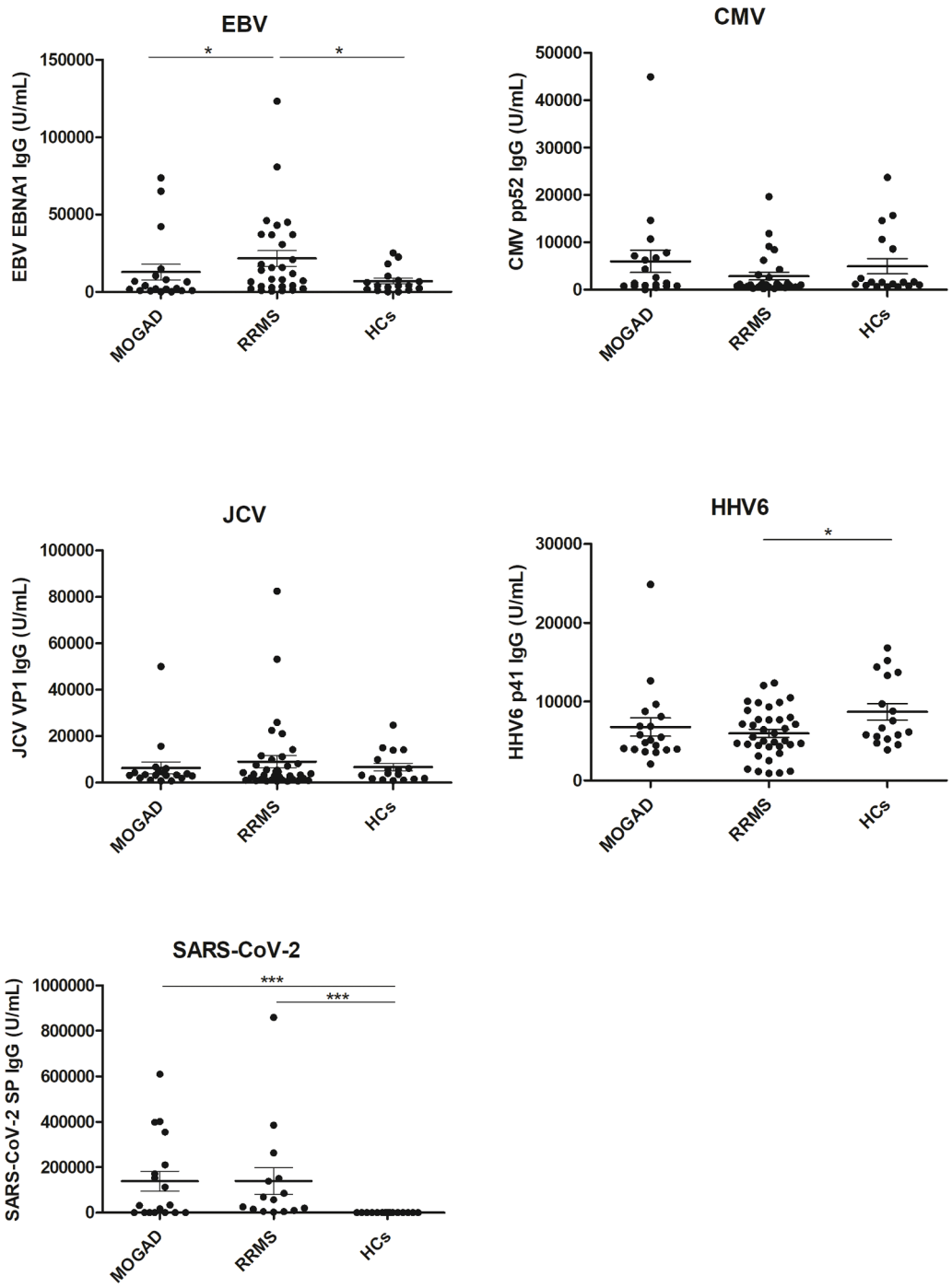

Fig. 3. Virus IgG levels in serum of patients with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD) and relapsing-remitting multiple sclerosis (RRMS) and healthy controls (HCs) analysed by enzyme-linked immunosorbent assay. Error bars present mean with standard deviation. Statistical analyses were conducted using Mann Whitney t tests, where a value of $p < 0.05$ was accepted as statistically significant. * = $p < 0.05$, ** = $p < 0.01$, *** $p < 0.001$. EBV, Epstein-Barr virus; CMV, Cytomegalovirus; JCV, John Cunningham virus, HHV, Human herpes virus, SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.Error bars present mean with standard deviation. Statistical analyses were conducted using Mann Whitney t tests, where a value of $p < 0.05$ was accepted as statistically significant. * = $p < 0.05$, ** = $p < 0.01$, *** $p < 0.001$.

MOGAD patients were positive for intrathecal synthesis to EBV EBNA1, whereas three out of 19 presented with a positive antibody index to HHV6 p41. None of the MOGAD patients presented with intrathecal antibody synthesis to SARS-CoV-2 SP.

3.3. Screening of total IgG levels in serum and cerebrospinal fluids

