Sero-epidemiological association between Epstein-Barr virus and autoimmune diseases

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Abstract

Background: The exact mechanisms of pathogenesis of several autoimmune diseases remain unclear. It has been suggested and researched thoroughly that infection with Epstein-Barr virus (EBV) plays a definite role in the development of these diseases, including rheumatoid arthritis (RA) and multiple sclerosis (MS), which are the focus of this review.

Objective: Determining whether prior infection with EBV occurs more frequently in patients with RA or MS compared to controls, was the purpose of this review.

Methods: Medline/Pubmed, Embase and Web of Science were systematically searched for several viruses (HSV, CMV, EBV) and their association in the development of several autoimmune diseases. Driven by our search, performed by three researchers independently, a majority of studies appeared to report on the association between Epstein-Barr virus and rheumatoid arthritis or multiple sclerosis, which became the focus of our review. The Newcastle-Ottawa scale was used for quality assessment.

Results: To demonstrate the link between EBV & RA 16 studies were included. In the same manner 15 were included for EBV & MS. The included studies, regarding RA, showed a trend of higher percentages of antibodies against EBV, more specifically for VCA and EA-D. For EBNA-1, the rate of seropositivity remained similar, partly due to the high global infection rate of EBV. A more prominent association was observed for EBV and MS. This effect becomes more significant the longer the period after primo-infection, becoming even more apparent if the infection occurs later in life.

Conclusion: For both RA and EBV, a direct causal relationship seems to be still lacking. Regarding MS, it can be more surely assumed that there seems to be a definite link with EBV seropositivity. Larger prospective studies for RA are necessary to provide a clear understanding of the direction and timing to demonstrate causality. In terms of MS, functional studies are needed to clarify disease mechanisms and possible cellular pathways.

Samenvatting

Achtergrond: De exacte mechanismen achter de pathogenese van verschillende auto-immuunziekten blijft onduidelijk. Uit eerder onderzoek en studies werd gezien dat infectie met Epstein-Barr virus (EBV) een cruciale rol speelt in de ontwikkeling van deze ziekten, waaronder ook reumatoïde artritis (RA) en multiple sclerose (MS), die de focus van deze review zijn.

Doel: Het bepalen of primaire infectie met EBV meer voorkomt bij RA- of MS-patiënten in vergelijking met de controles, was het doel van deze review.

Methodes: Medline/Pubmed, Embase en Web of Science werden systematisch onderzocht naar virussen (HSV, CMV, EBV) en hun link met het ontwikkelen van verschillende auto-immuunziekten. Gedurende onze zoekopdracht, uitgevoerd door drie onafhankelijke onderzoekers, werd het duidelijk dat er vooral goed onderbouwde studies bestaan rond Epstein-Barr virus en de eventuele link met reumatoïde artritis en multiple sclerose. De Newcastle-Ottawa schaal werd gebruikt voor kwaliteitsbeoordeling van de studies.

Resultaten: Het aantal van 16 studies die de link tussen EBV - RA en 15 die de link tussen EBV - MS aantoonden, werd geïncludeerd. De geïncludeerde studies, wat betreft RA, vertoonden een trend van hogere percentages antilichamen tegen EBV, meer specifiek voor VCA en EA-D. Voor EBNA-1 bleef de graad van seropositiviteit gelijkaardig, mede vanwege de hoge globale infectiegraad van EBV. Wat betreft EBV en MS werd een duidelijker verband waargenomen. Dit effect wordt het meest significant hoe langer de periode na de primo-infectie. Dit wordt nog duidelijker indien de infectie op latere leeftijd plaatsvindt.

Conclusie: Zowel voor RA als EBV ontbreekt er nog steeds een direct causaal verband. Wat betreft MS kan met meer zekerheid verondersteld worden dat er een duidelijke link bestaat met EBV seropositiviteit. Grotere prospectieve studies zijn nodig om bij RA een duidelijk inzicht te geven in de richting en timing van de relatie om op die manier causaliteit aan te kunnen tonen. Op vlak van MS zijn er functionele studies nodig om ziektemechanismen en mogelijke cellulaire cascades te verduidelijken.

Introduction

The aim of this review is to investigate the link between viral infections and autoimmune pathology. The most common virus examined in association with autoimmune diseases, is Epstein-Bar virus (EBV). The two autoimmune pathologies most frequently linked to EBV according to the search, rheumatoid arthritis (RA) and multiple sclerosis (MS), are further elaborated below.

Epstein-Barr virus, a human herpesvirus, typically causes asymptomatic primary infection and occurs in 90% of cases during childhood. Approximately 98% of the global population is infected with EBV(1). The virus is the causative agent of infectious mononucleosis (IM) and is also strongly associated with various malignancies such as nasopharyngeal carcinoma and Burkitt lymphoma(1). To assess the presence of EBV infection in RA and MS patients, antibodies against EBV antigens are typically detected using various laboratory techniques. These antigens include EBV nuclear antigen (EBNA)-1, viral capsid antigen (VCA), early antigen complex-diffuse (EA-D), and early antigen complex-restricted (EA-R). During the early phase of EBV infection, antibodies against VCA and EA-D are produced. Antibodies against EA-R and EBNA are produced weeks to months later. Antibodies against EBNA-2 precede those against EBNA-1(2), and the latter remains stably present throughout life, while antibodies against EBNA-2 decrease significantly after a few weeks. During the latent phase of the viral cycle, stable levels of IgG antibodies against EBNA-1 are present, along with limited levels against VCA and EA-R. Reactivation of the virus, due to factors such as decreased immunity, leads to a switch to the lytic phase, during which antibodies against both VCA and EA-D are produced(2).

Rheumatoid arthritis (RA) is one of the most common autoimmune conditions, with a global prevalence of 0.5% to 1%(3). Prevalence is two to four times higher in women than in men(4). RA is a chronic inflammatory polyarthritis that results in progressive joint destruction and can also have systemic implications. The exact aetiology of RA remains unclear, but it is known to involve a combination of genetic components and environmental factors. While evidence regarding the impact of factors such as oral contraceptives, breastfeeding(5) and diet factors like anti-oxidants(6) and fat consumption(7), is limited. There is substantial evidence implicating smoking(8) as a risk factor for RA and studies investigating a viral trigger for RA have also shown promising results(9, 10).

Multiple sclerosis (MS) is an autoimmune condition affecting the central nervous system, characterized by immune-mediated demyelination and axonal degeneration, leading to progressive neurological deterioration. Symptoms include muscle weakness, sensory disturbances, visual impairments, and ataxia, usually in a relapsing-remitting pattern. Globally, approximately 2.8 million people suffer from this condition(11), with higher prevalences in certain regions. At present, there is no treatment that effectively halts the progression of the disease, highlighting the importance of understanding its development for the implementation of preventive interventions. Both genetic and environmental factors, such as smoking(12), vitamin D deficiency(13) and allergen exposure(14), have been implicated in the aetiology of MS(15). Similarly to RA, there are numerous promising studies investigating the link between a viral trigger (specifically EBV) and the pathogenesis of MS.

The goal of this review is to establish a causal relationship between EBV and both RA and MS. A challenge in doing so is the high prevalence of EBV in the general population compared to the relatively lower prevalence of RA and MS.

Methods

Review question

This systematic review aims to answer the following question: 'Is there an association between common viruses and the development of autoimmune disorders?'

The components to translate this into an answerable research question for the population, exposure, and outcome (PEO) are as follows:

- Population: general population
- Exposure: infection by common viruses (HSV, CMV, EBV)
- Outcome: Standardised Incidence Ratios and percentages of people who developed an autoimmune disease (rheumatoid arthritis, diabetes mellitus (type 1), multiple sclerosis and lupus) after being infected by common viruses (HSV, CMV, EBV)

Search strategy

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines(16). Three databases (Medline via Pubmed, Embase and Web of Science) were searched on June 17, 2023, and the following selection was done by two independent researchers (Arno Dejaegere and Severino Montero). Two common conditions in general practice were chosen, specifically rheumatoid arthritis and diabetes. Two more interesting autoimmune disorders were added: multiple sclerosis and lupus. Those are two autoimmune conditions that are often part of a differential diagnosis during clinical reasoning. In terms of viral pathology, three of the most prevalent viruses pathogenic to humans were chosen. All belong to the herpesviruses, specifically the alpha herpesviruses (which include HHV-1 and -2), the Epstein-Barr virus and the cytomegalovirus.

The used concepts for the MeSH terms are the alphaherpesvirinae (simplexvirus, herpesvirus 1, herpesvirus 2, varicellovirus, varicella zoster virus), this is concept 1; cytomegalovirus (concept 2), Epstein-Barr virus (concept 3). Concept 4 consists of autoimmune diseases: rheumatoid arthritis, diabetes mellitus (type 1), multiple sclerosis and lupus. Terminology that was used for the full search strategy can be found in Appendix I. Duplicate papers were resolved using the Endnote X9 software. To minimise the risk of exclusion of relevant citations, the citation list of each included study was searched.

The selection process took place in two phases. In the first phase two independent researchers (AD and SM) screened all included studies on title and abstract, using the reference managing software Rayyan(17). After completion of this phase by both investigators, inconsistencies were resolved by consensus. During the second phase full-text articles were also reviewed by same two independent researchers (AD and SM). Discrepancies were resolved by discussion with a third reviewer (Jan Verbakel).

Determination of inclusion and exclusion criteria

The first and one of the main inclusion criteria, is the fact that it had to be an article where a viral infection (HSV, VZV, CMV, EBV) causes or increases the risk of developing an autoimmune disorder. We focused on including case control studies, cross-sectional studies, prospective and retrospective cohort studies. Nevertheless, there were no restriction based on study design, only conference abstracts were excluded before we start screening. We only included the original studies. Furthermore no restriction based on date were made.

Exclusion criteria were based on the fact that the study's outcome was something other than the association between a viral infection (HSV, VZV, CMV, EBV) that causes or increases the risk of developing an autoimmune disorder. For example: (1) Connection between viral infections and diseases other than autoimmune disease, e.g. malignancies. (2) The body response of immunocompromised/ patients with an autoimmune disease after infection with certain viruses. (3) Vaccinations against certain viral diseases in immunocompromised/ patients with an autoimmune disease. (4) Possible autoimmune reactions after vaccination against a viral condition. (5) The use of viral cell lines in studies concerning the approach, treatment or management of an autoimmune disease. Furthermore, we excluded papers written in languages other than English.

Ethical approval

This review underwent ethical approval, supervised by the Education Guidance Committee OBC (Appendix II). Since no personal data was collected, this research design was approved automatically. The application was originally for a review with title 'Viral infections and autoimmune disorders'. The final title was later specified to 'Sero-epidemiological association between Epstein-Barr virus and autoimmune diseases'.

Results

Study selection

The performed selection process is summarised in the flow diagram (Figure 1).

