scientific reports

Check for updates

OPEN

Associations between past infectious mononucleosis diagnosis and 47 inflammatory and vascular stress biomarkers

Ragnar P. Kristjánsson[1]✉, Jojo Biel-Nielsen Dietz[1], Ólafur B. Davíðsson[1], Bertram Kjerulff[2,3,4], Klaus Rostgaard[1,5], Joseph Dowsett[6], Signe Holst Søegaard[1,7], Emelie Curovic Rotbain[1,8], Michael Schwinn[6], Kristoffer Sølvsten Burgdorf[9,10], Jakob Thaning Bay[11], Christina Mikkelsen[6,12], Henrik Ullum[10], Søren Brunak[9], Erik Sørensen[6], Bitten Aagaard Jensen[13], Mie Topholm Bruun[14], Mette Nyegaard[10,15], Sisse Rye Ostrowski[6,16], Ole Birger Pedersen[11,16], Christian Erikstrup[2,3,4], Thomas Folkmann Hansen[9,17] & Henrik Hjalgrim[1,5,16]

Infectious mononucleosis (IM), predominantly caused by primary Epstein-Barr virus (EBV) infection, is a common disease in adolescents and young adults. EBV infection is nearly ubiquitous globally. Although primary EBV infection is asymptomatic in most individuals, IM manifests in a subset infected during adolescence or young adulthood. IM occurrence is linked to sibship structure, and is associated with increased risk of multiple sclerosis, other autoimmune diseases, and cancer later in life. We analyzed 47 biomarkers in 5,526 Danish individuals aged 18–60 years, of whom 604 had a history of IM, examining their associations with IM history up to 48 years after IM diagnosis. No significant long-term associations were observed after adjusting for multiple comparisons. When restricting the analysis to individuals measured within 10 years post-IM diagnosis, a statistically significant increase in CRP levels was observed in females. This association was not driven by oral contraceptive use. No significant associations between sibship structure and biomarker levels were detected. In conclusion, our study shows that while IM may lead to a transient increase in CRP levels in females, it does not result in long-term alterations in plasma biomarkers related to immune function, suggesting other mechanisms may be responsible for the long-term health impacts associated with IM.

Infectious mononucleosis (IM) is a disease predominantly caused by the Epstein-Barr virus (EBV), typically affecting adolescents and young adults[1]. EBV is transmitted via saliva, and primary infection typically occurs in early childhood or in adolescence. EBV infection is near-ubiquitous in the global population, with 90% of individuals testing positive for EBV antibodies by age 30[2–4]. While primary EBV infection is mostly asymptomatic in childhood, it is accompanied by IM in about 75% of individuals infected in adolescence or later[5]. IM itself is a

[1]Danish Cancer Society Research Center, Danish Cancer Society, Copenhagen, Denmark. [2]Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark. [3]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark. [4]BERTHA Big Data Centre for Environment and Health, Aarhus University, Aarhus, Denmark. [5]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark. [6]Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark. [7]Department of Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark. [8]Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. [9]Translational Disease Systems Biology, Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [10]Statens Serum Institut, Copenhagen, Denmark. [11]Department of Clinical Immunology, Zealand University Hospital, Køge, Denmark. [12]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [13]Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark. [14]Department of Clinical Immunology, Odense University Hospital, Odense, Denmark. [15]Department of Health Science and Technology, Aalborg University, Aalborg, Denmark. [16]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [17]NeuroGenomics group, Translational Research Centre, Copenhagen University Hospital, Rigshospitalet, Glostrup, Denmark. ✉email: ragkri@cancer.dk

self-limiting disease, and symptoms include sore throat, swollen lymph nodes, fatigue, myalgia, fever, and upper respiratory symptoms[5].

The pathophysiology of IM is closely tied to the immune system's interaction with EBV. Upon infection, EBV targets B-cells, exploiting the complement receptor 2 (CD21) receptor to gain entry. This leads to the activation and proliferation of monocytes, T-cells, in particular CD8 + cytotoxic T cells, and natural killer (NK) cells. As a result of this, acute increases in levels of interferons α2, β, and γ, interleukins 6, 10, and 12, TNFα, and TGFβ occur[5–7]. EBV then establishes latency, largely in B-cells, persisting as a life-long asymptomatic infection with occasional reactivation[8].

