Cytomegalovirus seropositivity is associated with reduced risk of multiple sclerosis—a presymptomatic case–control study

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Abstract

Background and purpose: Epstein–Barr virus (EBV) and human herpesvirus 6A (HHV-6A) are associated with increased risk of multiple sclerosis (MS). Conversely, infection with cytomegalovirus (CMV) has been suggested to reduce the risk of MS but supporting data from presymptomatic studies are lacking. Here, it was sought to increase the understanding of CMV in MS aetiology.

Methods: A nested case–control study was performed with presymptomatically collected blood samples identified through crosslinkage of MS registries and Swedish biobanks. Serological antibody response against CMV, EBV and HHV-6A was determined using a bead-based multiplex assay. Odds ratio (OR) with 95% confidence interval (CI) for CMV seropositivity as a risk factor for MS was calculated by conditional logistic regression and adjusted for EBV and HHV-6A seropositivity. Potential interactions on the additive scale were analysed by calculating the attributable proportion due to interaction (AP).

Results: Serum samples from 670 pairs of matched cases and controls were included. CMV seropositivity was associated with a reduced risk for MS (OR = 0.70, 95% CI 0.56–0.88, p = 0.003). Statistical interactions on the additive scale were observed between seronegativity for CMV and seropositivity against HHV-6A (AP 0.34, 95% CI 0.06–0.61).
INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease driven by inflammation against central nervous system antigens. The prevailing hypothesis is that environmental risk factors may trigger inflammatory processes resulting in demyelination in the central nervous system, which may be facilitated by certain genetic predispositions [1]. Epstein–Barr virus (EBV) is now firmly established as such a risk factor for MS [1]. Infection in early childhood is often asymptomatic and does not appear to increase the risk for MS, whilst symptomatic infection—‘infectious mononucleosis’—in adolescents and adults is strongly associated with increased MS risk [2]. EBV infection appears to be a prerequisite for MS in adults [3,4], and it was recently shown that the association of EBV seropositivity and MS risk is age-dependent; EBV seropositivity was associated with a decreased MS risk before 20 years of age and an increased risk after that age [5]. Human herpesvirus 6A (HHV-6A) has in recent years emerged as a risk factor for MS [6,7]. Contrary to EBV, HHV-6A appears to be associated with increased MS risk in all age groups [5]. Cytomegalovirus (CMV), another virus from the Herpesviridae family (denoted HHV-5), has been suggested to play a protective role in MS aetiology. A negative association of past CMV infection and risk of developing MS has been observed in serological studies on samples collected after MS diagnosis [8–12]. However, reverse causation may explain associations seen between exposures and diseases when samples are drawn after disease onset. Therefore, presymptomatic samples are preferred, but such previous studies on MS and CMV have been underpowered [13–16]. The aim of the present study was to increase the understanding of CMV in MS aetiology through a sufficiently powered study with serological analyses of presymptomatically collected serum samples.

METHODS

Trial design and patients

By crosslinkage of MS registries and microbiological biobanks, plasma or serum samples were identified and retrieved from individuals who later in life developed relapsing–remitting MS, as described elsewhere [17]. In short, the Swedish MS registry was crosslinked with five Swedish microbiological biobanks, containing remainders of sera from clinical analyses. An additional microbiological biobank was crosslinked with a local MS database at the University Hospital of Umeå, Sweden. All samples were donated before the age of 40 and prior to MS symptom onset. For each case, one control was randomly selected, matched for biobank, sex, date of blood sampling and date of birth (in order of priority).

Laboratory procedures

Serological responses to viruses were analysed using a bead-based multiplex assay, as previously described in detail [18]. Briefly, each bacterially expressed viral antigen was loaded onto a fluorescence-labelled glutathione-casein-coated bead set and presented to primary serum antibodies in a multiplex. Bound primary antibodies were detected using a biotinylated goat-α-human immunoglobulin G (IgG)/IgM/IgA secondary antibody and streptavidin-R-phycocyanin as reporter dye. A Lumixen 200 analyser was used to measure median fluorescence intensities (MFI) for each bead set, that is, antigen, thereby quantifying antibody levels. The following recombinantly expressed antigens were used to detect serum antibodies against CMV: pp28; pp52; and pp150 N-terminus (pp150-N) [19]. For EBV, the following antigens were used: EBV nuclear antigen 1 truncated, amino acids 325–641 (EBNA-1 trunc); EBV nuclear antigen 1 peptide, amino acids 385–420 (EBNA-1 pep); and viral capsid antigen p18 (VCA p18) [19]. For HHV-6A, truncated immediate-early protein 1 from HHV-6A (IE1A) was used [7]. Inter-batch controls and linear or modified logarithmic models were used to correct for batch-related variability.