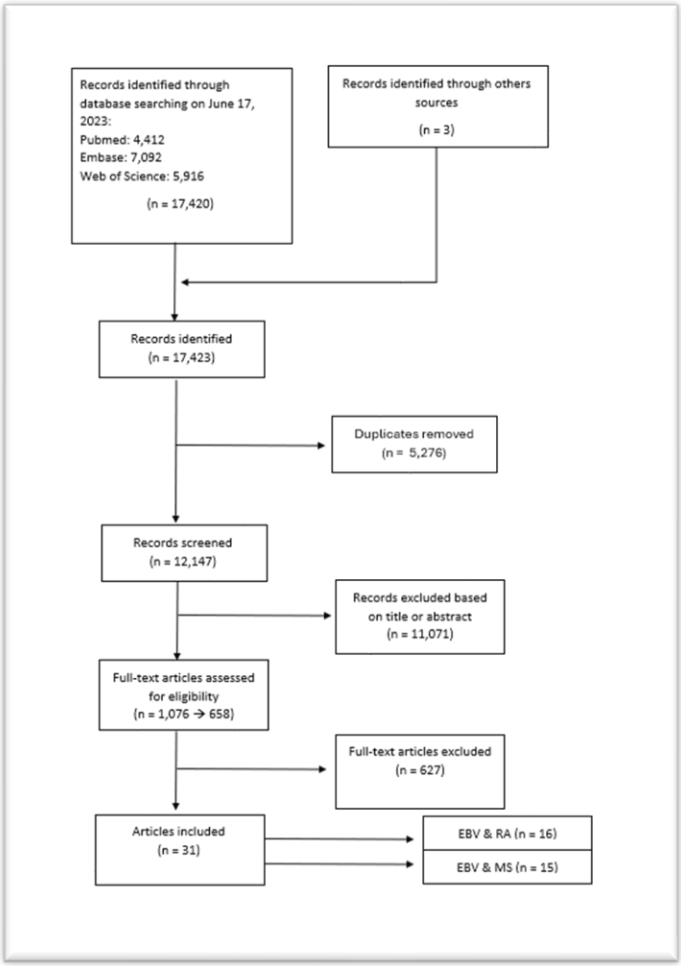


Figure 1: Study selection flow diagram according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines

The overall search identified 17,423 studies applicable for this review. After deduplication 12,147 articles remained. After the screening of all titles and abstracts (if available) 1,076 articles were investigated for further suitability. These articles were all labelled in groups by type of virus and by type of autoimmune condition. Since the association between EBV and autoimmune disorders, RA and MS in particular, seemed to be more prominent in the search, it was decided in mutual consultation with all parties to continue with these two topics. Given that the studies we selected showed significant heterogeneity, it was not possible to perform a representative meta-analysis. We found 658 articles that discussed the link between EBV and MS on the one hand, and between EBV and RA on the other hand. Out of the 658 articles, 31 articles were ultimately retained after full-text screening. For MS we retained 15 and for RA 16 articles.

Quality assessment

The methodological quality of the included studies were scored using a modified version of the Newcastle-Ottawa scale (NOS)(18). For matching case-control studies, sex was also included, next to age (C1). We also found importance in the fact that analysis was conducted in an independent laboratory, as a reliable marker for ascertainment of exposure (E1a). In the evaluation of this item, the use of explicit and definite laboratory cutoff values was also assessed (E1b). Reporting of missing data was also checked (E3).

Newcastle-Ottawa assessment scale: EBV & RA (Table 1) (18)

Authors, year	S1	S2	S3	S4	C1	C2	E1a	E1b	E2	E3	Total
Fechtner et al, 2022	*	-	-	-	-	-	-	-	*	-	2
Ferrell et al, 1981	*	-	-	-	*	-	-	-	*	-	3
Goldstein et al, 2012	*	-	-	*	*	*	*	*	*	-	7
Mahabadi et al, 2016	-	-	-	-	-	-	-	-	-	-	0
Marzoq et al, 2019	-	-	-	*	-	-	-	-	*	-	2
Masuoka et al, 2018	*	-	-	-	-	-	-	-	*	-	2
Musiani et al, 1987	*	-	*	*	-	-	-	*	*	-	5
Niedobitek et al, 2000	*	-	-	-	-	-	-	-	*	-	2
Saal et al, 1999	*	-	-	-	*	*	-	-	-	-	3
Takeda et al, 2000	*	-	-	-	*	*	-	-	*	-	4
Takei et al, 1997	*	-	-	-	-	-	-	-	*	-	2
Thomas et al, 1982	*	-	-	*	-	-	-	-	*	-	3
Venables et al, 1985	*	-	*	*	-	-	-	-	*	-	4
Westergaard et al, 2015	-	-	*	*	-	-	-	*	*	-	4
Yao et al, 1986	*	-	-	*	-	-	-	-	*	-	3
Yazbek et al, 2011	*	*	-	*	*	*	-	-	*	-	6

Newcastle-Ottawa assessment scale: EBV & MS (Table 2) (18)

Authors, year	S1	S2	S3	S4	C1	C2	E1a	E1b	E2	E3	Total
Abrahamyan et al, 2020	*	*	-	*	-	-	*	*	*	*	7
Biström et al, 2021	*	*	-	*	*	-	*	-	*	-	6
Bjornevik et al, 2022	*	*	*	*	*	*	*	*	*	*	10
Comabella et al, 2009	-	*	-	*	*	-	-	-	*	-	4
Goldacre et al, 2004	*	-	-	*	*	-	-	-	*	-	4
Gustavsen et al, 2014	*	*	-	*	-	-	-	-	*	-	4
Langer-Gould et al, 2017	*	*	*	*	*	*	-	-	*	-	7
Laribi et al, 2018	*	*	*	*	-	-	-	*	*	-	6
Levin et al, 2010	*	*	*	*	*	*	-	*	*	*	9
Loosen et al, 2022	*	*	*	*	*	-	-	-	*	*	7
Mameli et al, 2013	-	-	-	*	*	-	-	-	*	-	3
Munger et al, 2011	*	-	*	*	*	*	*	-	*	*	8
Nielsen et al, 2007	-	-	-	*	*	-	-	-	*	-	3
Nourbaksh et al, 2018	*	-	-	*	-	-	*	-	*	-	4
Xu et al, 2021	*	*	*	*	*	*	-	-	*	*	8

Table 1+ 2 Modified NOS

*: positive score, - : negative score, S1 adequate case definition, S2 cases consecutively recruited or obviously representative, S3 community controls, S4 controls with no disease history, C1 age and sex-matched controls, C2 controls matched for additional factors, E1a conducting analysis in an independent clinical laboratory, E1b explicit laboratory cutoffs for seropositivity were applied, E2 same method of ascertainment for cases and controls, E3 missing data reported

Results of the individual studies

1. EBV - RA

Table 3 shows the characteristics of the included studies for this review, arranged alphabetically. There were only two cohort studies from the 16 included. The remaining studies were all case control and cross-sectional studies. The methods and criteria used for diagnosing and characterising the type of RA were quite similar across all studies. Depending on the year of publication in which the article was published, the guidelines proposed by the 'American Rheumatism Association' were used. More recent studies used the guidelines and criteria suggested by the 'American College of Rheumatology' (ACR). Two of the included studies did not specify the method of diagnosis ('Mahabadi et al'(19), 'Westergaard et al'(20)), one study mentioned that the diagnoses in their study were made 'according to rheumatologist physicians'. Despite the heterogeneity in the data of the collected studies, they are grouped below based on what exactly was measured and which techniques were used for collecting data and the study design.

1.1 Cohort studies

In a first cohort study, 'Fechtner et al'(21) assessed the effects of EBV on the development of RA based on serum samples gained from military personnel. This was done by comparing 2 samples, the first sample before diagnosis with RA and the other sample after diagnosis. The researchers demonstrated the number of positive cases for a specific antibody, the total titres of this antibody, as well as specifically the titres of these antibodies pre- and post-RA. The results showed that the number of cases with EA IgG antibodies was significantly higher in the RA group than in the control group, unlike cases with EBNA-1 IgG and VCA IgG antibodies. However, the titres of EBNA-1 IgG and VCA IgG, like EA IgG, were significantly different between the RA and control groups. One drawback of this study is that the data come from military personnel, resulting in a higher number of men included than we would expect to represent the general population. 'Goldstein et al'(22) conducted a cohort study comparing anti-EBV serology before and after developing RA with serum samples taken concurrently from a control group. The data came from two large cohorts of female nurses, with 87 RA patients identified. Each of these patients was matched with a similar control, taking into account potential confounding factors such as smoking, alcohol intake, BMI, parity, and breastfeeding. However, no significant difference was found between the RA and control groups for any of the antibody titres (VCA, EA-D, EA-complex, EBNA-1, and EBNA-2). Two limitations of this study were the limited number of patients for a cohort and the fact that the results were based on a single blood draw (both pre- and post-RA).

1.2 Case control and cross-sectional studies

Some of the studies (5 out of 16) used Polymerase Chain Reaction (PCR) to quantitatively compare EBV DNA, often in a cross-sectional manner, between RA patients and a control group.
In the study of 'Mahabadi et al'(19), research was conducted to assess the number of RA patients in whom EBV DNA was found in their synovial fluid. It was concluded that EBV DNA was present in 60% of cases, which was significantly higher than in the control group ($p \leq 0.04$). However, no data on the

AUTHORS, YEAR	ORIGIN OF THE STUDY (COUNTRY)	NUMBER OF (RA-) PATIENTS (+ RACE OF POPULATION)	CONTROLS (+ RACE OF POPULATION)	AGE (YEARS), SEX	OTHER VIRUSES	WHAT IS MEASURED?	EBV-POSITIVE RESULTS (%)	TECHNIQUE USED	SPECIMENS USED/ CELLS LABELLED BY IN SITU HYBRIDIZATION	OUTCOME
Fechtner et al, 2022*	USA	RA: 83 (NS)	83 (NS)	NS	CMV	EBNA-1 IgG, VCA IgG, EA IgG	RA: EBNA-1 IgG 82/83 (98,8%), VCA IgG 79/83 (95,2%), EA IgG 28/83 (33,7%) Controls: EBNA-1 IgG 82/83 (98,8%), VCA IgG 81/83 (97,6%), EA IgG 9/83 (10,8%)	ELISA	Serum	EBNA-1 IgG: P = not significant (1) VCA IgG: P = not significant (0,68) EA IgG: P < 0,001
Ferrell et al, 1981	USA	RA: 89 (NS)	53 (NS)	RA: mean age 56, 42F Controls: mean age 51, 28F	No	Anti-VCA, anti-EA, anti-EBNA titers	RA: anti-EBNA 14/80 (17,6%), anti-VCA 27/86 (31,4%), anti-EA 22/86 (25,6%) Controls: anti-EBNA 10/51 (19,6%), anti-VCA 8/53 (15,1%), anti-EA 4/53 (7,5%)	Anti-complement immunofluorescent technique	Serum	Anti-EBNA: P = not significant Anti-VCA: P < 0,01 Anti-EA: P < 0,01
Goldstein et al, 2012*	USA	RA: 87 (Caucasian)	87 (85 Caucasian, 2 NS)	RA: mean age 55, 87F Controls: mean age 55, 87F	CMV	Anti-VCA, anti-EA D, anti-EBNA 1 & 2 titers	RA: anti-EBNA1 31/87 (35,6%), anti-EBNA2 21/87 (24,1%), anti-VCA 15/87 (17,2%), anti-EA D 7/87 (8,0%) Controls: anti-EBNA1 26/87 (29,9%), anti-EBNA2 19/87 (21,8%), anti-VCA 17/87 (19,5%), anti-EA D 3/87 (3,4%)	Immunofluorescence assay	Peripheral blood	P = not significant
Mahabadi et al, 2016	Iran	RA: 50 (NS)	NS	RA: mean age 49, 20F	No	EBV DNA	RA: 30/50 (60%)	PCR	Synovial fluid	P < 0,04
Marzoq et al, 2019	Iraq	RA: 74 (NS)	74 (NS)	RA: 15-75y, 58F Controls: 15-75y, NS	No	EBV DNA	RA: 23/74 (31,1%) Controls: 0/74	PCR	Peripheral blood	NR
Masuoka et al, 2018	Japan	RA: 128 (NS)	OA: 98 (NS)	NS	No	EBV DNA	RA: 42/128 (32,8%) Controls (OA): 15/98 (15,3%)	PCR	Synovial tissue	P < 0,01