The link between EBV infection and subsequent development of autoimmune diseases such as multiple sclerosis (MS), inflammatory bowel diseases, systemic lupus erythematosus, and rheumatoid arthritis, as well as depression, is also well-documented, highlighting the virus's potential long-term role in altering biological functions, particularly of the immune system[9–14]. Although these long-term sequelae are known, the underlying mechanistic effects pertaining thereto are not well understood[15,16]. The study of circulating proteins is therefore essential for understanding the impact of EBV and IM on the immune system, as well as their role in the aetiology of the aforementioned conditions.

Epidemiological studies have repeatedly shown that sibship composition - an individual's number of siblings and birth order - influences health not only in childhood but throughout life[17,18]. The biological underpinnings of such lasting consequences of sibship composition are not well-understood but are speculated to involve immunological mechanisms; sibship composition may affect both number, intensity, and/or age of typical childhood infections, which in turn may influence immune maturation and immune responses[19,20]. Whether this is reflected in biomarkers in adulthood has, to our knowledge, not been investigated. EBV is one such infectious agent for which both acute immunological responses to and long-term consequences of the infection vary significantly by age at first exposure and sibship structure[21,22].

Advancements in high-throughput technologies have significantly improved the sensitivity, specificity, and multiplexing capabilities of biomarker assays[23]. Platforms like Meso Scale are capable of detecting and quantifying a large number of different biomarkers in a single assay, enabling detailed profiling of inflammatory responses and vascular stress[24,25]. These developments have facilitated the discovery of novel biomarkers and enhanced our understanding of various diseases at a molecular level[26]. This capability is particularly valuable in the fields of immunology, oncology, and cardiovascular research, where understanding complex biomarker networks is crucial, and where the need for personalized medicine informed by integrated understanding is increasingly called for[26–29].

Leveraging these developments, we conducted a large-scale study on the long-term effects of IM on inflammatory and vascular stress biomarkers in a sub-cohort of the Danish Blood Donor Study[25,30,31]. This study aims to investigate whether IM causes long-term activation or alteration of the immune system measurable through inflammatory biomarkers, and the extent to which this is influenced by sibship structure. Understanding these mechanisms could potentially lead to the identification of new strategies to mitigate the long-term consequences of IM, including the serious diseases thought to result from this proposed permanent immune system dysfunction.

Results

Demographics

We analyzed levels of 47 inflammatory and vascular stress biomarkers in a sample of 5,526 Danish individuals originating from the Danish Blood Donor Study (DBDS). The demographics of the cohort are described in Table 1. We identified 604 individuals within this cohort who had received a diagnosis of infectious mononucleosis (IM), ascertained using hospital records or self-reported in questionnaires administered as part of the DBDS protocol[30].

Analysis of biomarker levels by IM history

First, we tested for association between past IM diagnosis and plasma biomarker concentration (Supplementary Table 1). When performing this analysis we adjusted both for demographic covariates (age, sex, BMI, smoking status, oral contraceptive use), and technical covariates (number of previous blood donations, time between sample collection and analysis, analysis date) (see Methods). After Bonferroni correction, we observed no significant associations between IM diagnosis and plasma biomarker concentration.

Time since diagnosis

Second, we stratified IM cases on time since diagnosis, assessing individuals who were sampled within 10 years post-IM diagnosis against all remaining individuals (Supplementary Table 2). This revealed a significant association with log median normalized CRP levels (Effect (95% CI) = 1.47 (1.24–1.67), p-value = 1.43×10^{-4}, adjusted p-value = 0.02). Sex-stratified analysis revealed this association to be driven by females ($\text{Effect}_{\text{Female}}$ (95% CI) = 1.87 (1.60–2.14), $\text{p-value}_{\text{Female}} = 6.62 \times 10^{-6}$, adjusted p-value = 9.73×10^{-4}), and absent in males ($\text{Effect}_{\text{Male}}$ (95% CI) = 1.05 (0.75–1.35), $\text{p-value}_{\text{Male}} = 0.75$, adjusted p-value = 1.00). This effect was also seen when analysing only the subset of females with no reported history of oral contraceptive use (Effect (95% CI) = 2.04 (1.67–2.41), p-value = 1.69×10^{-4}, $n = 2{,}703$). Normalized CRP levels per sex by time since diagnosis are depicted in Fig. 1a.