Antigen serostatus for each virus was assessed in accordance with previously validated cut-offs [19]. In the validation study, the CMV serological assay was validated against two reference assays on two independent reference serum panels. Sensitivity and specificity in comparison to both reference assays was high and resulted in two sets of cut-offs. The differences in the cut-offs were most probably driven by the different serum panels and reference assays used [19]. The antibody responses against CMV are nearly dichotomous [19] and the published cut-offs are probably influenced by a small number of samples with intermediate seroreactivity. After assessing the antibody reactivity distribution in the present study, the higher set of cut-offs was selected: pp28, 200 MFI; pp52, 1101 MFI; and pp150N, 655 MFI. A sensitivity analysis was performed with the lower set of cut-offs: pp28, 73 MFI; pp52, 854 MFI; and

and EBV antigen EBNA-1 (amino acid 385–420) at age 20–39 years (AP 0.37, 95% CI 0.09–0.65).

Conclusions: Cytomegalovirus seropositivity is associated with a decreased risk for MS. The protective role for CMV infection in MS aetiology is further supported by the interactions between CMV seronegativity and EBV and HHV-6A seropositivity.

KEYWORDS

case–control studies, cytomegalovirus, herpesviruses, multiple sclerosis, serology
used to assess a different hypothesis. These data have been expanded for the current study and were a covariate in a regression analysis to adjust for possible confounders. A subset of the data on CMV has been used in a previous study as EBV and HHV-6A serostatus have been published previously [5,7]. Sensitivity and specificity, as described elsewhere [5]. The data on infection assay is yet available for comparison studies. Therefore, seropositivity for HHV-6A was defined as IE1A over 50 MFI to maximize sensitivity and specificity, as described elsewhere [5]. The data on EBV and HHV-6A serostatus have been published previously [5,7]. A subset of the data on CMV has been used in a previous study as a covariate in a regression analysis to adjust for possible confounders [7]. These data have been expanded for the current study and were used to assess a different hypothesis.

Statistical analyses

Analyses were performed on the entire cohort and stratified based on age at blood sampling: <20 years and 20–39 years of age. Matched pairs with participants on different sides of the age limits were assigned to the younger group, which contained fewer individuals. Odds ratio (OR) for CMV seropositivity as risk factor for MS was calculated using conditional logistic regression and adjusted for EBV and HHV-6A by including serostatus for all three viruses in the model. A sensitivity analysis was performed for samples drawn >8 years before symptom onset. A subgroup analysis was also performed, stratified by sex.

Interactions between CMV and HHV-6A or EBV serostatus were analysed on an additive scale using conditional logistic regression, calculating attributable proportion (AP) due to interaction [21,22]. Confidence intervals for AP were calculated as described by Hosmer and Lemeshow [21]. The post hoc analysis assessed interactions between serostatus for CMV and EBV antigen EBNA-1 pep with regard to MS risk. Conditional logistic regression analyses of all combinations of serostatus for the three viruses were performed in the older group. For all interaction analyses, the exposure group with the lowest MS risk was used as reference category. Interactions on the multiplicative scale were assessed by modelling the CMV–EBV and CMV–HHV-6A product terms in conditional logistic regression. The distribution of categorical variables was analysed using Pearson’s χ² test or Fisher’s exact test, where appropriate.

RESULTS

In total, 670 pairs of matched cases and controls were included. Median age at the time of blood sampling was 25 years and median time from sampling to symptom onset of MS was 8 years. The absolute mean differences for sampling date and sampling age between cases and controls were 6 days and 152 days, respectively.

Viral serostatus as risk factor for MS

Seropositivity against CMV was more common amongst females than males (56.8% vs. 43.5%; p < 0.001) and increased by age (Table 1). CMV seropositivity was significantly associated with a decreased risk of developing MS at age 20–39 (Figure 1) and in the total cohort (unadjusted OR = 0.71, 95% CI 0.57–0.90, p = 0.003). The result was similar after adjustments for EBV and HHV-6A serostatus (OR 0.70, 95% CI 0.56–0.88, p = 0.003). The sensitivity analysis using the lower set of cut-offs for CMV antigens yielded similar results (Figure S1). The negative association of CMV and MS risk remained in the sensitivity analysis of samples drawn >8 years before symptom onset (OR 0.66, 95% CI 0.48–0.93, p = 0.016, n = 664). The result was also similar in the female subgroup (unadjusted OR = 0.69, 95% CI 0.54–0.88, p = 0.002) but not statistically significant amongst men (unadjusted OR = 0.91, 95% CI 0.51–1.65, p = 0.76).