Musiani et al, 1987	Italy	RA: 35 (NS)	40 (NS)	RA: 28-65y, NS Controls: NS, NS	CMV	Anti-VCA, anti-EA, anti-EBNA titers	RA: anti-VCA 18/35 (51,4%); anti-EA 15/35 (42,9%); anti-EBNA 19/35 (54,3%) Controls: anti-VCA 6/40 (15,0%); anti-EA 5/40 (12,5%); anti-EBNA 6/40 (15,0%)	Immunofluorescence assay	Synovial fluid, serum	P < 0,01
Niedobitek et al, 2000	Germany	RA: 37 (NS)	Other joint diseases*: 51 (NS)	RA: 23-76y, 29F Other joint diseases*: NS, NS	No	EBER	RA: 7/39 (17,9%) Other joint diseases*: 0/51	In situ hybridization	Synovial membrane	NR
Saal et al, 1999	Germany	RA: 84 (NS) Other arthritis: 24 (NS)	OA: 67 (NS)	RA: mean age 57, 59F Other arthritis: mean age 51, 6F Controls: mean age 64, 42F	No	EBV DNA	RA: 29/84 (34,5%) Other arthritis: 2/24 (8,3%) Controls (OA): 6/67 (9,0%)	PCR + Southern blot hybridization	Synovial fluid, peripheral blood	P < 0,001
Takeda et al, 2000	Japan	RA: 32 (NS)	OA: 30 (NS)	RA: mean age 60, 29F Controls: mean age 72, 26F	No	EBV DNA	RA: 15/32 (46,8%) Controls (OA): 0/30	PCR + Southern blot hybridization	Synovial tissue	P < 0,01
Takei et al, 1997	Japan	RA: 34 (NS)	OA: 20 (NS)	NS	No	EBER	RA: 8/34 (23,5%) Controls (OA): 0/20	In situ hybridization	Synovial cells	P < 0,05
Thomas et al, 1982	USA	RA: 61 (Mexico)	59 (Mexico)	NS	No	Anti-RANA, anti-VCA, anti-EA-D, anti-EBNA Ab	RA: anti-RANA 47/61 (77,0%), anti-VCA 32/61 (52,5%), anti-EA-D 12/61 (19,7%), anti-EBNA 13/61 (21,3%) Controls: anti-RANA 10/59 (16,9%), anti-VCA 19/59 (32,2%), anti-EA-D 2/59 (3,4%), anti-EBNA 12/59 (20,3%)	Double immunodiffusion (anti-RANA), Anti-complement immunofluorescent technique (anti-EBNA)	Serum	Anti-RANA, anti-EA: P < 0,001 Anti-VCA: P < 0,05 Anti-EBNA: P = not significant
Venables et al, 1985	UK	RA: 41 (NS)	26 (NS)	RA: mean age 61, 23F	CMV	EBVCA IgG antibodies	RA: 19/41 (46,3%) Controls: 12/26 (46,2%)	Indirect immunofluorescence	NS	P = not significant

				Controls: mean age 42, 23F						
Westergaard et al, 2015	Denmark	RA: 77 (NS)	28 (NS)	RA: mean age 55, 59F Controls: mean age 37, 27F	No	EBNA-1 IgG/IgM antibodies, EAD IgG/IgM antibodies	RA: EBNA-1 IgG 75/77 (97,4%)/ IgM 75/77 (97,4%); EAD IgG 15/77 (19,5%)/ IgM 21/77 (27,3%) Controls: EBNA-1 IgG 27/28 (96,4%)/ IgM 2/28 (7,1%); EAD IgG 3/28 (10,7%)/ IgM 2/28 (7,1%)	ELISA	Plasma	EBNA-1 IgG/IgM-Ag levels: P < 0,0001 EAD IgG/IgM-Ag levels: P < 0,0011
Yao et al, 1986	UK	RA: 33 (NS) SA: 15 (NS) OA: 15 (NS)	26 (NS)	RA/SA/OA: 28-76y, NS Controls : 22-64y, NS	No	Anti-VCA, anti-EA titers	RA: anti-VCA 28/33 (84,8%), anti-EA 18/33 (54,5%) SA: anti-VCA 13/15 (86,7%), anti-EA 6/15 (40,0%) OA: anti-VCA 10/15 (66,7%), anti-EA 6/15 (40,0%) Controls: anti-VCA 18/26 (69,3%), anti-EA 8/26 (30,8%)	NS	Saliva, peripheral blood	Ab titers RA compared with controls: Anti-VCA: P < 0,02 Anti-EA: P < 0,01
Yazbek et al, 2011	Brazil	RA: 140 (74 caucasians, 46 mulattoes, 20 blacks)	143 (83 caucasians, 39 mulattoes, 21 blacks)	25-82y RA: mean 54 years, 122F Controls: mean 56 years, 123F	No	Anti-EBNA-1 antibodies	RA: 127/140 (90,7%) Controls: 130/143 (90,9%)	ELISA	NS	P = not significant (0,955)

Table 3: Baseline characteristics of the included studies of Epstein-Barr virus and rheumatoid arthritis

*: Cohort study

Abbreviations: RA: rheumatoid arthritis; EBV: Epstein-Barr virus; NS: not specified; CMV: cytomegalovirus; EBNA: Epstein-Barr virus-(associated) nuclear antigen; (EB)VCA: (Epstein-Barr) viral capsid antigen; EA: early antigen; ELISA: enzyme-linked immunosorbent assay; F: females; EA(-)D: diffuse component of early antigen; PCR: Polymerase chain reaction; NR: not reported; OA : osteoarthritis; *: osteoarthritis, traumatic joint lesions, tuberculosis, other bacterial infections, chondrocalcinosis, gout, and seronegative spondylarthritis; EBER: EBV-encoded small RNA-1; RANA: Rheumatoid arthritis-associated nuclear antigen; Ab: antibodies; GMT: the $\log_2$ geometric mean titre; SA: seronegative spondyloarthropathy

control group were provided, neither on the size of this group nor on specific characteristics of these patients (possible other pathologies such as osteoarthritis, gender, etc.). In 'Marzoq et al'(23), again using PCR, it was shown that serum samples from RA patients had a significantly higher EBV DNA load (present in 31%) than those from the control group, where no EBV DNA was detected at all. 'Masuoka et al'(24) were able to demonstrate that EBV DNA was much more frequent in the synovial tissue of RA patients than in that of the control group, in this case osteoarthritis (OA) patients. In this study, they also examined HLA genotyping, specifically the HLA-DRB1-shared epitope. Shared epitope (SE) is a subset of HLA-DRB1 alleles, consisting of a five amino acid sequence. It was found that the group that was SE positive was significantly larger in RA patients than in OA patients. In other words, HLA-DRB1-SE was seen as a genetic risk factor for developing RA. However, the occurrence of EBV did not differ between SE-positive and SE-negative RA patients. From this, it could be concluded that EBV and SE are likely two independent risk factors for developing RA. 'Saal et al'(25) also concluded that EBV DNA occurred significantly more often in synovial fluid of RA patients than in the control group. People with EBV-positive synovial membranes had a 5.47 times higher risk of developing RA than EBV DNA-negative individuals. A significant difference was also found between the two groups in the analysis of peripheral blood; however, the EBV DNA titres were lower than in synovial fluid. Like 'Masuoka et al'(24), the researchers went beyond just looking at the effect of EBV on RA; they also looked at genetic factors (HLA genotype). HLA genotyping taught 'Saal et al'(25) that patients with synovial EBV DNA and positive genotyping for HLA-DRB1-SE had a significantly higher risk of developing RA than patients who scored negative on one or both of these characteristics. The last study to demonstrate EBV DNA in RA patients using PCR was that of 'Takeda et al'(26). They found that EBV DNA occurred in 47% of RA patients and in none of the tested control patients with osteoarthritis. The researchers also demonstrated that the group of EBV-positive RA patients was significantly younger than the group of EBV-negative RA patients.

'Niedobitek et al'(27) and 'Takei et al'(28) studied EBV infection by examining the presence of EBV-encoded RNA (EBER). Both studies detected EBER using in situ hybridization. From the research of 'Niedobitek et al'(27) that compared biopsies of synovial membranes from RA patients and a control group (with other joint pathologies), no significant difference between the two groups could be demonstrated. EBER-positive cells were found in only 17.9% of the biopsies, while they were absent in any of the tested control patients. With this data, it was concluded that EBV infection of the synovial membranes does not play a role in the pathogenesis of RA. 'Takei et al'(28) also detected EBV in RA patients by examining the presence of EBER in synovial cells. Here, it was not only found that EBER occurred significantly more often in the RA patient group than in the control group (patients with osteoarthritis), but also that EBER was detected mainly in specific locations. The localization of the occurrence of EBER was exclusively in the proliferative sites of the apex of the synovial villi. It is striking that despite similar data, both studies draw different conclusions. 'Niedobitek et al'(27) themselves claim not to be able to fully explain their difference in outcome with 'Takei et al'(28), but suspect that the difference in population (Western vs. Japanese) could play an important role.

Two studies that used the ELISA technique to determine the titres of EBNA-1 antibodies in RA patients were those of 'Westergaard et al'(20) and 'Yazbek et al'(29). In Westergaard's study, both the titres of EBNA-1 and EA-D were examined and compared between a group of RA patients and a control group. 'Westergaard et al'(20) demonstrated that RA patients had a higher percentage of antibodies for EA-D IgM/IgG than healthy controls. Also, the EBNA-1 IgM and IgG titres were significantly higher in RA patients. Regarding the effective presence of EBNA-1, only significant higher percentages were found with IgM and not with EBNA-1 IgG. Due to the high infection rate of EBV in approximately 95% of the general population (>40 years old), similar percentages of EBNA-1 IgG positive cases were expected in both the RA group and the control group. 'Yazbek et al'(29) investigated whether the titres of anti-EBNA-1 antibodies could be linked to RA positivity to demonstrate a link between EBV and RA. From their study, it became very clear that there was no link between either the effective presence of anti-EBNA-1 or the titres of anti-EBNA-1 antibodies and the risk of developing RA. This is because the percentages of anti-EBNA-1 antibody positivity in the RA group and in the control group were almost identical. Unlike 'Westergaard et al'(20), no specification was made here about the exact type of antibodies involved.

In the last five studies, researchers investigated multiple types of antibodies against EBV-related antigens using various laboratory techniques. It is also noteworthy that all five of these studies date back to the 1980s. In the study of 'Ferrell et al'(30), a group of RA patients was compared with a control group in terms of both the presence and titres of antibodies against EBV-related antigens (anti-EBNA, anti-VCA, anti-EA). For anti-EBNA, both a similar presence of anti-EBNA and similar titres were found in both the RA group and the control group, logically resulting in no significant difference to be demonstrated. For anti-VCA, a similar presence of anti-VCA was found in both groups, showing that there were as many people infected with EBV in the RA group as in the control group, and thus no difference in exposure to EBV. However, the titres of anti-VCA were significantly different between the two groups. For anti-EA, both the presence of anti-EA and the titres were significantly different between the RA group and the control group. 'Musiani et al'(31) were able to demonstrate that antibody titres against EBV-induced VCA, EA, and EBNA were significantly higher in RA patients compared to healthy control patients. Although the cutoff values for both anti-VCA, anti-EA, and anti-EBNA were different, a significant difference was always found compared to the control group. In fact, antibodies against VCA and EBNA were found in all RA and control patients, again indicating that all had previously been infected with EBV. It was also the case that among the RA patients, 14 out of 35 (40%) showed serological characteristics of a reactivated EBV infection (VCA ≥ 1/320, EA ≥ 1/20, and EBNA ≥ 1/10). While in the control group, only 5 out of 40 (12.5%) individuals showed these serological characteristics. The study by 'Thomas et al'(32), conducted in 1982, examined multiple types of antibodies in the sera of a Mexican group of RA patients and compared them with a control group. Specifically, antibodies against RANA, VCA, EA (diffuse and restricted component), and EBNA were examined. 'Thomas et al'(32) saw a significantly higher occurrence of antibodies against RANA, VCA, and EA (diffuse component) in the RA group than in the control group of patients. No significant difference was found between the two groups in the antibodies against the restricted component of EA and EBNA. The penultimate study, by 'Venables et al'(33), again could not demonstrate a significant difference. The number of patients who were above a certain titre of antibodies for VCA were almost identical between a population of RA patients and a control population. From the last study by 'Yao et al'(34), it appeared that depending on the predetermined cutoff values, the number of patients who tested positive for the presence of both anti-VCA and anti-EA antibodies was distributed quite similarly

between the RA/OA/SA (spondylarthrosis) patients and the healthy controls. ‘Yao et al’(34) were able to demonstrate in their study that RA patients showed a higher level of anti-VCA titres and a significantly higher level of anti-EA titres ($p < 0.01$) compared to healthy controls. However, there was no clear difference with the SA and OA patients, as high anti-VCA and anti-EA titres were also found in these patients.