To replicate these findings, we investigated CRP levels in a non-overlapping set of 16,801 individuals ($n_{\text{IM history}} = 1{,}834$) from the DBDS for whom CRP was specifically measured (Methods). After adjusting for age at sampling, we again found a significant association between CRP levels and IM diagnosis within 10 years prior to sampling (Effect (95%CI) = 0.27 mg/L (0.09–0.45), p-value = 2.96×10^{-3}). Upon stratification by sex, this effect was again found to be driven by females ($\text{Effect}_{\text{Female}}$ (95%CI) = 0.36 mg/L (0.07–0.65), $\text{p-value}_{\text{Female}} = 1.44 \times 10^{-2}$; $\text{Effect}_{\text{Male}}$ (95%CI) = 0.05 mg/L (-0.15–0.25), $\text{p-value}_{\text{Male}} = 0.62$). Females with an IM diagnosis

	All Individuals			Individuals with IM History			Individuals without IM History		
	F	M	Total	F	M	Total	F	M	Total
N	2703	2823	5526	311	293	604	2392	2530	4922
Age (mean [SD])	40.51 [11.52]	40.62 [11.38]	40.56 [11.45]	36.21 [10.18]	38.59 [10.19]	37.37 [10.24]	41.07 [11.57]	40.85 [11.49]	40.96 [11.53]
BMI (mean [SD])	25.12 [4.38]	25.85 [3.52]	25.49 [3.98]	24.66 [4.41]	25.50 [3.05]	25.07 [3.83]	25.18 [4.38]	25.89 [3.57]	25.54 [4.00]
IM history (ICD-10)*	311 (31)	293 (40)	604 (71)	311 (31)	293 (40)	604 (71)	-	-	-
Current Smokers	391 (14.6%)	402 (14.3%)	793 (14.4%)	52 (16.8%)	40 (13.8%)	92 (15.4%)	339 (14.3%)	362 (14.3%)	701 (14.3%)
Oral Contraceptives**	584 (21.6%)	-	584	85 (27.3%)	-	85	499 (20.9%)	-	499
Number of Siblings* (mean [SD])**	1.49 [0.86]	1.54 [0.85]	1.52 [0.86]	1.37 [0.83]	1.47 [0.78]	1.41 [0.81]	1.51 [0.86]	1.55 [0.86]	1.53 [0.86]

Table 1. Demographic information pertaining to the final cohort. Information is presented for the entire cohort, as well as stratified on both sex and IM history. * Total number of individuals with a history of IM diagnosis. The number within parentheses represents those for whom IM history was ascertained by ICD-8/ICD-10 code from the Danish Patient Register (Methods). ** Filled a prescription for ATC code G03A and all subcodes covering a period of time ending between 0 days and 3 months prior to sample donation *** Sibship information was available for 3,012 individuals (n_{female} = 1,453, n_{male} = 1,559). Of these, 415 had a history of IM (n_{female} = 218, n_{male} = 197), and 2,597 did not (n_{female} = 1,235, n_{male} = 1,362).

within the past 10 years were observed to have 35% increased likelihood of presenting with clinically elevated CRP levels, defined as > = 3.0 mg/L (OR [95% CI] = 1.35 [1.00–1.81], p-value = 0.043). CRP levels per sex by time since diagnosis for this cohort are depicted in Fig. 1b.

Sibship structure

To assess the effect of birth order on long-term IM-mediated biomarker changes, we assessed the association between number of younger and older siblings and measured biomarker levels (Supplementary Table 3). To this end, we constructed sibships based on common maternity for all individuals for whom this information was available (n = 3,012; 415 IM cases and 2,597 controls). Treating the number of younger or older siblings as factors (1, 2, or 3 + younger or older siblings, for a total of 6 factors), we observed no significant associations between sibship structure and levels of the 47 measured plasma biomarkers.

Discussion

In our analysis of 5,526 Danish individuals recruited as part of the Danish Blood Donor Study, we observed no long-term changes in plasma levels of 47 inflammatory and vascular stress biomarkers associated with having any prior IM diagnosis. When limiting our analysis to samples donated within 10 years of IM diagnosis, we observed a significant association with increased CRP levels in females (Effect (95%CI) = 1.87 (1.60–2.14), p-value = 6.62×10^{-6}). It is established that CRP levels are elevated in the acute phase of IM[32] but, to our knowledge, long-term CRP elevations following IM have not been previously described.

As IM typically occurs in late childhood or early adulthood, females reporting IM within the 10 years preceding donation are likely to be premenopausal and may show increased rates of oral contraceptive use. Oral contraceptives have been reported by numerous studies, including one study of the DBDS cohort, to be associated with increased circulating CRP levels[33–35]. In this study, we adjusted for the use of oral contraceptives within 3 months prior to sample donation to account for both usage and a wash-out effect. However, the time required for CRP levels to return to baseline following oral contraceptive cessation has, to our knowledge, not been investigated. We further assessed whether adjusting for oral contraceptive use in the 6 months and 1 year preceding donation attenuated the observed signal, and found that it did not. The observed association is therefore unlikely to be driven by this factor. However, CRP levels are known to fluctuate significantly throughout the menstrual cycle[36]. Information was not available on menstrual cycle phase at time of sampling and represents a factor for future study, but is unlikely to be a confounder in this case since we expect menstrual cycle phase at time of sampling to be uniformly distributed throughout the two groups.