Epstein–Barr virus seropositivity was associated with an increased MS risk in the older group and a non-significant decreased MS risk in the younger group (Figure 1). HHV-6A seropositivity was consistently associated with an increased risk for MS in both age groups and in the total cohort (Figure 1).

Interactions of viral serostatus on MS risk

Compared to controls, a significantly higher proportion of cases were both seropositive for EBV and seronegative for CMV. This difference was observed at sampling age 20–39 and in the whole cohort (Table 2). The interaction analysis was complicated by the high proportion of EBV seropositive individuals. At age 20–39, the reference category of EBV seropositive and CMV seronegative individuals contained only one case and five controls (Table 2). Therefore, no interaction analysis could be performed for CMV and EBV serostatus.
CMV ASSOCIATED WITH REDUCED RISK OF MS

In the post hoc analysis of CMV and EBNA-1 pep, a significant additive interaction was observed with regard to MS risk in the older stratum (Figure 2a).

As for CMV and HHV-6A serostatus, additive interactions with regard to MS risk were observed at sampling age 20–39 years and in the whole cohort (Figure 2b). The same trend was present in the younger group, although not statistically significant.

When all combinations of viral serostatus were analysed simultaneously, the highest MS risk was observed at age 20–39 for those seronegative for CMV and seropositive for both HHV-6A and EBNA-1 pep (OR = 16.4, 95% CI 5.7–47, p < 0.0001; Figure 3), although the confidence interval was very wide and some of the exposure groups were small.

No interactions were observed on the multiplicative scale between CMV and EBV or HHV-6A with regard to MS risk.

DISCUSSION

The key result of this study is the association between CMV seropositivity and a reduced risk of developing MS in a large presymptomatic cohort. In addition, additive interactions were observed with regard to MS risk between serostatus for CMV and the EBV antigen EBNA-1 pep, as well as for CMV and HHV-6A. Altogether, these findings indicate a role for CMV in MS aetiology.

The primary strength of this study is the large number of cases (n = 670) with samples collected before symptom onset. This study design contradicts previous concerns that the negative association of CMV and MS could be affected by reverse causation. A relatively large group of young cases were included, which allowed for analysis of risk factors for MS in two separate age groups. Cases and controls were matched on sex, age and date of sampling and the matching was kept intact through all statistical analyses. The method for assessment of CMV serostatus has recently been validated and demonstrated high sensitivity and specificity to detect antibodies against CMV [19]. The observed rates of CMV seropositivity, as well as the sex differences in this regard, are consistent with previous investigations of CMV seroprevalence [23,24].

Still, some limitations must be acknowledged. The mean time from sampling to MS symptom onset was 8 years. Even this latency might not be sufficient to ensure that samples are presymptomatic, considering the emerging evidence of a long prodromal phase of MS [25,26]. However, the negative association of CMV serostatus and MS risk remained in the sensitivity analysis of samples drawn more than 8 years before MS onset.

Sex stratified subgroup analyses were performed, but the low number of male cases (n = 108) limits the possibility of drawing conclusions from such assessments. Whether the association of CMV serostatus and reduced MS risk applies to men remains to be evaluated, but would require a larger sample.

Whilst the study was adequately powered for the regression analyses of antiviral serostatus and MS risk, the interaction analyses were
TABLE 2 Comparison of viral serostatus between cases and controls