2. EBV - MS

The characteristics of the patients in the selected studies are presented in Table 4 and 5. The included studies were of both case-control and cohort designs. Out of the 15 included studies, eight were cohort studies. Most studies determined both VCA IgG and EBNA IgG antibodies to assess EBV status. Two studies ('Xu et al'(35) and 'Loosen et al'(36)) used ICD10 coding to identify patients diagnosed with infectious mononucleosis (IM). 'Goldacre et al'(37) identified patients through registered hospital admissions with IM, although this is not further specified. 'Gustavsen et al'(14) assessed the history of IM using a questionnaire, while 'Nielsen et al'(38) used the Paul-Bunnell test to determine IM.

Ten out of the 15 studies serologically assessed EBV status using immunofluorescence, ELISA, or CLIA. The majority of studies determined both EBNA and VCA IgG antibodies. 'Nourbaksh et al'(39) determined only VCA antibodies, 'Mameli et al'(40) only EBNA-1 antibodies. Six out of the ten studies used ELISA for analysis. One of these six studies was conducted by ‘Bjornevik et al’(41), a large cohort study consisting of over 10 million young adult military personnel. In this study, the group of MS patients numbered 955, while the control group consisted of 1,566 individuals. Three serum samples were analysed for each MS patient: the earliest available, the last one before MS onset, and one between these two samples. Serum samples from the control group were matched at comparable time periods. 35 cases and 107 controls were EBV seronegative at the first sample. All cases seroconverted, except for one, during the follow-up period. In the control group, slightly over half seroconverted, representing a statistically significant difference. They also analysed CMV seropositivity in their study, but no association with an increased risk of developing MS was found. ‘Mameli et al’(40) studied the relationship between EBV and MS in patients from Sardinia. Despite being located further south, Sardinia has one of the highest prevalences of MS worldwide(42), contradicting the assumed north-south distribution of MS(43). The higher prevalence of MS in Sardinia is presumably explained by the characteristic genetics of this population. Type 1 diabetes is also believed to be more prevalent in Sardinia(44). In this study, they also found a significantly higher seropositivity for EBV in MS patients, analysed solely through EBNA-1 IgG antibodies, as described earlier. The higher the antibody titre, the higher the risk of MS. This represented a 4.6-fold increase in risk in patients in the highest quartile of IgG titres. ‘Langer-Gould et al’(45) focused more on the potential role of ethnicity in modulating the risk of developing MS due to EBV. They distinguished between three groups: Black, Hispanic/Latin American, and Caucasian. They also used ELISA to serologically determine the presence of IgG antibodies for VCA and EBNA-1. A statistically significant difference was observed in seropositivity as well as EBNA-1 titres, which were higher in the MS group. VCA titres were also determined, but no statistical data were documented for these. CMV was also analysed in this study, but there was no significant difference between the two groups. ‘Laribi et al’(46) conducted a smaller study with 68 MS patients and 20 controls to demonstrate whether EBV antibodies and titres differed between different clinical patterns of MS. Serum from both groups was analysed to determine seropositivity and titres of VCA and EBNA-1 antibodies. They found

that all MS patients were seropositive for at least one EBV antibody. Additionally, the titres in MS patients were significantly higher than in control patients. They also conducted analysis on several cellular immune responses in different MS subtypes. This is the only study we included that also emphasised this aspect. T-helper cells play a significant role in immunity against EBV. These cells produce various cytokines, including IFN-gamma, IL-12, and IL-4. The concentrations of these cytokines were significantly increased in all MS patients compared to controls. From this, one could conclude that cellular immune response could indeed play a causative role. We also included a study on MS in paediatric patients conducted by 'Nourbaksh et al'(39), examining the role of EBV seropositivity as well as HSV 1-2 and CMV seropositivity in the development of MS. As stated earlier, they only researched VCA seropositivity. In the MS group, they observed much more seropositivity for EBV, with odds of having MS increasing up to 7.4 times. A significant difference was also seen for HSV-1, but only in Caucasians and patients negative for HLA-DRB*1501. This is a gene variant that is the major genetic risk factor for developing MS(47). There was no statistically significant difference for CMV between the two groups, just like in the studies described earlier. The last study that used ELISA to assess EBV status was performed by 'Comabella et al'(48). They studied the virus antigen-specific IgG responses to EBV-derived gene products, as well as to HHV6, CMV and measles, in MS patients and their healthy siblings. Seroprevalence for EBV, HHV6, CMV, and measles was similar in both groups, which was to be expected. In the serum of MS patients, they could demonstrate a statistically significant increased IgG response to EBNA-1 ($p = 0.0007$). In this study, however, no difference was found regarding anti-VCA titres.

Three out of the 10 studies that serologically assessed EBV status did so using immunofluorescence. These included two American studies ('Munger et al'(15) and 'Levin et al'(49)) and one Swedish study ('Bistrom et al'(50)). In the case-control study according to 'Levin et al'(49), all MS patients became EBV-positive during follow-up, and only 10 out of 28 initially seronegative control patients seroconverted. Therefore, it was not possible to calculate a relative risk in their study, as it would involve division by 0, making the relative risk infinite. However, the difference in seroconversion between the two groups was statistically significant (p-value of 0.0008). ‘Munger et al’(15) investigated, in addition to the risk modulation of EBV on MS development, potential influences of vitamin D, gender and race. They also wanted to determine if EBNA antibodies could be suitable as a marker for MS disease activity. It was noted that all 7 cases seroconverted before MS onset, as in the study described by ‘Levin et al’(49). For 17 out of 25 control patients, serum remained EBV-negative. Statistical analysis showed a risk increase up to 36,1-fold with high EBNA IgG titres, compared to lower titres. Positive associations were also found for IgG antibody titres to EBNA-2 and VCA, however weaker. Gender, race, and vitamin D status had little influence on the risk of developing MS.

AUTHORS, YEAR	ORIGIN OF THE STUDY (COUNTRY)	CASES: TOTAL	CASES: GENDER/AGE	CONTROLS: TOTAL	CONTROLS: GENDER/AGE	OTHER VIRUSES	WHAT IS MEASURED?	TECHNIQUE USED	SEROCONVERSION/SEROPOSITIVITY EBV	OUTCOME
Comabella et al, 2009	Spain	25	36% M Mean age 36.2y	49	47.8% M Mean age 36.3y	HHV6, CMV, measles	EBVCA IgG antibodies and EBNA IgG antibodies	ELISA	NS	P = 0.0007
Gustavsen et al, 2014	Norway	756	26.2% M Mean age 50.5y	1090	41.7% M Mean age 44y	No, but other exposures included	Subjective recognition to exposures	Verified questionnaire	N/A	IM: p = 0.016
Langer-Gould et al, 2017	USA	519	28.7% M Age NS (3 groups)	571	29.8% M Age NS (3 groups)	CMV	EBVCA IgG antibodies and EBNA IgG antibodies	ELISA	NS	EBNA1: P < 0.001
Laribi et al, 2018	Iran	68	41.1% M Mean age 35.3y	20	40% M Mean age 30y	No	EBVCA IgG antibodies and EBNA IgG antibodies	ELISA	All MS-pts: minimally 1 of 2 EBV-antibodies. Higher grade seropositivity and titres in MS-pts	EBNA1: p = 0.04 VCA: p = 0.04
Mameli et al, 2013	Italy	125	29.6% M Mean age 41y	140	32.8% M Mean age 44y	No	EBNA-1 IgG antibodies	ELISA	MS: 114/125 EBNA-1 IgG+ (91%) Controls: 96/140 EBNA-1 IgG + (69%)	P < 0.0001 RR = 4.6 with CI 95%: 1.5-14
Munger et al, 2011	USA	222	66% M, M:F ratio 2 Mean age 23.4y	444	66% M, M:F ratio 2 Mean age 23.4y	No	EBVCA IgG antibodies and EBNA IgG antibodies	Immunofluorescence	MS: 100% seroconversion (7/7) Controls : 32% seroconversion (8/25)	RR 36.1 (95% CI: 9.6-136) with high anti-EBNAc titres RR 7.7 (95% CI: 2.6-23) with high anti-EBNA1 titres
Nourbaksh et al, 2018	USA	356	35.7% M Mean age 15.2y	493	48.1% M Mean age 14.2y	CMV HSV1/2	EBVCA IgG antibodies	ELISA	EBVCA+: 91% cases, 58.2% controls HSV1+: 26.4% cases, 20% controls HSV2+: 18.7% cases, 12.6% controls CMV+: 38% cases, 32% controls	EBVCA: p < 0.001 HSV1: p = 0.025

Table 4: Baseline characteristics of the included case control studies of Epstein-Barr virus and multiple sclerosis

Abbreviations: MS: multiple sclerosis; IM: infectious mononucleosis; EBV: Epstein-Barr virus; CMV cytomegalovirus; HSV: herpes simplex virus; HHV: human herpesvirus; ; EBNA: Epstein-Barr virus-(associated) nuclear antigen; (EB)VCA: (Epstein-Barr) viral capsid antigen; ELISA: enzyme-linked immunosorbent assay; NS: not specified; N/A: not applicable; M: male; F: female; y: years; RR: relative risk; CI: confidence interval

AUTHORS, YEAR	ORIGIN OF THE STUDY (COUNTRY)	COHORT	GENDER/AGE	CASES	CONTROLS	OTHER VIRUSES	WHAT IS MEASURED?	TECHNIQUE USED	SEROCONVERSION/SERO-POSITIVITY EBV	OUTCOME
Abrahamyan et al, 2020	Germany	2 separate cohorts	30.1% M Mean age 33y (cases) 52% M	901	16.163	No	EBVCA IgG antibodies and EBNA IgG antibodies	CLIA	EBNA-1+: 93.1% of cases Total seropositivity EBV: 100% of cases	P < 0.0001
Bistrom et al, 2021	Sweden	NS	19% M Mean age 25y	670	670	HHV6	EBVCA IgG antibodies and EBNA IgG antibodies	Immunofluorescence	MS: EBNA-1 613/670; VCA 598/670 Controls: EBNA-1 600/670; VCA 575/670	OR 3.5, 95% CI 1.7-7.1
Bjornevik et al, 2022	USA	Total > 10.000.000 Study population 955 cases – 1843 controls	83.1% M, 4.91 M:F ratio 45.7% <26y, 21.2% 26-30y 67.3% M, 2.05 M:F ratio 78.1% >26y, 13.1% 26-30y	955 of which 801 with serum samples available for EBV-serology analysis	1566 with serum samples available for EBV-serology analysis	CMV	EBVCA IgG antibodies and EBNA IgG antibodies sNfL-levels	ELISA	35 cases and 107 controls EBV- at start 33/35 cases vs 90/107 controls EBV+ in 3 samples 2nd sample: 28/33 vs 40/90 3rd sample 32/33 vs 51/90 (P < 0.0001)	EBV- seropositivity at 1st sample = 26-fold risk (P < 0.01) EBV-seroconversion at 3rd sample = 32-fold risk (P < 0.001)
Goldacre et al, 2004	UK	2.767 patients with IM; 640.163 patients in reference cohort	Gender NS; Mean age 18y	6 patients with MS after IM	N/A	No	NS	NS	N/A	Significant difference 10y after IM: RR 4.01 with 95% CI 1.48-8.93
Levin et al, 2010	USA	Approx. 8.000.000	66% M Mean age 23.5y	305	610	CMV	EBVCA IgG antibodies and EBNA IgG antibodies CMV IgG	Immunofluorescence ELISA	MS: 10/10 EBV+ (100%) Controls: 10/28 EBV+ (35.71%)	RR incalculable, due to no EBV- MS patients