CRP is a widely recognized marker of inflammation[37,38], and the observation that women exhibit heightened CRP levels for up to a decade post-IM suggests a sustained inflammatory response. This persistent inflammation may contribute to the increased susceptibility to autoimmune conditions observed in women, notable for diseases such as MS, systemic lupus erythematosus, and rheumatoid arthritis[39,40]. This raises the possibility of an underlying sex-specific difference in the immune response to EBV. Understanding these sex-based immunological differences, such as through genetic studies, may eventually lead to targeted prevention or treatment strategies to mitigate long-term effects of IM, particularly in women.

Sibship structure has previously been identified as an important factor in modulating risk of IM, most likely by affecting age at primary EBV infection[21]. This risk-modulation has been shown to exist for both IM, an acute result of EBV infection, as well as MS, a much later complication[22]. In the current study we did not find

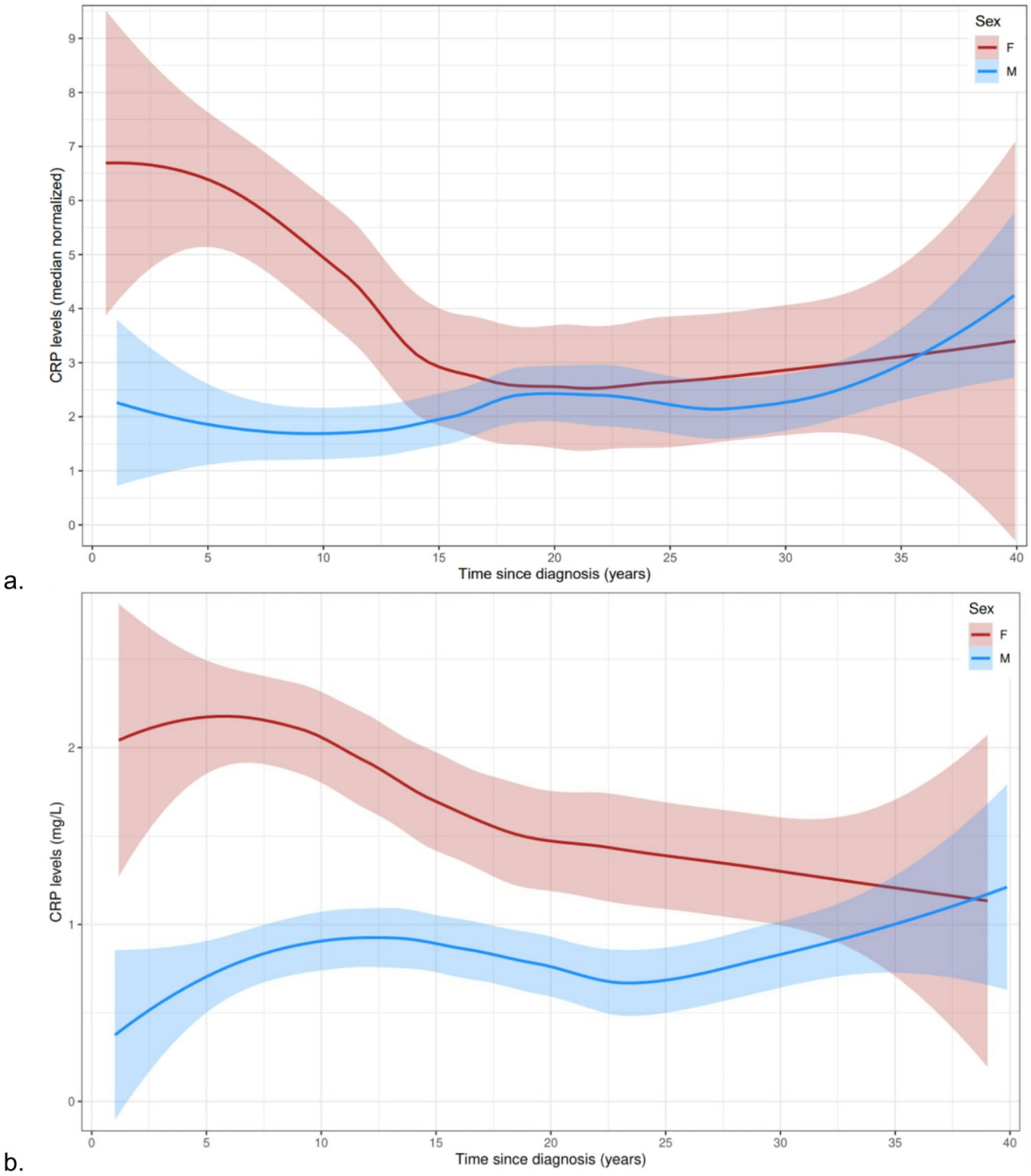

Fig. 1. CRP levels among individuals with a history of IM, with LOESS-smoothed values and corresponding confidence bounds plotted against time since diagnosis and stratified by sex. **(a)** Normalized CRP levels among individuals in the main analysis (Meso Scale). A total of 574 individuals with history of IM had CRP measurements that passed QC filtering. The red line represents females ($N = 294$) and the blue line represents males ($N = 280$). **(b)** Measured CRP levels in the CRP replication set. The red line represents females ($N = 929$) and the blue line represents males ($N = 905$).

sibship structure to correlate in any way with plasma biomarker levels, reinforcing the hypothesis that the effects conferred by siblings are mediated by age at primary EBV infection, rather than an alternate and unknown biological pathway. It must be noted, however, that birth order's effects on exposure may be somewhat attenuated in Denmark due to high rates of daycare attendance and resultant early social contact[41–43].

Prolonged elevation of CRP levels has been observed in various disease states, including chronic inflammatory and infectious diseases[44]. For instance, conditions such as rheumatoid arthritis[45] and inflammatory bowel disease[46] are associated with persistently elevated CRP levels due to ongoing inflammation. Similarly, chronic infections can lead to sustained CRP elevation as the body continuously responds to the persistent pathogen[47]. The exact mechanisms behind the prolonged CRP elevation following IM remain unclear, but it is possible that persistent immune activation or low-grade inflammation contributes to this phenomenon. Ultimately, further research is necessary to elucidate these mechanisms.