|                      | Case, n (%) | Controls, n (%) | p     |
|----------------------|-------------|----------------|-------|
| **CMV and EBV**      |             |                |       |
| All ages             |             |                |       |
| CMV+, EBV−           | 11 (1.6%)   | 10 (1.5%)      | 0.83  |
| CMV−, EBV−           | 30 (4.5%)   | 36 (5.4%)      | 0.45  |
| CMV+, EBV+           | 329 (49.1%) | 382 (57.0%)    | 0.004 |
| CMV−, EBV+           | 300 (44.8%) | 242 (36.1%)    | 0.001 |
| **Age <20**          |             |                |       |
| CMV+, EBV−           | 10 (7.0%)   | 5 (3.5%)       | 0.19  |
| CMV−, EBV−           | 22 (15.4%)  | 17 (11.9%)     | 0.39  |
| CMV+, EBV+           | 51 (35.7%)  | 61 (42.7%)     | 0.23  |
| CMV−, EBV+           | 60 (42.0%)  | 60 (42.0%)     | 1.00  |
| **Age 20–39**        |             |                |       |
| CMV+, EBV−           | 1 (0.2%)    | 5 (0.9%)       | 0.22† |
| CMV−, EBV−           | 8 (1.5%)    | 19 (3.6%)      | 0.03  |
| CMV+, EBV+           | 278 (52.8%) | 321 (60.9%)    | 0.007 |
| CMV−, EBV+           | 240 (45.5%) | 182 (34.5%)    | 0.0003|
| **CMV and EBNA-1 pep** |          |                |       |
| All ages             |             |                |       |
| CMV+, EBNA−          | 27 (4.0%)   | 41 (6.1%)      | 0.08  |
| CMV−, EBNA−          | 43 (6.4%)   | 61 (9.1%)      | 0.07  |
| CMV+, EBNA+          | 313 (46.7%) | 351 (52.4%)    | 0.04  |
| CMV−, EBNA+          | 287 (42.8%) | 217 (32.4%)    | <0.0001|
| **Age <20**          |             |                |       |
| CMV+, EBNA−          | 16 (11.2%)  | 9 (6.3%)       | 0.14  |
| CMV−, EBNA−          | 29 (20.3%)  | 25 (17.5%)     | 0.55  |
| CMV+, EBNA+          | 45 (31.5%)  | 57 (39.9%)     | 0.14  |
| CMV−, EBNA+          | 53 (37.1%)  | 52 (36.4%)     | 0.90  |
| **Age 20–39**        |             |                |       |
| CMV+, EBNA−          | 11 (2.1%)   | 32 (6.1%)      | 0.001 |
| CMV−, EBNA−          | 14 (2.7%)   | 36 (6.8%)      | 0.001 |
| CMV+, EBNA+          | 268 (50.9%) | 294 (55.8%)    | 0.11  |
| CMV−, EBNA+          | 234 (44.4%) | 165 (31.3%)    | <0.0001|
| **CMV and HHV-6A**   |             |                |       |
| All ages             |             |                |       |
| CMV+, HHV-6A−        | 208 (31.0%) | 289 (43.1%)    | <0.0001|
| CMV−, HHV-6A−        | 199 (29.7%) | 215 (32.1%)    | 0.34  |
| CMV+, HHV-6A+        | 132 (19.7%) | 103 (15.4%)    | 0.04  |
| CMV−, HHV-6A+        | 131 (19.6%) | 63 (9.4%)      | <0.0001|
| **Age <20**          |             |                |       |
| CMV+, HHV-6A−        | 40 (28.0%)  | 51 (35.7%)     | 0.16  |
| CMV−, HHV-6A−        | 56 (39.2%)  | 63 (44.1%)     | 0.40  |
| CMV+, HHV-6A+        | 21 (14.7%)  | 15 (10.5%)     | 0.29  |
| CMV−, HHV-6A+        | 26 (18.2%)  | 14 (9.8%)      | 0.04  |

(Continues)

|                      | Case, n (%) | Controls, n (%) | p     |
|----------------------|-------------|----------------|-------|
| **Age 20–39**        |             |                |       |
| CMV+, HHV-6A−        | 168 (31.9%) | 238 (45.2%)    | <0.0001|
| CMV−, HHV-6A−        | 143 (27.1%) | 152 (28.8%)    | 0.54  |
| CMV+, HHV-6A+        | 111 (21.1%) | 88 (16.7%)     | 0.07  |
| CMV−, HHV-6A+        | 105 (19.9%) | 49 (9.3%)      | <0.0001|

Notes: Serostatus for cases who later developed MS and matched (1:1) controls.

Statistically significant results in bold text. p values were calculated with Pearson’s χ² test, with the exception of CMV+, EBV− at age 20–39 (marked †) where Fisher’s exact test was used.