Loosen et al, 2022	Germany	32.116 patients	41.4% M Mean age 32y	16.058 with IM	16.058 without IM	No	Diagnoses in outpatients	Diagnosis by ICD-10 coding B27	N/A	HR 1.86 with 95% CI 1.09-3.16 (p = 0.021) Group IM 14-20y: HR 3.52, 95% CI 1.00-12.37 (p = 0.033)
Nielsen et al, 2007	Denmark	25,234 patients with IM	NS	104 cases in the group with positive Paul Bunnell	68 cases in the group with negative Paul Bunnell	No	IgM heterophilic antibodies	PB	N/A	SIR MS 2.27 with 95% CI: 1.87-2.75 with pos. PB SIR MS 1.23 with 95% CI: 0.97-1.56 with neg. PB
Xu et al, 2021	Sweden	2.492.980 patients	52.68% M Age NS	5.867 MS	N/A	No	IM diagnosis before MS onset	Diagnosis by coding ICD-9 075 and ICD-10 B27	N/A	IM as child: HR 1.98 with 95% CI 1.21-3.23 IM as adolescent: HR 3.00 with 95% CI 2.48-3.63

Table 5: Baseline characteristics of the included cohort studies of Epstein-Barr virus and multiple sclerosis

Abbreviations: MS: multiple sclerosis; IM: infectious mononucleosis; EBV: Epstein-Barr virus; CMV cytomegalovirus; ; HHV: human herpesvirus; ; EBNA: Epstein-Barr virus-(associated) nuclear antigen; (EB)VCA: (Epstein-Barr) viral capsid antigen; sNfl: serum neurofilament light chain; ELISA: enzyme-linked immunosorbent assay; CLIA: chemiluminescent immunoassay; NS: not specified; N/A: not applicable; M: male; F: female; y: years; approx.: approximately; RR: relative risk; OR: odds ratio; HR; hazard ratio; SIR: standardized incidence ratio; CI: confidence interval; pos.: positive (+); neg.: negative (-); PB: Paul Bunnell

‘Bistrom et al’(50) also looked at the effect of age on the risk of MS due to EBV infection in their study. As previously described, primary EBV infection has been shown to be a risk factor for developing MS, especially infection at older ages. In this study, they also investigated whether HHV6A interacts with EBV and changes the risk of MS. The study population was divided into three age categories (<20 years, 20-29 years, 30-39 years), depending on the age at which the serum sample was taken (before symptom onset). It was found that the risk of developing MS increased especially with increasing age regarding seropositivity for EBV. Seropositivity for HHV6A showed an age-independent increase in MS risk across all three groups.

One study used CLIA (Chemiluminescence Immunoassay) for their serological analysis. 'Abrahamyan et al'(51) investigated the prevalence of EBV antibodies in MS patients in a recent study in a German cohort. All 901 MS patients ultimately tested positive for EBV. A control cohort consisting of patients who had been hospitalised or attended outpatient clinics was used for comparison. Stratification by age group revealed a significant difference in EBV seropositivity in the age group 20-40 years. The difference was more significant in the 20-24 age group and decreased with increasing age.

The remaining five studies assessed EBV status in ways other than analysing IgG antibodies. 'Xu et al'(35) and 'Loosen et al'(36) based on registered diagnoses in their databases, according to ICD coding (Xu: ICD-9 075 and ICD-10 B27, Loosen: ICD-10 B27). In their study, performed in Sweden, 'Xu et al'(35) also considered potential confounding of maternal/paternal MS and birth order (1st, 2nd, ... child) as well as maternal age at birth. The statistical analysis divided the group with IM diagnosis before the age of 25 into three groups (birth to 10 years, 11 to 19 years, 20 to 24 years), done to distinguish between IM in childhood, adolescence, and young adulthood. They found an association between IM and the development of MS in the childhood and adolescent age groups. An effect was also seen for infection with IM at young adulthood, but this effect was no longer statistically significant after stratified Cox regression to control for the above-mentioned confounding factors. The same regression for younger ages changed little, these differences remained statistically significant. The other aforementioned, also large European study, that made use of ICD coded diagnoses was conducted by ‘Loosen et al’(36), covering a follow-up period of ten years in 16,058 patients with IM. They used the IQVIA database(52), which also registers diagnoses according to ICD-10 coding, among other useful medical data of outpatients in Germany. Here too, they clearly saw how important the age at which a primary EBV infection occurs is for potentially developing MS. The greatest increase in risk was seen in the 14-20 age group, and with increasing age, the difference in risk became less significant. Regarding age, these findings are consistent with those of ‘Abrahamyan et al’(51) and ‘Xu et al’(35), which were described earlier. However, ‘Biström et al’(50) concluded that EBV seropositivity from the age of 20 onwards poses a higher risk of developing MS. In a slightly older cohort study by ‘Nielsen et al’(38), it was studied whether a priori IM is a risk for developing MS, also with calculations of standardised incidence ratios (SIRs). This is the only included study that calculated SIRs. Their study also considered gender, age, severity of IM, and the time interval between IM and MS. To establish their cohort of IM patients, they used the Paul-Bunnell test. The control group consisted of patients with a negative test around the same time. There was a significant difference in the development of MS between the two groups, the IM group had a statistically significantly higher risk. Compared to the general population, the control group did not have a different risk of developing MS. They assessed this by calculating SIRs, as described above. These were also calculated for the IM group, which was

statistically significantly different. This difference was significant for both men and women. The SIR for MS in people with a positive Paul-Bunnell test after the age of 30 was not significant (0.91 [0.23-3.65]). In the penultimate study, ‘Gustavsen et al’(14) used questionnaires to assess exposure to certain factors and the development of MS. The questionnaire included elements about smoking, environmental factors, demographics, and comorbidities. To verify the quality of the questionnaire, a test-retest was performed with an interval of 4 weeks and this for about 50 control patients. After processing and statistically analysing the data from the questionnaires, a significant difference was observed regarding smoking and history of infectious mononucleosis. It was also noted that exposure to cats in childhood could be protective against MS ($p = 0.001$). This could be supported by the fact that the risk of autoimmune diseases increases with higher hygiene standards. However, having a dog as a pet could not show a significant difference ($p = 0.06$). The last study we discussed that did not work with serological data, is the one of ‘Goldacre et al’(37). They conducted a study to see if IM is a risk factor for developing MS, with a closer look on the timeframe, referring to the time between exposure and the first disease manifestation of MS. Patients with a hospital admission for IM in an NHS hospital in the Oxford region were included in their IM patient group. This is not further specified, except that this data comes from the 'Oxford record linkage study'(53). Across all time intervals, the effect of IM on developing MS was not significant (95% confidence interval is [0.79-4.77]). However, all cases of MS in patients with IM were diagnosed only after an interval of more than ten years. The relative risk of developing MS after IM in this interval group was significant. Because the effect of EBV only becomes noticeable after a long period of time regarding the onset of MS, it could be stated that EBV infection is more of an initiator of development than an activator of latent pathology.

Discussion

Summary of main findings

The exact aetiology of RA and MS remains unclear to date. According to our research, the link between EBV and RA remains indistinct. Regarding MS, there is more correlation between EBV and specifically infectious mononucleosis. Only the mechanisms and the way in which EBV plays a role remain unclear. However, it is certain that a combination of genetic components and environmental factors underlie the disease.

Genetic factors certainly play a role in RA pathology. Familial clustering of RA has been described, with a concordance rate for RA in monozygotic twin pairs ranging from 15 to 30%. In other words, genetic factors cannot fully explain the occurrence of RA(54). An important role in RA pathology is undoubtedly attributed to cross-reactivity between viral and human proteins, specifically the 'molecular mimicry hypothesis'. This would contribute to the autoimmune aspect of RA. One type involves glycine-alanine repeat sequences found in EBNA-1, which are also expressed in cytoskeleton proteins such as type 2 collagen and cytokeratin(55). A second type of molecular mimicry was observed between the QKRAA amino acid sequence of the beta chain of human leukocyte antigen (HLA-DRB1*0401) and the EBV glycoprotein 110 (gp110). EBV gp110 is an antigen produced during the lytic phase of EBV replication. Antibodies against EBV gp110 are produced during a normal EBV infection. So, both T and B cells cross-react with the QKRAA sequence in both HLA-DRB1*0401 and gp110. As a result, EBV infection triggers an immune response against HLA-DR molecules with this epitope(56). Two studies previously discussed ('Masuoka et al'(24), 'Saal et al'(25)) demonstrated that HLA-DRB1-SE positivity was an independent risk factor for developing RA. 'Saal et al'(25) concluded that patients with synovial EBV DNA and positive genotyping for the shared epitope had a significantly higher risk of developing RA than patients who scored negative for one or both of these characteristics. In MS as well, the phenomenon of molecular mimicry appears to play a role in pathogenesis. For example, EBNA-1 IgG bears many similarities to glialCAM, the glial cell adhesion molecule(57). The immune system may therefore interpret white matter tissue as foreign and initiate an immune response. The HLA-DRB*1501 allele with its protein form the basis of the genetic component that contributes to MS pathogenesis. Proteins transcribed from HLA genes form the MHC (major histocompatibility complex)(47), these play a major role in immunity. They bind antigens and present them to receptors on T cells, specifically to CD4+ T cells. The HLA-DRB*1501 protein is known to bind well with, for example, myelin basic protein (MBP), presenting them to the aforementioned T cells and thus eliciting an inflammatory reaction with demyelination(58).

There are other environmental factors associated with MS, including smoking and vitamin D status. An interesting note within environmental factors consists of the 'hygiene hypothesis'. It is stated that a late exposure to antigens can modulate the immune system and lead to autoimmune diseases. This was also proven by 'Gustavsen et al'(14) for example, through analysis about exposure to cats. Another equally important factor in RA pathology is cell-mediated immune response. Several findings indicate weak control of EBV infection in RA patients. It has been shown that the percentage of cultured B cells transformed into lymphoblastoid cell lines is higher in RA patients than in healthy controls(59). This can be explained by the less efficient ability of RA patients' T cells to control EBV-induced overgrowth(59). The reduced T-cell function is linked more to disease activity than genetic

factors, as it is not present in the early stages of the disease(60). The first pathophysiological link between RA and EBV infection was suggested in 1976 by 'Alspaugh et al'(61). Antibodies against an antigen in the nucleus of EBV-transformed B cells were measured. These antibodies were found to be elevated in RA patients, and this antigen was called RANA (rheumatoid arthritis nuclear antigen). Later, it was found that RANA was the same molecule as EBNA-1(62). Therefore, only older studies, such as those by 'Ferrell et al'(30) and 'Thomas et al'(32), still refer to RANA.

Several techniques have been used to detect EBV in synovial fluid, synovial tissue or serum of RA patients, including ELISA, in situ hybridization, and PCR (see Table 3), each with its specific properties. EBV infection is typically identified by antibodies against EBV antigens, namely: EBNA-1, EA-D, EA-R, and VCA. PCR studies (see Table 3) have shown that RA patients have significantly more EBV DNA in their synovial fluid, synovial tissue and serum than control patients. To specifically identify which cells in the synovial tissue EBV DNA was amplified from, in situ hybridization is used. In situ hybridization detects the presence of EBV-encoded small RNA (EBER) in synovial cells. 'Takei et al'(28) found that the occurrence of EBER was limited to the proliferative sites of the apex of the synovial villi using this method. However, another study ('Niedobitek et al'(27)) with a similar setup failed to confirm this. It was explained that the difference in outcome likely had to do with a difference in the included population (Western vs. Japanese), although this cannot be the complete explanation. The results of 'Takei et al'(28) were, however, confirmed by two other studies, 'Takeda et al'(26) and 'Blaschke et al'(63). Several included studies detected a trend where RA patients had higher percentages of antibodies against EBV than the control group. Often, percentages of EBNA-1 positive cases in the RA group and the control group were similar due to the high infection rate of EBV in the world population. However, significant differences were more often found in antibody determination for VCA and EAD. Based on these cross-sectional findings, it is impossible to speak of a causal relationship. Two longitudinal preclinical RA cohorts attempted to further clarify a possible causal relationship. However, here too, no consistent result was found. 'Fechtner et al'(21) detected a significant difference between antibody titres both pre- and post-RA, between the RA group and the control group. On the contrary, 'Goldstein et al'(22) did not find a significant result. A possible explanation for this difference could be found in the population of the cohorts. 'Goldstein et al'(22) studied a cohort of nurses, predominantly women, while 'Fechtner et al'(21) studied a cohort of military personnel, almost exclusively men. The included studies generally had small sample sizes, the cut-off levels for viral seropositivity were often different, there was a lack of reporting on patient recruitment, heterogeneity in terms of research material (synovial fluid, tissue, blood, serum), and techniques used, leading to heterogeneous outcomes.