To evaluate the blood-brain barrier (BBB) status of MOGAD patients, albumin and total IgG levels were determined in serum and CSF of the enrolled cohorts and compared (Fig. 5).

No difference in serum IgG and serum albumin was observed between RRMS, MOGAD, and HCs ($p > 0.05$). In contrast, CSF IgG levels were significantly elevated in RRMS patients when compared to MOGAD patients ($p = 0.0024$) and HCs ($p = 0.0017$), whereas no difference in CSF IgG was observed between MOGAD patients and HCs ($p = 0.7862$). Similarly, CSF albumin levels were elevated in RRMS patients

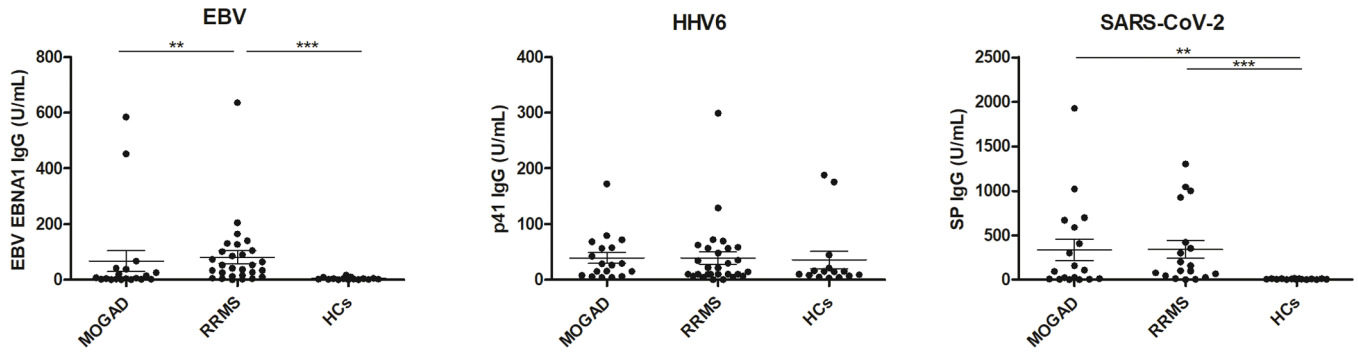

Fig. 4. Virus IgG levels in cerebrospinal fluids (CSF) of myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), relapsing-remitting multiple sclerosis (RRMS) and healthy controls (HCs) analysed by enzyme-linked immunosorbent assay. Error bars present mean with standard deviation. Statistical analyses were conducted using Mann Whitney t tests, where a value of $p < 0.05$ was accepted as statistically significant. * = $p < 0.05$, ** = $p < 0.01$, *** $p < 0.001$. EBV, Epstein-Barr virus; HHV, Human herpes virus; SARS-CoV-2, Severe acute respiratory syndrome coronavirus-2.