Abbreviations: CMV, cytomegalovirus; CMV+, positive seroresponse for ≥2 cytomegalovirus antigens (pp28, pp52, pp150N); EBNA, EBV nuclear antigen 1 pep; EBNA+, positive seroresponse for EBNA-1 pep; EBV, Epstein–Barr virus; EBV+, positive seroresponse for ≥1 Epstein–Barr virus antigens (EBNA-1 trunc, EBNA-1 pep, VCA p18); HHV-6A, human herpesvirus 6A; HHV-6A+, positive seroresponse against human herpesvirus 6A antigen (IE1A > 50 median fluorescence intensity).

insufficiently powered. This especially accounts for the analyses in the youngest group, where few individuals were present in some of the exposure combinations (Table 2). The interaction analyses of CMV and EBV were also affected by the shifting effect of EBV with age, from protective factor to risk factor for MS [5], explaining why the effect of EBV dissolved when analysed in the whole cohort. Due to the low number of EBV negative individuals, the reference category only contained a few individuals. Altogether, this rendered the common methods for interaction analysis unsuitable for CMV and EBV serostatus.

To enable CMV and EBV serostatus interaction analysis, a post hoc analysis was performed using the single EBV antigen EBNA-1 pep, thereby increasing the number of negative samples. The single antigen performance of EBNA-1 pep has been validated and demonstrated high specificity and sensitivity for detection of EBV antibodies [19]. In previous studies, EBNA-1 pep had a stronger connection to MS risk than other EBV antigens [27,28]. A significant additive interaction with regard to MS risk was observed for CMV and EBNA-1 pep serostatus at age 20–39 years.

Unlike EBV, the effect of HHV-6A on MS risk was constant throughout all age groups. This facilitated the interaction analysis of CMV and HHV-6A serostatus concerning MS risk. Significant additive interactions between serostatus were observed for these viruses with regard to MS risk. These interactions were present in the whole cohort, as well as in the older group.

The relationship between CMV and MS has been addressed previously in a few presymptomatic serological studies, but none with significant results [13–16]. The previous studies were all relatively small, consisting of 18 to 305 presymptomatic cases, and their lack of significant results might be due to insufficient power. A meta-analysis using raw data from three presymptomatic studies [13,15,16] showed a negative association of CMV and MS risk with OR = 0.73, 95% CI 0.59–0.91, p = 0.005 [29]. This is similar to the results from a Swedish study on samples drawn after MS diagnosis.
In the present study, validated cut-offs were used for each CMV antigen [7]. In the worldwide, about 60% of the adult population in developed countries and almost 100% in developing countries are seropositive for CMV, HHV-6A, and a validated definition of CMV seropositivity [19].

7215 controls, no significant interaction between CMV and HHV-6A on samples drawn after disease onset, containing 8742 cases and 7215 controls, no significant interaction between CMV and HHV-6A concerning MS risk have not been studied previously in presymptomatically collected samples. In a study on samples drawn after disease onset, containing 8742 cases and 7215 controls, no significant interaction between CMV and HHV-6A was found using a different measure of antibody response [7]. In the present study, validated cut-offs were used for each CMV antigen and a validated definition of CMV seropositivity [19].

Cytomegalovirus infects the majority of the adult population worldwide. About 60% of the adult population in developed countries and almost 100% in developing countries are seropositive for CMV [30]. After primary infection, CMV establishes a latent infection through several immune evasive mechanisms, affecting both innate and adaptive immune responses [30,31]. Immunocompetent hosts manage to control both the primary and the latent infection and rarely develop symptoms of the disease. In order to control the latent infection, a gradually increasing proportion of the immune system is committed to CMV. After years of latency, the immune response against CMV constitutes a major proportion of the immune system [30,32,33].

Whether this results in a reduced immunocompetence against other viruses remains unclear [32,34]. However, CMV seropositivity may reduce the immune response to EBV infection [35]. Against this background, it has been hypothesized that immune competition between CMV and EBV could be a possible mechanism behind the observed risk reduction for MS in CMV seropositive individuals [10]. The latent CMV infection is suggested to preempt a large proportion of the immune system, thereby reducing the adverse immune reaction against EBV that could lead to MS. Alternative mechanisms have also been suggested. For example, CMV infection promotes expansion of a subset of mature natural killer cells, which could modulate the control of EBV [36,37]. Interestingly, both the above hypotheses involve the immune response against EBV [36,37].