One aspect that has not been extensively addressed in previous reviews/studies was research into the risk of MS in other ethnic groups. 'Langer-Gould et al'(45) for example identified this gap in evidence and included analysis across ethnic groups. From the studies we selected, it appeared that the risk of MS due to EBV is also evident in other ethnic groups, in almost the same order of magnitude. This was also noticed in the large cohort study by 'Bjornevik et al'(41). Another trend observed is that in the study groups of most studies, cases tend to have a higher proportion of women than men, although there is no greater risk of MS due to EBV among women than men overall(15, 51). A common notice is also the fact that there seems to be an interval between possible primo-infection with EBV and disease onset of MS, as seen by 'Goldacre et al'(37) and the other included longitudinal studies(41, 49). It is also noticeable that the timing of seroconversion seems to be of great importance, raising

questions about the specific role of EBV and its mechanism in the development of MS. In some studies, it was sometimes difficult for us to differentiate between EBV seropositivity and IM. Not all patients with EBV seroconversion will develop IM, although the likelihood of this increases with age at seroconversion. It is precisely in that group that the risk of developing MS is highest in the selected studies, especially in those of 'Xu et al'(35) and Biström et al'(50). With these considerations in mind, the question then arises whether only EBV seroconversion is responsible for the increased risk of MS or a past history of IM. EBV seropositivity is very high in the general population, while MS is a rare condition. In some studies, there were also EBV-negative MS patients. Studies also mention being mindful of "reverse causation". This means that in the preclinical phase of MS, there may be immune dysregulation, making EBV infection more likely. However, there are enough studies that do not observe a difference in the frequency of infections in the years before MS onset or during the course of untreated MS. The time interval between IM and MS also seems to argue against such reverse causation(41). For RA too, reverse causation appears plausible, but since we have limited longitudinal data, this assumption is difficult to refute.

Strengths and limitations

A notable strength of our search lies in its comprehensive scope of viruses and autoimmune diseases from the outset. We promptly identified the viruses and autoimmune diseases that had been extensively researched and had ample evidence available. This initial approach also highlighted certain gaps in current literature and research. However, it's important to acknowledge a potential limitation: the risk of selection bias among researchers, possibly favouring topics with the highest number and possible positive correlation. Since the association between EBV and autoimmune disorders, RA and MS in particular, seemed to be more prominent in the search, it was decided in mutual consultation with all parties to continue with these two topics. While studies on RA displayed some degree of heterogeneity, it was less prominent in those concerning MS, likely due to the clearer association between EBV and MS. Given that the studies we selected showed significant heterogeneity, it was not possible to perform a representative meta-analysis.

Implications for routine care

Potential notes for the future that could play a role in the treatment of RA include immunotherapy(64) or pharmalogical treatment that could prevent/reduce the (re)activation of EBV in RA to lower the EBV load. It was observed that DMARDs, disease-modifying antirheumatic drugs, (such as methotrexate) could reduce the EBV load in RA patients. TNF-alpha antagonists, on the other hand, increased the EBV load(65). From a primary care perspective, it is also interesting to consider preventive measures, such as potential vaccination against EBV(66), and early diagnosis using specific markers. Currently, there is a vaccine in trial for EBV, which seems to lower the incidence of IM but not the number of EBV infections(67, 68). Since the mechanism of MS development by EBV is not fully understood, a vaccine cannot be developed to directly play a role. However, if it can indeed reduce the incidence of IM, which does increase the risk of developing MS, it could indirectly reduce the risk of MS. One of the most effective current treatments for MS is anti-CD20 monoclonal antibodies(69). These lead to B-cell depletion, the primary site of latent EBV infection. The fact that this treatment is effective also argues for a role of EBV, not only in development but also in the course of the disease. Therefore, it seems plausible that antiviral therapy could potentially play a role. Also, the fact that humans are the only natural host of EBV makes representative animal experiments difficult. In the

future, this problem could be partially overcome through the use of humanized mice(70), allowing for more representative animal experiments to be conducted. Disturbed EBV serology is not limited solely to RA and MS but is also present in many other autoimmune pathologies (SLE, Sjögren's syndrome) as well as nasopharyngeal carcinoma and Burkitt's lymphomas. Analogous to nasopharyngeal carcinoma, certain antibodies could serve as screening tools in RA(71). In various Asian countries where nasopharyngeal carcinoma is endemic, IgA anti-VCA is used as a screening tool because these titers often exceed ten times the titers in normal individuals years before disease onset(72).

Implications for future research

To further establish causality of EBV in MS, functional studies are needed to clarify disease mechanisms, especially considering the presence of EBV-negative MS patients in some studies. Additionally, larger prospective studies for RA are necessary to provide a clear understanding of the direction and timing of the EBV-RA relationship in order to demonstrate causality. As mentioned above, in the future, it is plausible that appropriate vaccines will be developed. Research will then be needed to determine whether these vaccines also play a beneficial role in preventing the development of autoimmune diseases or other comorbidities linked to the viruses they target.

Conclusion

There are certainly many arguments supporting a link between RA and EBV, but a direct causal relationship is still lacking. For MS, it can be surely assumed that there is an association between EBV seropositivity and the development of the disease, across the board. The relationship between EBV and RA is complex, and besides genetic predisposition, molecular mimicry and T-cell dysfunction also play a role in pathology. On the other hand, it could also be argued that an innate or acquired immunodeficiency in patients with RA causes these patients to have difficulty suppressing a chronic viral disease like EBV. In MS, the phenomenon of reverse causation is refuted by the demonstrated time interval between primary infection and onset of MS symptoms.

In conclusion, a combination of genetic and environmental factors underlies both pathologies. "Genetics load the gun, lifestyle/environment pull the trigger"(73).

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Appendix

I. Search strategy

Pubmed

Concept 1: Alphaherpesvirinae (simplexvirus, herpesvirus 1, herpesvirus 2, varicellovirus, varicella zoster virus)
"Alphaherpesvirinae"[Mesh] OR "Alphaherpesvirinae"[tiab] OR "Simplexvirus"[Mesh] OR "Simplexvirus*"[tiab] OR "Herpesvirus Homini*"[tiab] OR "Herpes Simplex Virus*"[tiab] OR "Herpes Labialis Virus*"[tiab] OR "Herpes-T Virus*"[tiab] OR "Herpes T Virus*"[tiab] OR "Herpesvirus 1, Human"[Mesh] OR "Herpesvirus 1, Human"[tiab] OR "Human Herpesvirus 1"[tiab] OR "HHV-1"[tiab] OR "Herpes Simplex Virus Type 1"[tiab] OR "Herpes Simplex Virus 1"[tiab] OR "HSV-1"[tiab] OR "Herpesvirus 2, Human"[Mesh] OR "Herpesvirus 2, Human"[tiab] OR "Herpes Simplex Virus Type 2"[tiab] OR "Herpes Simplex Virus 2"[tiab] OR "Human Herpesvirus 2"[tiab] OR "HHV-2"[tiab] OR "HSV-2"[tiab] OR "Varicellovirus"[Mesh] OR "Varicellovirus*"[tiab] OR "Herpesvirus 3, Human"[Mesh] OR "Herpesvirus 3, Human"[tiab] OR "Human Herpesvirus 3"[tiab] OR "Chickenpox Virus*"[tiab] OR "HHV-3"[tiab] OR "Ocular Herpes zoster Virus"[tiab] OR "Varicella-Zoster Virus*"[tiab] OR "Varicella Zoster Virus"[tiab] OR "VZ Virus*"[tiab] OR "Herpesvirus Varicellae"[tiab] OR "Herpes zoster Virus*"[tiab] OR "alpha herpes virus"[tiab] OR "herpes hominis"[tiab] OR "herpes hominis virus"[tiab] OR "herpes virus hominis"[tiab] OR "herpesvirus hominis"[tiab] OR "hsv"[tiab] OR "Herpes zoster Virus"[tiab] OR "human herpes virus"[tiab] OR "herpes simplex virus i"[tiab] OR "herpesvirus 1"[tiab] OR "herpes virus 1"[tiab] OR "herpes virus type 1"[tiab] OR "hsv 1"[tiab] OR "Human herpes virus 1"[tiab] OR "herpes simplex type 2 virus"[tiab] OR "Herpes zoster Virus"[tiab] OR "herpes simplex virus ii"[tiab] OR "herpes simplex virus type 2"[tiab] OR "herpes virus 2"[tiab] OR "herpesvirus 2"[tiab] OR "herpes virus hominis type 2"[tiab] OR "herpes virus type 2"[tiab] OR "hsv 2"[tiab] OR "human herpes virus 2"[tiab] OR "varicella herpes zoster virus"[tiab] OR "varicella virus"[tiab] OR "varicella zoster"[tiab] OR "varicella zoster herpetovirus"[tiab] OR "varicella zona virus"[tiab] OR "varicella herpetovirus"[tiab] OR "varicella foster virus"[tiab] OR "vzv"[tiab] OR "zona virus"[tiab] OR "herpes virus varicellae"[tiab] OR "herpes zoster varicella virus"[tiab] OR "chicken pox virus"[tiab] OR "shingles virus"[tiab] OR "herpes simplex"[tiab] OR "varicella"[tiab] OR "varicello"[tiab]

Concept 2: Cytomegalovirus (Betaherpesvirinae)
"Cytomegalovirus"[Mesh] OR "Cytomegalovirus*"[tiab] OR "Salivary Gland Virus*"[tiab] OR "Human Herpesvirus 5"[tiab] OR "HHV 5"[tiab] OR "cytomegalovirus infection*"[tiab] OR "CMV disease"[tiab] OR "CMV infection*"[tiab] OR "CMV syndrome"[tiab] OR "Cytomegalovirus disease"[tiab] OR "congenital cytomegalic inclusion body disease"[tiab] OR "cytomegaloviral infection"[tiab] OR "cmv"[tiab]

Concept 3: Epstein-Barr virus (Gammaherpesvirinae)
"Epstein-Barr Virus Infections"[Mesh] OR "Epstein-Barr Virus Infection*"[tiab] OR "Epstein Barr Virus Infection*"[tiab] OR "EBV Infection*"[tiab] OR "Infectious Mononucleosis"[tiab] OR "Human Herpesvirus 4 Infection*"[tiab] OR "Human Herpes Virus 4 Infection*"[tiab] OR "EB virus"[tiab] OR "human herpes virus 4"[tiab] OR "epstein barr herpetovirus"[tiab] OR "Infectious mononucleosis

herpetovirus"[tiab] OR "infectious mononucleosis virus"[tiab] OR "mononucleosis infectiosa virus"[tiab] OR "Epstein-Barr"[tiab] OR "EBV"[tiab]

Concept 4: Autoimmune diseases: rheumatoid arthritis, diabetes mellitus (type 1), multiple sclerosis and lupus