Our analyses employed Bonferroni correction to minimize type I errors at the expense of statistical power, a conservative approach particularly vulnerable to missing weaker associations. This stringency must be interpreted in the context of both potential misclassification and our sample size limitations. We have presented unadjusted p-values for all tested biomarkers so that these can be assessed independently and used in any eventual meta-analyses. However, we note that, besides the reported CRP association, only the association between IL-8 levels and IM history in individuals with two younger siblings approached nominal significance post-adjustment ($p_{adj} = 0.08$); an association that would not survive correction unless testing burden were reduced by about 40%. These observations suggest that while type II errors remain possible, they are unlikely to mask substantial biomarker associations. Ultimately, prospective studies with serological confirmation of IM exposure and longitudinal biomarker assessment will be required to definitively address these limitations.

Should any true associations be overlooked due type II errors, it would likely be due to significant decreases in statistical power due to case-status misclassification. IM cases were ascertained either through self-reported questionnaires, where subjects provided a yes/no answer to whether they had ever been diagnosed with IM as well as the age at which this occurred, or through hospital discharge diagnoses. This dataset could therefore be subject to some recall bias, most likely manifesting as enrichment for those who experienced particularly severe or late IM. A recent publication analyzing the self-reported data in the UK Biobank demonstrated that this data type is subject to non-trivial amounts of error, leading to substantially reduced statistical power[48]. This underscores how measurement error may have exacerbated type II error rates in our study. In any case, the observed 11% prevalence of self-reported IM aligns with findings from a different sub-sample of the same DBDS cohort, where the validity of this estimate was extensively discussed[49]. Furthermore, distinguishing between all IM and the supposedly more severe hospital-diagnosed IM has been shown to not lead to altered associations with familial exposures[21,50]. Finally, the concordance between the hospital-ascertained and self-reported IM diagnoses (85%) is on par with the estimated precision of the Danish National Patient Register itself[51]. However, robust population-level estimates of IM prevalence remain unavailable for Denmark, precluding direct external validation.

Further contributing to possible type II errors in this study is the fact that the cohort, drawn from the DBDS, consists of relatively young and healthy individuals[52]. This could lead to a general underestimation of effect sizes, as any major health events could lead to an individual being precluded from donating blood. Finally, we acknowledge that the Meso Scale panel used in this study only assesses a limited number of inflammatory biomarkers. Interrogating a broader range of biomarkers could help identify any further long-term effects of IM, as well as shed light on any biological mechanisms underlying the observed CRP association.