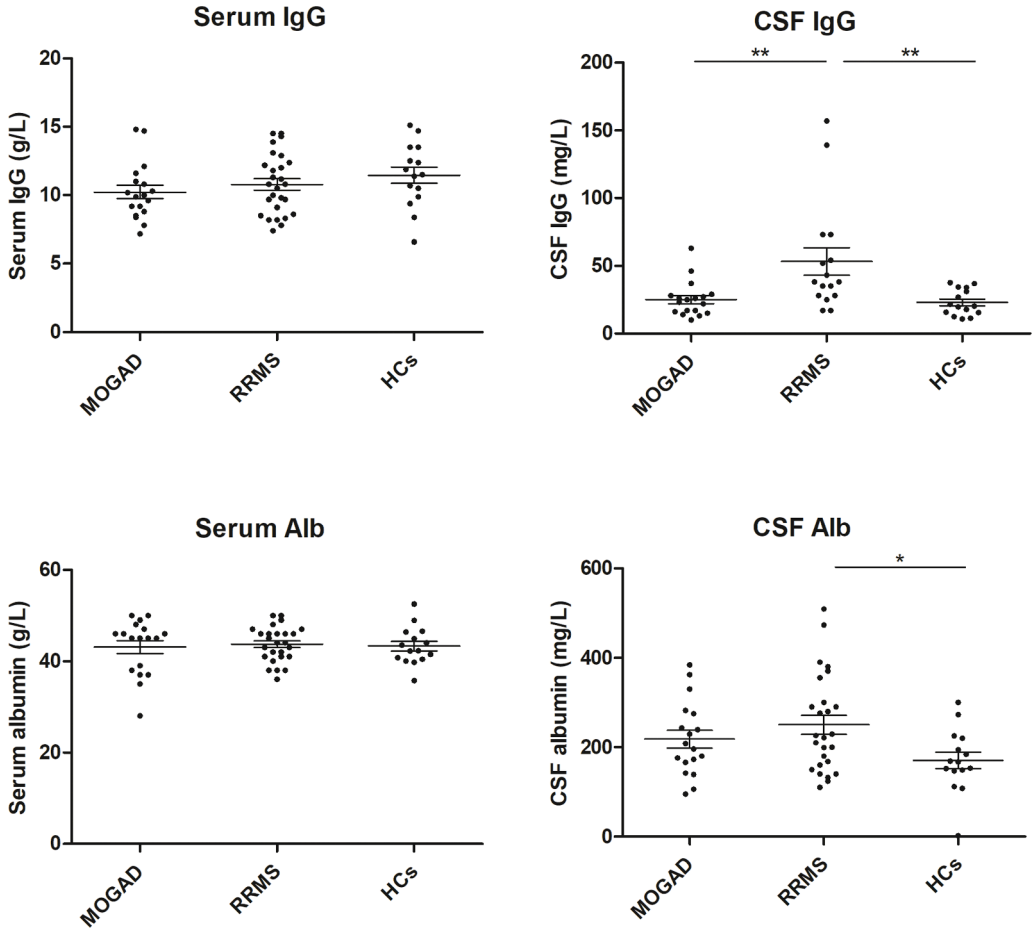

Fig. 5. Total IgG and albumin (Alb) levels in serum and cerebrospinal fluid (CSF) of patients with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), relapsing-remitting multiple sclerosis (RRMS) and healthy controls (HCs). Error bars present mean with standard deviation. Statistical analyses were conducted using Mann Whitney t tests, where a value of $p < 0.05$ was accepted as statistically significant. * = $p < 0.05$, ** = $p < 0.01$, *** $p < 0.001$.

compared to HCs ($p = 0.0325$), whereas no significant difference was observed between MOGAD and RRMS patients ($p = 0.4238$) and HCs ($p = 0.1431$).