"Arthritis, Rheumatoid"[Mesh] OR "Rheumatoid Arthritis"[tiab] OR "Diabetes Mellitus, Type 1"[Mesh] OR "Diabetes Mellitus Type 1"[tiab] OR "Type 1 Diabetes Mellitus"[tiab] OR "Type 1 Diabetes"[tiab] OR "Insulin-Dependent Diabetes Mellitus"[tiab] OR "Insulin Dependent Diabetes Mellitus 1"[tiab] OR "Insulin-Dependent Diabetes Mellitus 1"[tiab] OR "Juvenile-Onset Diabetes Mellitus"[tiab] OR "IDDM"[tiab] OR "Juvenile Onset Diabetes"[tiab] OR "Sudden-Onset Diabetes Mellitus"[tiab] OR "Autoimmune Diabetes"[tiab] OR "Brittle Diabetes Mellitus"[tiab] OR "Ketosis-Prone Diabetes Mellitus"[tiab] OR "Juvenile-Onset Diabetes"[tiab] OR "Multiple Sclerosis"[Mesh] OR "Multiple Sclerosis"[tiab] OR "Disseminated Sclerosis"[tiab] OR "Lupus Erythematosus, Systemic"[Mesh] OR "Lupus Erythematosus, Systemic"[tiab] OR "Systemic Lupus Erythemato*"[tiab] OR "lupus erythematosus disseminatus"[tiab] OR "arthritis deformans"[tiab] OR "arthrosis deformans"[tiab] OR "chronic articular rheumatism"[tiab] OR "chronic polyarthritis"[tiab] OR "rheumatic arthritis"[tiab] OR "rheumatic polyarthritis"[tiab] OR "sclerosis multiplex"[tiab] OR "disseminated lupus"[tiab] OR "disseminated lupus erythematodes"[tiab] OR "disseminated lupus erythematosis"[tiab] OR "disseminated lupus erythematosus"[tiab] OR "erythematodes visceralis"[tiab] OR "lupovisceritis"[tiab] OR "lupus erythematodes disseminatus"[tiab] OR "lupus erythematosus visceralis"[tiab] OR "sle"[tiab] OR "s.l.e"[tiab] OR "insulin dependent diabetes mellitus"[tiab] OR "diabetes type 1"[tiab] OR "diabetes mellitus type i"[tiab] OR "diabetes type I"[tiab] OR "dm 1"[tiab] OR "early onset diabetes mellitus"[tiab] OR "insulin dependent diabetes"[tiab] OR "juvenile diabetes"[tiab] OR "juvenile diabetes mellitus"[tiab] OR "juvenile onset diabetes mellitus"[tiab] OR "ketoacidotic diabetes"[tiab] OR "labile diabetes mellitus"[tiab] OR "T1DM"[tiab] OR "type I diabetes"[tiab] OR "type I diabetes mellitus"[tiab] OR "brittle diabetes"[tiab]

Embase

Concept 1: Alphaherpesvirinae: simplexvirus, herpesvirus 1, herpesvirus 2, varicellovirus, varicella zoster virus

'Alphaherpesvirinae'/exp OR 'Alphaherpesvirinae':ti,ab,kw OR 'alpha herpes virus':ti,ab,kw OR 'Simplexvirus'/exp OR 'Simplexvirus':ti,ab,kw OR 'Herpes simplex virus'/exp OR 'Herpes simplex virus*':ti,ab,kw OR 'herpes hominis':ti,ab,kw OR 'herpes hominis virus':ti,ab,kw OR 'herpes virus hominis':ti,ab,kw OR 'herpesvirus hominis':ti,ab,kw OR 'hsv':ti,ab,kw OR 'human herpes virus':ti,ab,kw OR 'Human alphaherpesvirus 1'/exp OR 'Human alphaherpesvirus 1':ti,ab,kw OR 'Herpes simplex virus 1':ti,ab,kw OR 'herpes simplex virus i':ti,ab,kw OR 'herpes simplex virus type 1':ti,ab,kw OR 'herpes virus 1':ti,ab,kw OR 'herpesvirus 1':ti,ab,kw OR 'herpes virus type 1':ti,ab,kw OR 'hsv 1':ti,ab,kw OR 'Human herpes virus 1':ti,ab,kw OR 'human herpesvirus 1':ti,ab,kw OR 'Herpes simplex virus 2'/exp OR 'Herpes simplex virus 2':ti,ab,kw OR 'herpes simplex type 2 virus':ti,ab,kw OR 'herpes simplex virus ii':ti,ab,kw OR 'herpes simplex virus type 2':ti,ab,kw OR 'herpes virus 2':ti,ab,kw OR 'herpesvirus 2':ti,ab,kw OR 'herpes virus hominis type 2':ti,ab,kw OR 'herpes virus type 2':ti,ab,kw OR 'hsv 2':ti,ab,kw OR 'human herpes virus 2':ti,ab,kw OR 'human herpesvirus 2':ti,ab,kw OR 'Varicellovirus'/exp OR 'Varicellovirus*':ti,ab,kw OR 'Varicella zoster virus'/exp OR 'Varicella zoster virus':ti,ab,kw OR 'varicella herpes zoster virus':ti,ab,kw OR 'Varicella virus':ti,ab,kw OR 'Varicella

zoster':ti,ab,kw OR 'varicella zoster herpetovirus':ti,ab,kw OR 'varicella zona virus':ti,ab,kw OR 'varicella-zoster virus*':ti,ab,kw OR 'Varicella herpetovirus':ti,ab,kw OR 'varicella foster virus':ti,ab,kw OR 'vzv':ti,ab,kw OR 'zona virus':ti,ab,kw OR 'herpes virus varicellae':ti,ab,kw OR 'herpes zoster varicella virus':ti,ab,kw OR 'Herpes zoster virus*':ti,ab,kw OR 'human herpesvirus 3':ti,ab,kw OR 'shingles virus':ti,ab,kw OR 'chicken pox virus':ti,ab,kw OR 'chickenpox virus*':ti,ab,kw OR 'Herpes Labialis Virus*':ti,ab,kw OR 'Herpes-T Virus*':ti,ab,kw OR 'Herpes T Virus*':ti,ab,kw OR 'HHV-1':ti,ab,kw OR 'HSV-1':ti,ab,kw OR 'HHV-2':ti,ab,kw OR 'HSV-2':ti,ab,kw OR 'HHV-3':ti,ab,kw OR 'Ocular Herpes zoster Virus':ti,ab,kw OR 'VZ Virus*':ti,ab,kw OR 'Herpesvirus Varicellae':ti,ab,kw OR 'herpes simplex':ti,ab,kw OR 'varicella':ti,ab,kw OR 'varicello':ti,ab,kw

Concept 2: Cytomegalovirus (Betaherpesvirinae)
'cytomegalovirus infection'/exp OR 'cytomegalovirus infection*':ti,ab,kw OR 'cmv disease':ti,ab,kw OR 'cmv infection*':ti,ab,kw OR 'cmv syndrome':ti,ab,kw OR 'cytomegalovirus disease':ti,ab,kw OR 'congenital cytomegalic inclusion body disease':ti,ab,kw OR 'cytomegaloviral infection':ti,ab,kw OR 'cytomegalovirus*':ti,ab,kw OR 'salivary gland virus*':ti,ab,kw OR 'human herpesvirus 5':ti,ab,kw OR 'hhv 5':ti,ab,kw OR 'cmv':ti,ab,kw

Concept 3: Epstein-Barr virus (Gammaherpesvirinae)
'Epstein Barr virus'/exp OR 'Epstein Barr virus':ti,ab,kw OR ‘EB virus’:ti,ab,kw OR ‘ebv’:ti,ab,kw OR ‘epstein-barr virus’:ti,ab,kw OR ‘human herpes virus 4’:ti,ab,kw OR ‘human herpesvirus 4’:ti,ab,kw OR ‘epstein barr herpetovirus’:ti,ab,kw OR ‘Infectious mononucleosis herpetovirus’:ti,ab,kw OR ‘infectious mononucleosis virus’:ti,ab,kw OR ‘mononucleosis infectiosa virus’:ti,ab,kw OR ‘Epstein-Barr Virus Infection*’:ti,ab,kw OR ‘Epstein-Barr Virus Infection*’:ti,ab,kw OR ‘Infectious Mononucleosis’:ti,ab,kw OR ‘Epstein-Barr’:ti,ab,kw OR ‘EBV’:ti,ab,kw

Concept 4: Autoimmune diseases: rheumatoid arthritis, diabetes mellitus (type 1), multiple sclerosis and lupus
'rheumatoid arthritis'/exp OR 'rheumatoid arthritis':ti,ab,kw OR 'arthritis deformans':ti,ab,kw OR 'arthrosis deformans':ti,ab,kw OR 'chronic articular rheumatism':ti,ab,kw OR 'chronic polyarthritis':ti,ab,kw OR 'rheumatic arthritis':ti,ab,kw OR 'rheumatic polyarthritis':ti,ab,kw OR 'insulin dependent diabetes mellitus'/exp OR 'insulin dependent diabetes mellitus':ti,ab,kw OR 'diabetes mellitus type 1':ti,ab,kw OR 'diabetes type 1':ti,ab,kw OR 'diabetes mellitus type i':ti,ab,kw OR 'diabetes type I':ti,ab,kw OR 'dm 1':ti,ab,kw OR 'early onset diabetes mellitus':ti,ab,kw OR 'iddm':ti,ab,kw OR 'insulin dependent diabetes':ti,ab,kw OR 'insulin-dependent diabetes mellitus':ti,ab,kw OR 'juvenile diabetes':ti,ab,kw OR 'juvenile diabetes mellitus':ti,ab,kw OR 'juvenile onset diabetes':ti,ab,kw OR 'juvenile onset diabetes mellitus':ti,ab,kw OR 'ketoacidotic diabetes':ti,ab,kw OR 'labile diabetes mellitus':ti,ab,kw OR 'T1DM':ti,ab,kw OR 'type 1 diabetes':ti,ab,kw OR 'type 1 diabetes mellitus':ti,ab,kw OR 'type I diabetes':ti,ab,kw OR 'type I diabetes mellitus':ti,ab,kw OR 'brittle diabetes':ti,ab,kw OR 'brittle diabetes mellitus':ti,ab,kw OR 'multiple sclerosis'/exp OR 'multiple sclerosis':ti,ab,kw OR 'disseminated sclerosis':ti,ab,kw OR 'sclerosis multiplex':ti,ab,kw OR 'systemic lupus erythematosus'/exp OR 'systemic lupus erythemato*':ti,ab,kw OR 'disseminated lupus':ti,ab,kw OR 'disseminated lupus erythematodes':ti,ab,kw OR 'disseminated lupus erythematosis':ti,ab,kw OR 'disseminated lupus erythematosus':ti,ab,kw OR 'erythematodes visceralis':ti,ab,kw OR 'lupovisceritis':ti,ab,kw OR 'lupus erythematodes disseminatus':ti,ab,kw OR 'lupus erythematosus disseminatus':ti,ab,kw OR 'lupus

erythematosus visceralis':ti,ab,kw OR 'sle':ti,ab,kw OR 's.l.e.':ti,ab,kw OR 'Insulin Dependent Diabetes Mellitus 1':ti,ab,kw OR 'Insulin-Dependent Diabetes Mellitus 1':ti,ab,kw OR 'Juvenile-Onset Diabetes Mellitus':ti,ab,kw OR 'Sudden-Onset Diabetes Mellitus':ti,ab,kw OR 'Autoimmune Diabetes':ti,ab,kw OR 'Ketosis-Prone Diabetes Mellitus':ti,ab,kw OR 'Juvenile-Onset Diabetes':ti,ab,kw

Web of Science

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☐ Social Sciences Citation Index (SSCI)--1956-presentSocial Sciences Citation Index (SSCI)--1956-present

☐ Arts & Humanities Citation Index (AHCI)--1975-presentArts & Humanities Citation Index (AHCI)--1975-present

☐ Conference Proceedings Citation Index – Science (CPCI-S)--1990-presentConference Proceedings Citation Index – Science (CPCI-S)--1990-present

☐ Conference Proceedings Citation Index – Social Science & Humanities (CPCI-SSH)--1990-presentConference Proceedings Citation Index – Social Science & Humanities (CPCI-SSH)--1990-present

☐ Emerging Sources Citation Index (ESCI)--2018-presentEmerging Sources Citation Index (ESCI)--2018-present

Concept 1: Alphaherpesvirinae: simplexvirus, herpesvirus 1, herpesvirus 2, varicellovirus, varicella zoster virus