In conclusion, when assessing plasma biomarker levels in individuals with a history of IM diagnosis, we observed a transient association with increased CRP levels in females. No long-term changes in plasma biomarker levels were observed beyond 10 years post-diagnosis. Despite reported associations between IM diagnosis and a number of subsequent disease processes, often many years later, our analysis shows no evidence for long-term effects on immune function as measured by the studied biomarkers. Therefore, there is no evidence that future pathophysiologies caused by IM are mediated through altered levels of commonly assessed plasma biomarkers of immune functioning. It is most likely that biological processes initiated in puberty or later, leading to said pathophysiologies, are mainly characterized by qualitative features, such as the emergence of cells targeting myelin nerve sheaths as per the molecular mimicry theory of MS[53]. Our contribution is concordant with a general trend of not finding evidence for associations between common immune system characteristics and IM[54–57].

Methods

Participants

The Danish Blood Donor Study (DBDS) is a nationwide open cohort of blood donors, aged 18–70 years at inclusion[30,31]. In Denmark, blood donors must be aged 17–70 years, weigh more than 50 kg (60 kg for plasma donors), and be generally healthy. Additionally, select diseases and medical treatments cause temporary or permanent deferral. Upon donation, DBDS participants provide informed consent, answer a questionnaire[58], and allow their answers to be linked with data from national registers using their unique personal identification number[59,60]. Currently, more than 165,000 donors are included in the DBDS, and more than 450,000 questionnaires, distributed across several versions, have been answered[30], covering a wide range of phenotypic features. Currently, the DBDS represents a biobank of over two million blood samples.

The participants included in this study represent the DBDS 10 K Inflammatory Biomarker Cohort[25], a subset of 9,876 blood donors selected to ensure equal representation of the sexes, as well as age-stratified groups (18–29, 30–39, 40–49, 50–59, and 60–69). Figure 2 illustrates the selection and exclusion of subjects for the current study. Of the 9,876 individuals initially available for analysis, 9,810 samples remained after quality control (QC) and withdrawn consent. A further 2,157 individuals were removed as they lacked DBDS questionnaire data ($n = 193$) or were over 60 years of age at the time of donation and therefore deemed unrepresentative of the general population ($n = 1,964$). Lastly, we excluded a further 2,127 individuals with uncertain IM history. In total, 5,526 individuals were included for analysis. Demographic information for these participants is presented in Table 1.