Furthermore, correlations between serum and CSF levels were determined (Fig. 6). As presented, no correlation between serum and CSF IgG in MOGAD ($r = -0.0206$, $p = 0.9353$) and RRMS ($r = -0.0169$, $p = 0.9349$) was observed. In contrast, a significant correlation was determined in HCs ($r = 0.6472$, $p = 0.009$). A similar correlation was determined in HCs for albumin levels in serum and CSF, as a correlation of $r = 0.6816$ was determined ($p = 0.0051$), whereas MOGAD and RRMS groups obtained correlations of $r = 0.3820$ ($p = 0.1177$) and $r = 0.2873$ ($p = 0.1546$), respectively.

Similarly, correlations between measured virus IgG levels in MOGAD patients and clinical characteristics such as expanded disability status scale (EDSS), TP CSF and CSF cells were analysed (Supplementary C). No correlation between HHV6 p41 IgG, JCV VP1 IgG and clinical

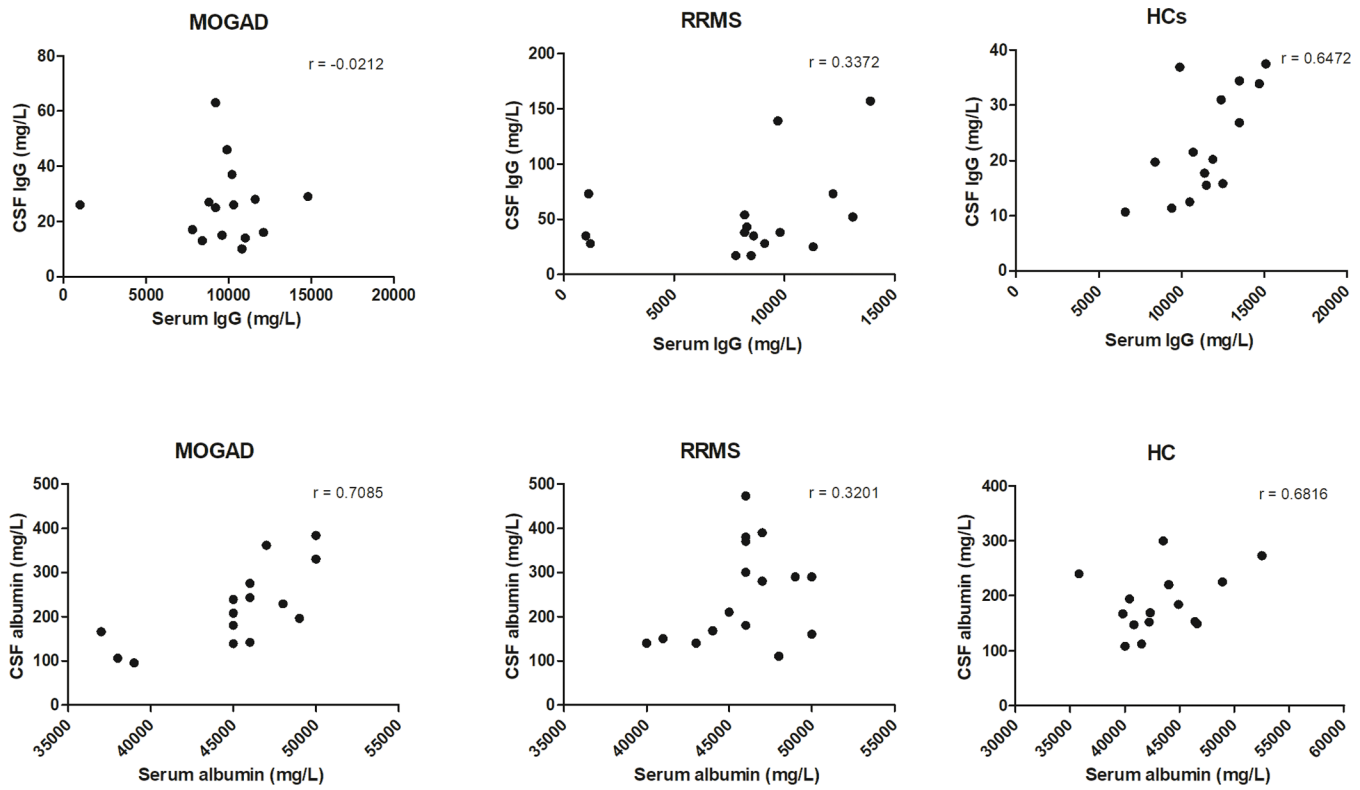

Fig. 6. Correlations between total IgG and albumin levels in serum and cerebrospinal fluid (CSF) of patients with myelin oligodendrocyte glycoprotein antibody-associated disease (MOGAD), relapsing-remitting multiple sclerosis (RRMS) and healthy controls (HCs). Correlations between serum and CSF IgG and albumin levels were determined using Pearson r correlations, where the correlation (r) was defined as follows: 0-0.25 = no correlation, 0.25-0.5 = weak positive correlation, 0.5-0.75 = moderate positive correlation, and 0.75-1.0 = strong positive correlation.

characteristics were determined, however a strong negative correlation was found between the number of cells in CSF and CMV pp52 IgG ($r = -0.76, p < 0.001$), whereas a strong positive correlation was determined for CSF EBV EBNA1 IgG and CSF cells ($r = 0.75$, $p < 0.001$) (supplementary C).

4. Discussion

MOGAD is a recently described inflammatory disorder of the CNS and only limited data on the association of some of the most common infections and MOGAD has been described [Fadda, 2024; Maniscalco, 2025; Molenaar, 2025].

For initial studies patient pools were analysed to screen for the presence of virus antibodies, as pools often indicate a general trend in the population. A drawback of pool screenings can be that outliers are ignored or that a few outliers may modify the final result. Based on this, it can be necessary to follow up with screening of individual samples. Hence, based on pool screenings, individual patient samples were tested for antibody reactivity.