TS=(“Alphaherpesvirinae” OR “alpha herpes virus” OR “Simplexvirus” OR “Herpes simplex virus*” OR “herpes hominis” OR “herpes hominis virus” OR “herpes virus hominis” OR “herpesvirus hominis” OR “hsv” OR “human herpes virus” OR “Human alphaherpesvirus 1” OR “Herpes simplex virus 1” OR “herpes simplex virus i” OR “herpes simplex virus type 1” OR “herpes virus 1” OR “herpesvirus 1” OR “herpes virus type 1” OR “hsv 1” OR “Human herpes virus 1” OR “human herpesvirus 1” OR “Herpes simplex virus 2” OR “herpes simplex type 2 virus” OR “herpes simplex virus ii” OR “herpes simplex virus type 2” OR “herpes virus 2” OR “herpesvirus 2” OR “herpes virus hominis type 2” OR “herpes virus type 2” OR “hsv 2” OR “human herpes virus 2” OR “human herpesvirus 2” OR “Varicellovirus*” OR “Varicella zoster virus” OR “varicella herpes zoster virus” OR “Varicella virus” OR “Varicella zoster” OR “varicella zoster herpetovirus” OR “varicella zona virus” OR “varicella-zoster virus*” OR “Varicella herpetovirus” OR “varicella foster virus” OR “vzv” OR “zona virus” OR “herpes virus varicellae” OR “herpes zoster varicella virus” OR “Herpes zoster virus*” OR “human herpesvirus 3” OR “shingles virus” OR “chicken pox virus” OR “chickenpox virus*” OR “Herpes Labialis Virus*” OR “Herpes-T Virus*” OR “Herpes T Virus*” OR “HHV-1” OR “HSV-1” OR “HHV-2” OR “HSV-2” OR “HHV-3” OR “Ocular Herpes zoster Virus” OR “VZ Virus*” OR “Herpesvirus Varicellae” OR “herpes simplex” OR “varicella” OR “varicello”)

Concept 2: Cytomegalovirus (Betaherpesvirinae)
TS=("cytomegalovirus infection*" OR "cmv disease" OR "cmv infection*" OR "cmv syndrome" OR "cytomegalovirus disease" OR "congenital cytomegalic inclusion body disease" OR "cytomegaloviral infection" OR "cytomegalovirus*" OR "salivary gland virus*" OR "human herpesvirus 5" OR "hhv 5" OR "cmv")

Concept 3: Epstein-Barr virus (Gammaherpesvirinae)
TS=("Epstein Barr virus" OR "EB virus" OR "ebv" OR "epstein-barr virus" OR "human herpes virus 4" OR "human herpesvirus 4" OR "epstein barr herpetovirus" OR "Infectious mononucleosis herpetovirus" OR "infectious mononucleosis virus" OR "mononucleosis infectiosa virus" OR "Epstein-Barr Virus Infection*" OR "Epstein-Barr Virus Infection*" OR "Infectious Mononucleosis" OR "Epstein-Barr" OR "EBV")

Concept 4: Autoimmune diseases: rheumatoid arthritis, diabetes mellitus (type 1), multiple sclerosis and lupus
TS=("rheumatoid arthritis" OR "arthritis deformans" OR "arthrosis deformans" OR "chronic articular rheumatism" OR "chronic polyarthritis" OR "rheumatic arthritis" OR "rheumatic polyarthritis" OR "insulin dependent diabetes mellitus" OR "diabetes mellitus type 1" OR "diabetes type 1" OR "diabetes mellitus type i" OR "diabetes type I" OR "dm 1" OR "early onset diabetes mellitus" OR "iddm" OR "insulin dependent diabetes" OR "insulin-dependent diabetes mellitus" OR "juvenile diabetes" OR "juvenile diabetes mellitus" OR "juvenile onset diabetes" OR "juvenile onset diabetes mellitus" OR "ketoacidotic diabetes" OR "labile diabetes mellitus" OR "T1DM" OR "type 1 diabetes" OR "type 1 diabetes mellitus" OR "type I diabetes" OR "type I diabetes mellitus" OR "brittle diabetes" OR "brittle diabetes mellitus" OR "multiple sclerosis" OR "disseminated sclerosis" OR "sclerosis multiplex" OR "systemic lupus erythemato*" OR "disseminated lupus" OR "disseminated lupus erythematodes" OR "disseminated lupus erythematosis" OR "disseminated lupus erythematosus" OR "erythematodes visceralis" OR "lupovisceritis" OR "lupus erythematodes disseminatus" OR "lupus erythematosus disseminatus" OR "lupus erythematosus visceralis" OR "sle" OR "s.l.e." OR "Insulin Dependent Diabetes Mellitus 1" OR "Insulin-Dependent Diabetes Mellitus 1" OR "Juvenile-Onset Diabetes Mellitus" OR "Sudden-Onset Diabetes Mellitus" OR "Autoimmune Diabetes" OR "Ketosis-Prone Diabetes Mellitus" OR "Juvenile-Onset Diabetes")

II. OBC approval

Viral infections and autoimmune disorders

Dossiernr.:	MP023476
Masterproef:	Masterproef I HAIO, 2021-2024
Faculteit/opleiding:	Faculteit Geneeskunde - Master in de huisartsgeneeskunde
Studiefase:	eerste fase
Academiejaar:	2022-2023
Korte omschrijving / abstract:	Recent scientific advances have brought to light the prominent role of viral infections in the development of certain autoimmune disorders. This study will aim to establish an up-to-date literature review of the associations between a range of viral infections and autoimmune disorders.
Toegepaste technieken:	Systematic review
Gewenste taal van communicatie:	nl
Promotor:	Prof Dr Jan Verbakel (u0042064)
Organisatie:	KU Leuven
Dienst:	Academisch Centrum voor Huisartsgeneeskunde
Discipline:	Masterproef
Extra groepsleden:	Arno Dejaegere arno.dejaegere@student.kuleuven.be r0635411

De verstrekte informatie toont aan dat het onderzoek in het kader van de masterproef een zuivere literatuurstudie betreft en dat er op geen enkele wijze gebruik wordt gemaakt van proefdieren, proefpersonen of menselijk lichaamsmateriaal.

Dit type onderzoek vereist geen goedkeuring van een gemachtigde commissie voor medische ethiek.

Onder voorbehoud van de volledigheid en correctheid van de door u verstrekte gegevens, krijgt u hierbij het akkoord om het onderzoek in het kader van de masterproef te starten. Deze mail dient als bewijs van goedkeuring mocht u uw onderzoek wensen te publiceren.

Wij willen u erop attent maken dat u zelf verantwoordelijk blijft voor uw onderzoek. Bovendien doet elke wijziging aan de door u verstrekte gegevens omtrent de onderzoeksopzet deze goedkeuring vervallen. U dient in dat geval een amendement te maken aan uw huidig dossier.

III. Persoonlijk reflectieverslag

Het verloop van het groepsproces

Initieel heb ik deze thesis alleen aangevat met als algemene zoekvraag de link tussen virale aandoeningen en auto-immune pathologieën. In onderling overleg met professor dokter Verbakel werd besloten de zoekstrategie vrij breed te houden om op die manier de mogelijkheden open te houden. De zoekstrategie werd meermaals aangepast na feedback van de promotor. Na het indienen van de onderzoeksopzet via Prospero, werd ook ongeveer gelijktijdig de zoekstrategie gefinaliseerd. Het uiteindelijke resultaat leverde 17,423 hits op. Om te voldoen aan de voorwaarden van een systematic review, moet het screeningsproces echter minstens door twee onafhankelijke personen gebeuren. Ter zelfde tijd was er een andere HAIO (Montero Severino) die ook door professor dokter Verbakel begeleid werd en die een systematic review ging schrijven met een gelijkaardige insteek, met name de link tussen virale aandoeningen en kanker. Severino zou dan mijn 17,423 artikels moeten screenen en ik zijn vergelijkbaar aantal artikels. Gezien de omvang van deze opdracht, kwam er het voorstel van professor dokter Verbakel om eventueel samen te werken. Enerzijds om maar eenmalig dergelijke uitgebreide screening te moeten uitvoeren, maar anderzijds uiteraard ook om de grote hoeveelheid resultaten samen te kunnen verwerken. Door het feit dat ik al verder zat in het proces van mijn systematic review en de screening ook direct kon starten, terwijl er bij Severino nog enkele fases moesten doorlopen worden, werd er in onderling overleg beslist om met mijn onderwerp verder te gaan. Toen we in mei 2023 de beslissing hadden genomen om vanaf dat moment te gaan samenwerken, heb ik geprobeerd om onszelf meerdere tussentijdse deadlines op te leggen. In het begin verliep de samenwerking niet zo vlot en had ik wel het gevoel dat het initiatief telkens van mijn kant moest komen. Na enkele maanden keerde dit wel. De tijdslijn die we voor onszelf opgesteld hadden, hebben we grotendeels kunnen volgen. De laatste maanden hoorden we elkaar minstens wekelijks, spraken we af in Leuven of online om elkaar op de hoogte te brengen van onze vorderingen. Nieuwe ideeën maar ook onzekerheden werden vlot gedeeld. Ook konden we steeds laagdrempelig met onze vragen bij professor dokter Verbakel terecht, waarvoor we hem via deze weg nogmaals graag zouden willen bedanken. Samenvattend denk ik dat we kunnen spreken van een vlotte samenwerking die voor ons beiden een resultaat heeft opgeleverd waar we tevreden op terug kijken.

Het persoonlijke aandeel

De planning, het opstellen van interne deadlines en initieel ook het ruimere initiatief bestond, zoals hierboven reeds vermeld werd, vooral uit mijn aandeel. De zoekstrategie, die dateert uit de periode dat we nog elk individueel werkten, heb ik in alle respectievelijke databanken ingegeven om daarna via Endnote alle duplicaten te verwijderen. Deze 12,147 hits werden dan overgeplaatst naar Rayyan alwaar Severino en ikzelf geblindeerd en onafhankelijk van elkaar konden screenen. Toen we beiden dit proces afgerond hadden, hebben we in onderling overleg alle verschillen in dit resultaat besproken en weggewerkt. Al deze resultaten heb ik gelabeld en ingedeeld in verschillende groepen, dit volgens het type virus en het type auto-immune pathologie. Toen in onderling overleg met professor dokter Verbakel werd beslist om verder te gaan met de twee meest courant voorkomende linken tussen een virus en auto-immune pathologie, met name Epstein-barr virus (EBV) en de link met respectievelijk reumatoïde artritis (RA) en multiple sclerosis (MS), besloten we het werk op te delen. Zowel in de introductie, resultaten als de discussie werden de delen over EBV en RA door mij geschreven, ook de bijhorende tabellen werden door mij gemaakt. Severino maakte dan op zijn beurt alles over EBV en de link met MS, zowel in de introductie, resultaten (met bijhorende tabellen) als in de discussie. De

abstract, alsook grotendeels de discussie hebben we samen geschreven. In de inleiding heb ik naast het stuk over RA, ook het gemeenschappelijke deel over EBV voor mijn rekening genomen, ook de methodes werden voornamelijk door mij geschreven. Over de volledige masterthesis heen mag ik wel zeggen dat ik iets meer geschreven heb, echter is het wel zo dat ik regelmatig volledige stukken in het Nederlands heb geschreven die Severino, wegens zijn sterke kennis van het Engels, dan vertaald heeft.

Betekenis voor het werkveld

Het opstellen van deze systematic review heeft ervoor gezorgd dat we kritisch moesten nadenken over de gevonden resultaten. Dit betrof literatuur die vaak complex, technisch en vrij heterogeen was. Voor mij persoonlijk heeft deze thesis ook bijgedragen tot de gewaarwording van de complexiteit van auto-immune pathologieën. Als huisarts komen we toch vrij frequent in aanraking met auto-immune aandoeningen waarbij er nog geen of slechts een gedeeltelijke etiologische verklaring is.

Interessante pistes voor ons als toekomstige huisarts, die in onze master-na-masterpaper naar boven kwamen, waren vooral de mogelijke rol van screening en preventie. Screening aan de hand van bepaalde antilichamen die reeds jaren voor de klinische manifestatie van de auto-immune pathologie al aanwezig zijn in het bloed van de patiënten en preventie door te gaan vaccineren tegen EBV. Ook aan de hand van antivirale medicatie de virale lading in patiënten met auto-immune pathologieën naar beneden proberen te halen, is een mogelijke piste. In deze context kunnen vrij banale virale aandoeningen toch gevolgen hebben in een groter geheel waar we als huisarts rekening zullen moeten mee houden.