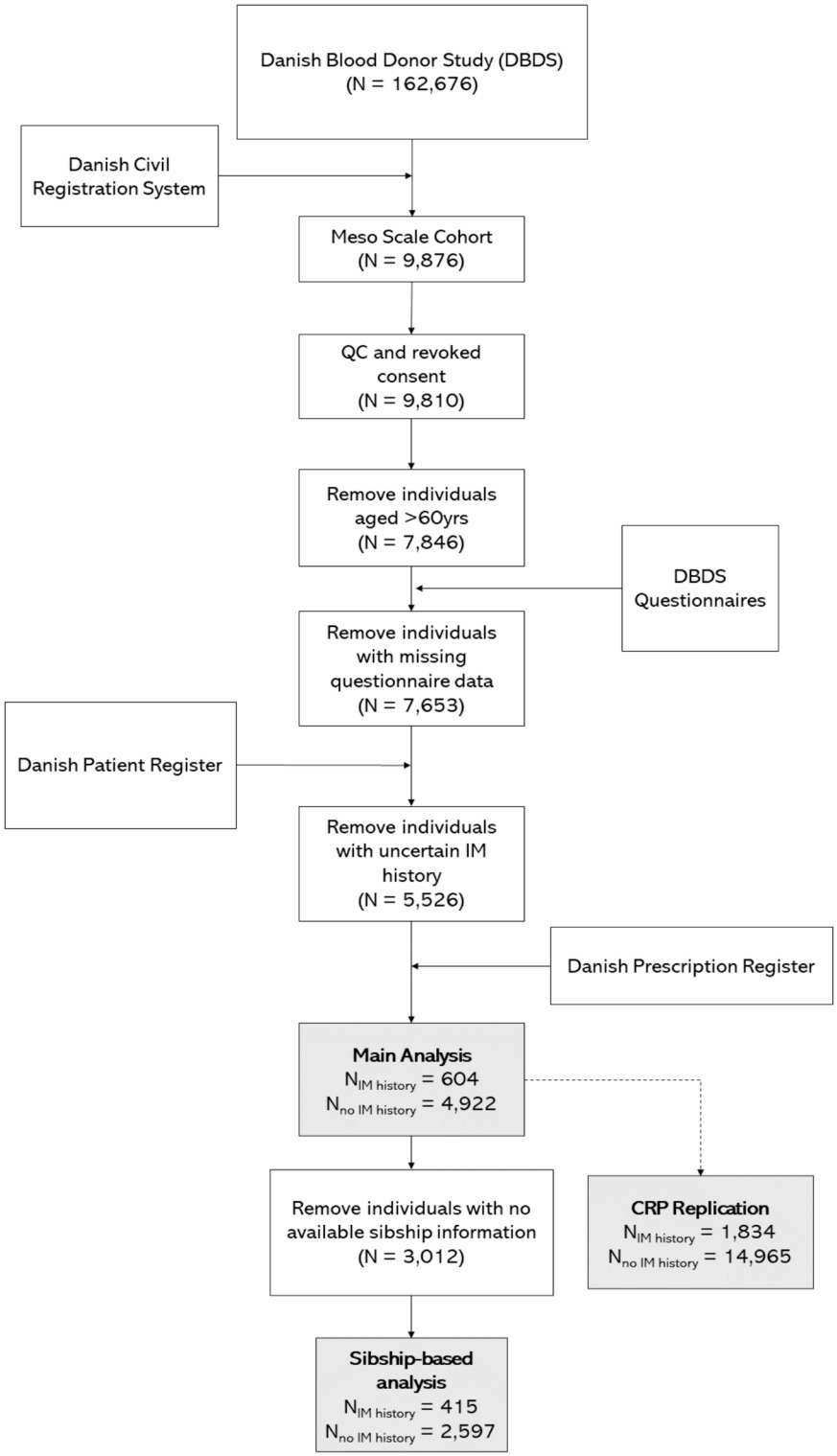

Fig. 2. Flowchart depicting the selection and exclusion of study subjects for the study population. DBDS = Danish Blood Donor Study, QC = quality control, IM = infectious mononucleosis.

Infectious mononucleosis cases

We identified individuals with a history of IM diagnosis in two ways: first, through hospital discharge codes (International Classification of Diseases, Eighth and Tenth Revisions (ICD-8/ICD-10)) ascertained through the National Patient Register[61]. Second, through self-reports ascertained from the questionnaires administered as part of the DBDS protocol. All reported dates of diagnosis occurred prior to both interview date and date of sample collection. Individuals with conflicting questionnaire answers pertaining to IM history were excluded ($n = 6$). Of

the 604 total IM cases, 71 were ascertained through ICD-10 discharge code B27 and all subcodes ($n = 47$), and ICD-8 code 075 and all subcodes ($n = 24$), and 573 were ascertained through the DBDS questionnaires. There were 31 hospital-diagnosed cases for whom no questionnaire data was available - these individuals were included in the study as cases. All individuals with no hospital diagnosis and either no questionnaire data, or who had not responded to the IM-specific questions, were excluded from the analysis. The total number of hospital diagnosed cases for whom questionnaire data was available was 47. Of these, 40 self-reported IM (85%). For the remaining 7, hospital diagnoses were considered the more reliable indicator, and these individuals were classified as cases.

Biomarker measurement

Biomarker measurements were performed using the electrochemiluminescence-based Meso Scale Discovery (MSD) V-PLEX Human Biomarker 54-plex kit. Measurements were performed from October 2020 to February 2022. The methodology has been extensively described in a recent publication[25]. In short, measurements of plasma biomarker concentrations were made using the aforementioned Meso Scale kit, which included 49 assays. Two different assays measured levels of IL-8 and VEGF-A and, therefore, the levels of a total of 47 biomarkers were assessed. Measurements below the range of the corresponding assay's fit curve were imputed from a uniform distribution between 0 and the lower limit of sample detection for that given assay. Not all individuals passed QC for all assays. These measurements were then median normalized.

Statistical analysis

Analysis of biomarker levels by IM history

Linear regression models were used to assess the association between IM history and biomarker concentrations. The median-normalized biomarker concentrations were log-transformed for the analysis. The following covariates, measured at sampling, were included in the models based on their potential association with both IM and biomarker concentrations: sex, age at sampling (as a continuous variable), BMI (as a continuous variable), smoking status at sampling, oral contraceptive use, measurement date, and sample age (time stored in freezer from donation to analysis). Current oral contraceptive use was defined as having filled a prescription for ATC code G03A and all subcodes covering a period of time ending between 0 days and 3 months prior to sample donation. These analyses were performed for the group as a whole, and after stratifying on sex (in which case sex was not included as a covariate). All results were Bonferroni adjusted to correct for multiple testing (number of tests = 147).