Cytomegalovirus infection can be acquired throughout the entire life span. During infancy, infection is often transferred through breastfeeding. Viral reactivation and viral shedding in breast milk

which also reported a negative association of CMV and MS risk with OR = 0.73, 95% CI 0.58–0.92, \( p = 0.005 \) [10]. Both results are consistent with that of the present study, where the OR for CMV as a risk factor for MS was 0.70, 95% CI 0.56–0.88, \( p = 0.003 \).

To our knowledge, interactions on the additive scale between CMV and EBV or HHV-6A concerning MS risk have not been studied previously in presymptomatically collected samples. In a study on samples drawn after disease onset, containing 8742 cases and 7215 controls, no significant interaction between CMV and HHV-6A was found using a different measure of antibody response [7]. In the present study, validated cut-offs were used for each CMV antigen and a validated definition of CMV seropositivity [19].

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Cytomegalovirus infection can be acquired throughout the entire life span. During infancy, infection is often transferred through breastfeeding. Viral reactivation and viral shedding in breast milk
occur for over 90% of seropositive mothers, which leads to subclinical infection of many infants [38]. Accumulating evidence indicates that breastfeeding may reduce the risk for MS in the offspring [39,40]. Considering the results from the present study, the transmission of CMV through breast milk could contribute to the observed risk reduction for MS in individuals that were breastfed.

When acquired during adolescence or later, CMV infection can cause mononucleosis with symptoms almost indistinguishable from those caused by EBV. Hypothetically, the interaction between CMV and EBNA-1 pep serostatus that was observed in the present study could reflect a decreased risk for EBV induced infectious mononucleosis in individuals already infected with CMV. Past CMV infection would then add to the hypothesis that young individuals with immunity against EBV are protected against infectious mononucleosis, which could be the key event that triggers the adverse immune reaction causing MS.

A statistical interaction was also observed between CMV and HHV-6A serostatus, with the combination of CMV seronegativity and HHV-6A seropositivity inferring the highest risk for MS development. Similar to the case for CMV and EBV, past CMV infection could reduce the risk for an HHV-6A-related autoimmune response. These two statistical interactions are suggestive of biological interaction and may relate to their evolutionary relationship. All three viruses belong to the Herpesviridae family and are thus genetically homologous. Still, the genetic distances between these three viruses are considerable, and future research will tell whether the relationship has a bearing on their interaction.

In conclusion, our results provide further evidence of the negative association between CMV serostatus and MS risk, as well as the significance of HHV-6A and EBV in MS aetiology.

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AUTHOR CONTRIBUTIONS
Viktor Grut: Formal analysis (lead); writing—original draft (lead); writing—review and editing (lead). Martin Biström: Data curation (lead); formal analysis (supporting); supervision (supporting); writing—original draft (supporting); writing—review and editing (supporting). Jonatan Salzer: Formal analysis (supporting); supervision (supporting); writing—original draft (supporting); writing—review and editing (supporting). Pernilla Strid: Formal analysis (supporting); supervision (supporting); writing—original draft (supporting); writing—review and editing (supporting). Daniel Jons: Investigation (supporting); writing—review and editing (supporting). Rasmus Gustafsson: Investigation (supporting); writing—review and editing (supporting). Anna Fogdell-Hahn: Investigation (supporting); writing—review and editing (supporting). Jesse Huang: Investigation (supporting); writing—review and editing (supporting). Nicole Brenner: Formal analysis (supporting); investigation (supporting); writing—review and editing (supporting). Julia Butt: Investigation (supporting); writing—review and editing (supporting). Noemi Bender: Investigation (supporting); writing—review and editing (supporting). Anna Lindam: Formal analysis (supporting); software (supporting); writing—review and editing (supporting). Lucia Alonso-Magdalena: Validation (equal); writing—review and editing (supporting). Martin Gunnarsson: Validation (supporting); writing—review and editing (supporting). Magnus Vrethem: Validation (supporting); writing—review and editing (supporting). Tomas Bergström: Formal analysis (supporting); writing—review and editing (equal). Oluf Andersen: Investigation (supporting); Writing—review and editing (supporting). Ingrid Kockum: Investigation (supporting); writing—review and editing (supporting). Tim Waterboer: Investigation (supporting); writing—review and editing (supporting). Tomas Olsson: Investigation (supporting); writing—review and editing (supporting). Peter Sundström: Conceptualization (lead); formal analysis (supporting); funding acquisition (lead); supervision (lead); writing—original draft (supporting); writing—review and editing (equal).

DATA AVAILABILITY STATEMENT
Anonymized data are available from the corresponding author upon request.

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### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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