ISRCTN69709064 https://doi.org/10.1186/ISRCTN69709064

Is Epstein-Barr Virus (EBV) the cause of multiple sclerosis? A preliminary study

Submission date	Recruitment status	[X] Prospectively registered
21/05/2022	No longer recruiting	[ ] Protocol
Registration date	**Overall study status**	[ ] Statistical analysis plan
21/07/2022	Completed	[ ] Results
Last Edited	**Condition category**	[ ] Individual participant data
18/12/2023	Nervous System Diseases	[ ] Record updated in last year

Plain English summary of protocol

Background and study aims
Multiple sclerosis (MS) is an autoimmune disorder affecting the central nervous system. Data supports Epstein Barr Virus (EBV) as being necessary, but not sufficient, for someone to develop MS. The memory B-cell population is a subset of immune cells that has been suggested to be the cell population driving MS. The memory B cell population, which contains the subset of cells that are latently infected with EBV, is critical in the development of MS and is likely to be the main target of licensed MS treatments. The aim of this study is to see if people with MS have evidence of ongoing EBV replication compared to healthy volunteers, and whether or not MS treatments impact EBV replication.

Who can participate?
People with MS and healthy volunteers aged 18 years or above.

What does the study involve?
Participants will be asked to provide up to three extra blood and saliva samples. They will be asked to complete a questionnaire regarding their demographics, medical history, current medications and natural history of MS. No personal identifiers will be collected as part of the questionnaire.

What are the possible benefits and risks of participating?
Blood tests are a safe procedure but there can be complications, such as bruising at the site of puncture and excessive bleeding. The researchers will minimise the risk of complications by having trained personnel perform the blood extraction in accordance with infection control guidelines. They are not aware of any risk linked to taking saliva samples. There is no immediate benefit to participants, but it is hoped that the findings from this study will help with the development of new MS treatments in the future.

Where is the study run from?
Queen Mary University of London and Barts NHS Trust (UK)

When is the study starting and how long is it expected to run for?
October 2021 to February 2026

Who is funding the study?
Horne Family Foundation (UK)

Who is the main contact?
1. Dr Louisa James, louisa.james@qmul.ac.uk
2. Prof. David Baker, david.baker@qmul.ac.uk
3. Prof. Gavin Giovannoni, g.giovannoni@qmul.ac.uk
4. Adekunle Adeyinka Adeniran, a.a.adeniran@qmul.ac.uk

Contact information

Type(s)
Principal investigator

Contact name
Prof Gavin Giovannoni

ORCID ID
https://orcid.org/0000-0001-9995-1700

Contact details
Barts Health NHS Trust and Queen Mary University of London
4 Newark St
London
United Kingdom
E1 2AT
+44 20 7882 8954
g.giovannoni@qmul.ac.uk

Type(s)
Scientific

Contact name
Prof David Baker

Contact details
Queen Mary University of London
4 Newark St
London
United Kingdom
E1 2AT
+44 20 7882 2485
david.baker@qmul.ac.uk

Type(s)
Scientific

Contact name
Dr Louisa James

Contact details
Queen Mary University of London
4 Newark St
London
United Kingdom
E1 2AT
+44 207 882 2329
louisa.james@qmul.ac.uk

Type(s)
Public

Contact name
Mr Adekunle Adeyinka Adeniran

Contact details
Queen Mary University of London
4 Newark St
London
United Kingdom
E1 2AT
+44 207 882 2329
a.a.adeniran@qmul.ac.uk

Type(s)
Public

Contact name
Prof Louisa James

Contact details
Queen Mary University of London
4 Newark St
London
United Kingdom
E1 2AT
+44 207 882 2329
louisa.james@qmul.ac.uk

Additional identifiers

Integrated Research Application System (IRAS)
313742

Study information

Scientific Title
EBV and Memory B-cells study in patients with multiple sclerosis

Acronym

EBV-Mems

Study objectives
Multiple sclerosis (MS) is an autoimmune disorder affecting the central nervous system. Epidemiological data support Epstein Barr Virus (EBV) as being necessary, but not sufficient, for someone to develop MS. The memory B-cell population is a subset of immune cells that has been suggested to be the pathogenic cell population driving MS. The memory B cell population, which contains the subset of cells that are latently infected with EBV, is critical in the pathogenesis of MS and likely to be the main therapeutic target of licensed MS disease-modifying therapies. We propose testing this hypothesis, using a range of technologies, to see if people with MS (pwMS), compared to normal controls, have evidence of ongoing EBV replication and whether or not MS disease-modifying therapies impact EBV replication.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 22/11/2022, West of Scotland REC 5 (Research Ethics, Clinical Research and Development, Dykebar Hospital, Grahamston Road, Paisley, PA2 7DE, United Kingdom; +44 (0) 141 314 0214; WoSREC5@ggc.scot.nhs.uk), ref: 22/WS/0150

Approved 22/11/2022, West of Scotland REC 5 (Research Ethics, Clinical Research and Development, Dykebar Hospital, Grahamston Road, Paisley, PA2 7DE, UK; +44 (0)141 314 0214; WoSREC5@ggc.scot.nhs.uk), ref: 22/WS/0150