Screening of individual serum samples suggest that development of MOGAD is neither associated with HHV6, CMV nor JCV, as no differences in serum antibody levels could be determined between patients with MOGAD and HCs. Serum EBNA1 IgG levels were sporadically increased in a limited number of MOGAD patients, however, in general no difference has determined in antibody levels between the MOGAD patients and the HC group. Collectively, these findings are in accordance with a recent study, testing serum samples from pediatric patients in a Dutch cohort, where the seroprevalence of EBV, CMV and HHV6 was tested in patients with MOGAD and other neurological diseases [Molenaar et al., 2025]. Here 88 %, 50 %, and 58 % of MOGAD patients tested positive for HHV6, EBV, and CMV antibodies, respectively, which however did not separate patients with MOGAD from patients with MS or other neurological diseases [Molenaar et al., 2025].

In contrast, significantly increased EBV EBNA1 IgG levels were observed in serum and CSF of RRMS patients when compared to patients with MOGAD and HCs, which is accordance to the literature [Bjornevik, 2022; Fadda et al., 2024; Gåsland, 2023; Houen, 2020a; Molenaar et al., 2025; Munger et al., 2011].

Although no difference in serum EBV EBNA1 IgG levels was observed between MOGAD patients and HCs, a few MOGAD patients presented with increased EBV EBNA1 IgG levels in CSF when compared to HCs. These results may indicate that EBNA1 IgG has crossed the BBB or that EBNA1 IgG is produced intrathecally. To analyze this further, AIs of the respective MOGAD samples were determined. As observed, approximately 21 % of samples presented with a positive AI to EBV EBNA1, indicating that EBV-specific B cells may have entered the CNS, where EBNA1 IgGs are produced. These findings are confirmed by a positive correlation between the number of CSF cells and CSF EBV EBNA1 IgG levels, although increased albumin levels in CSF of MOGAD patients may indicate that MOGAD patients are subject to increased albumin transport across the BBB. Hence, increased EBNA1 IgG levels in CSF may be a combination of intrathecal synthesis and influx across the BBB. These findings remain to be elaborated.