Primary study design
Observational

Study design
Single-centre longitudinal biomarker and laboratory study

Study type(s)
Other

Health condition(s) or problem(s) studied
Multiple sclerosis

Interventions
This longitudinal study, with the option to retest study subjects, will examine peripheral blood and saliva samples of people with Multiple Sclerosis (pwMS) and normal control subjects. EBV viral loads and EBV genome sequencing from spontaneously derived lymphoblastoid cell lines (LCLs) will be compared. Participants will also be asked to consent if they are prepared to return for repeated sampling (both blood and saliva) for the study over a period of 3 years. This will be a maximum of three time points at least 12 months apart (12, 24 and 36 months). We only anticipate recalling subjects who produce spontaneous LCLs, 12-24 months later, to ascertain whether the ability of their cells to spontaneously form LCLs is a stable phenomenon and to reassess the stability of the EBV genome and B-cell and T-cell repertoire between two different time points. Number of participants: up to 200 people with MS (pwMS) and up to 400 healthy subjects, with the aim of generating 100 LCLs from study subjects with MS and 30 LCLs from healthy subjects.

Comparisons of EBV viral loads will be done using a two-sided unpaired T-test if the data follows a normally distributed. The normality of the distribution will be measured using the Kolmogorov–Smirnov test. If the data is shown to be nonparametric standard transformation methods will be applied to the data. If transformation methods are unsuccessful the groups will be compared using Wilcoxon rank-sum test depending on the distribution.

Intervention Type
Other

Primary outcome(s)
EBV viral loads in cell-free plasma, peripheral blood mononuclear cells, B-cells, memory B-cells and saliva measured using a standard EBV-specific RT-qPCR assay at time zero, 12 and 24 months

Key secondary outcome(s)
1. EBV genome analysis and B cell repertoire analysis using DNA extraction, library construction, targeted enrichment and sequencing at time zero, at 12 months and at 24 months?
2. Proportion of subjects with MS who generate LCLs compared to healthy control subjects measured using LCLs ex vivo in cell culture lines numbers
Time points are not relevant as this is a cross-sectional biomarker study with the option of calling back the LCL+ve subjects to assess how reproducible the findings are.
3. Relative number of EBV-associated LCLs generated by pwMS on each DMT measured using LCLs ex vivo in cell culture lines numbers.
Time points are not relevant as this is a cross-sectional biomarker study with the option of calling back the LCL+ve subjects to assess how reproducible the findings are.
4. Mutations, deletions and insertions in the EBV genomes from pwMS LCLs and normal controls, measured using principal component analysis as described by Palser and colleagues (Palser et al. 2015).
Time points are not relevant as this is a cross-sectional biomarker study with the option of calling back the LCL+ve subjects to assess how reproducible the findings are.
5. Proportion of subjects with MS who have type 2 EBV genome compared to healthy control subjects measured using principal component analysis as described by Palser and colleagues (Palser et al. 2015)
Time points are not relevant as this is a cross-sectional biomarker study with the option of calling back the LCL+ve subjects to assess how reproducible the findings are.
6. The relative number of genetic polymorphisms in the B-cell repertoire (IgG sequences) of pwMS on each DMT measured using [method] Antibody genes will be sequencing by high-throughput Illumina Miseq sequencing (using 300bp paired-end reads).
Time points are not relevant as this is a cross-sectional biomarker study with the option of calling back the LCL+ve subjects to assess how reproducible the findings are.
7. EBV-specific T-cell response measured using EBV-specific tetramers after antigen-specific stimulation with intracellular cytokine staining, elispot gamma-interferon production analysis and proliferative responses in subjects that produce spontaneous LCLs compared to subjects who do not, at time zero, at 12 months and at 24 months

Completion date
01/02/2026

Eligibility

Key inclusion criteria

People with MS (pwMS):
1. Diagnosis of Multiple Sclerosis according to the McDonald criteria (Thompson et al. 2018)
2. Age above 18 years
3. Informed consent

Normal controls (healthy volunteers):
1. Age above 18 years
2. Informed consent
3. Absent of a pre-existing autoimmune disease, for example, type diabetes mellitus, autoimmune thyroid disease, inflammatory bowel disease, psoriasis, etc

Participant type(s)
Mixed

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
18 Years

Sex
All

Key exclusion criteria
People with MS and healthy subjects:
1. Other pre-existing autoimmune diseases, for example, type I diabetes mellitus, autoimmune thyroid disease, inflammatory bowel disease, psoriasis, etc
2. Unable to comply with study requirements
3. Unable to give informed consent to participate

Date of first enrolment
01/09/2022

Date of final enrolment
01/08/2025

Locations

Countries of recruitment
United Kingdom

England

Study participating centre
The Royal London Hospital Laboratory
The Royal London Hospital

Alexandra House
London
United Kingdom
E1 1BB

Sponsor information

Organisation
Queen Mary University of London

ROR
https://ror.org/026zzn846

Funder(s)

Funder type
Charity

Funder Name
Horne Family Foundation

Results and Publications

Individual participant data (IPD) sharing plan
The datasets generated and/or analysed during the current study during this study will be included in the subsequent results publication

IPD sharing plan summary
Published as a supplement to the results publication

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
HRA research summary			28/06/2023	No	No