Although a few MOGAD patients presented with intrathecal antibody synthesis to EBV, the current findings do not indicate that EBV plays a central role for disease development, which in great contrast to MS, where EBV is central for disease development [Bjornevik 2022; Houen, 2020b; Vietzen, 2023]. These findings are in accordance to recently published studies using larger cohorts, describing the difference in EBV serology between MS and MOGAD patients (74 MS, 55 MOGAD) by Maniscalco et al. (2025) and in pediatric cohorts (31 MS, 36 MOGAD) by Molenaar et al. (2025), and (31 MS, 105 MOGAD) by Fadda et al. (2024). Collectively, these findings suggest a different role of EBV in the pathogenesis of the two conditions, pointing to that the etiology of

MOGAD is different from the etiology of RRMS.

Recent studies have proposed that CMV rather than EBV infection appear to be more common in MOGAD patients compared to EBV [Molenaar et al., 2025]. Our findings did not confirm this hypothesis, as MOGAD patients did not present with elevated CMV pp52 IgG levels in serum. Furthermore, these findings were confirmed by a strong negative correlation between serum CMV pp52 IgG levels and CSF cells. Thus, the precise role of CMV infections in MOGAD remains to be elaborated.

Finally, SARS-CoV-2 IgG levels were determined in MOGAD and RRMS samples. As observed, MOGAD patients presented with SARS-CoV-2 IgG levels in both serum and CSF, but the results were not significantly different from RRMS patients. These findings may indicate that SARS-CoV-2 does not contribute exclusively to the development of MOGAD when compared to RRMS. It remains to be determined whether the SARS-CoV-2 SP IgG levels were significantly elevated when compared to HCs, as samples from the HCs were collected prior to the Corona pandemic. Case studies have previously been presented, describing that SARS-CoV-2 infection may trigger development of MOGAD [Johnsson, 2022; Lotan, 2022]. Our findings do not support that this is a general trend.

In this study, results obtained in larger studies were confirmed, despite the used cohort size (Fadda et al., 2024; Maniscalco et al., 2025; Molenaar et al., 2025). To confirm these results, a larger study should be conducted using an expanded MOGAD group in combination with a larger set of virus antibodies. Furthermore, all of the MOGAD patients included presented with monophasic disease. It remains to be determined, whether patients with relapsing MOGAD differ in their serologic virus antibody profile. It is tempting to speculate, whether monophasic MOGAD disease resembles a disease state similar to clinically isolated syndrome (CIS), whereas relapsing disease resembles a disease state similar to relapsing MS. This remains to be elaborated on. However, findings describing antibody response to EBV proteins in patients with MOGAD do not support this hypothesis, as patients with CIS presented with elevated antibody levels to EBV when compared to HCs [Schlemm, 2016].

With that being said, one should be cautious in the comparison of the diseases, as findings within this study conform to that RRMS and MOGAD are two different diseases. While MOGAD clearly is a B-cell mediated disease specifically targeting MOG proteins, RRMS appears primarily to be a T-cell mediated disease, which conforms to that the disease etiology of the 2 neurological diseases most likely differs. This terminology is further complicated as studies have found that some patients with MS and HCs can also have very low MOG antibodies, which may lead to confusion about the diagnosis.

Collectively, current findings do not support that EBV, CMV, HHV6, JCV or SARS-CoV-2 plays a direct role in the onset of MOGAD, indicating that the etiology of MOGAD differs from MS etiology.

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CRediT authorship contribution statement

JL Frederiksen: Writing – original draft, Resources, Project administration, Investigation, Conceptualization. **D Žiogienė:** Writing – review & editing, Resources. **R Slibinskas:** Writing – review & editing, Resources. **E Ciplys:** Writing – review & editing, Resources. **G Houen:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Formal analysis. **NH Trier:** Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2026.107014.

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