Time since diagnosis

Information on the date of IM diagnosis was available for all 604 individuals with a reported history of IM. In the case of the 71 individuals who were ascertained through hospital discharge diagnosis, this date represents the date of the admission. For the remaining subjects for whom IM history was ascertained through the DBDS questionnaires, an age at diagnosis was self-reported. The approximate date of diagnosis was thus calculated by adding the age, in days, to the birthdate of the individual.

To assess transient changes in plasma biomarker levels we performed another analysis in the same manner as outlined above, using only IM cases who were diagnosed within the 10-year window preceding sample donation ($n_{Total} = 112$, $n_{Female} = 63$, $n_{Male} = 49$). These analyses were performed for the group as a whole, and after stratifying on sex. All results were Bonferroni adjusted to correct for multiple testing (number of tests = 147).

Birth order / Sibship structure

To assess whether birth order associated with long-term changes in biomarker levels, we gathered all available data on sibship structure for the individuals in the DBDS 10 K Inflammatory Biomarker Cohort. As information on paternity was systematically missing, and is subject to significant error even when complete[62], sibships were constructed based only on common maternity. We were able to construct sibships for a total of 3,012 individuals from the cohort (Table 1), of whom 415 were IM cases and 2,597 were controls.

For each individual, the total number of siblings and birth order were ascertained. This information was used to determine the total number of younger and older siblings, which was then treated as factors in linear regression models estimating average normalized biomarker concentrations associated with sibship structure: 1, 2, or 3 + younger or older siblings, for a total of 6 factors in linear regression models performed in the same way as prior. These analyses were performed for the group as a whole, and after stratifying on sex. All results were Bonferroni adjusted to correct for multiple testing (number of tests = 882).

CRP replication

To replicate our CRP findings, we scrutinized CRP levels in a non-overlapping set of individuals from the DBDS for whom CRP was specifically measured[63]. Measured levels were reported in mg/L. In total, 18,448 individuals had their CRP levels measured using a commercially available, high-sensitivity assay on an automated system (Ortho Vitros 5600, Ortho Clinical Diagnostics, Rochester, NY, USA), performed between December 2010 and September 2011. From this set, we were able to ascertain IM history for a total of 16,799 individuals ($n_{female} = 7,970$), 1,834 of whom had a history of IM ($n_{female} = 929$) and 14,965 of whom did not. Of those with a history of IM, 446 ($n_{female} = 257$) received that diagnosis within 10 years prior to sample donation. Statistical analysis was performed in the same way as for the main analysis. The OR for clinically elevated CRP levels was based on a cutoff of 3.0 mg/L, and was ascertained through logistic regression.

Ethics

Oral and written informed consent was obtained from all study participants. The DBDS was approved by the Danish Data Protection Agency (P-2019-99) and the Committees on Health Research Ethics in the Central

Denmark Region (1-10-72-95-13) and the Zealand Region (SJ-740). The ethical approvals covered the measurement of biomarkers in the DBDS, and additional ethical approval was therefore not required. All methods were performed in accordance with relevant guidelines and regulations.

Data availability

The DBDS is a platform for studies carried out by the Danish blood centers and collaborators. The study is managed by a steering committee who respond to enquiries regarding collaboration. The blood donors participate in the DBDS to increase the scope of their donation, i.e. to help produce valuable research for the benefit of future patients. Additional information can be found on our home page [http://www.dbds.dk]. We invite researcher s to collaborate by contacting the steering committee [info@dbds.dk]. Data access requires that projects and applicants obtain permission from the Regional Committees on Health Research Ethics and the Danish Data Protection Agency [http://www.datatilsynet.dk].

Received: 24 October 2024; Accepted: 20 March 2025
Published online: 02 April 2025

References

1. Dunmire, S. K., Hogquist, K. A. & Balfour, H. H. Infectious mononucleosis. *Curr. Top. Microbiol. Immunol.* **390**, 211–240 (2015).
2. de-Thé, G. et al. Sero-epidemiology of the Epstein-Barr virus: preliminary analysis of an international study - a review. *IARC Sci. Publ* **11**(2), 3–16 (1975).
3. Venkitaraman, A. R., Lenoir, G. M. & John, T. J. The seroepidemiology of infection due to Epstein-Barr virus in Southern India. *J. Med. Virol.* **15**, 11–16 (1985).
4. Levin, L. I., Munger, K. L., O'Reilly, E. J., Falk, K. I. & Ascherio, A. Primary infection with the Epstein-Barr virus and risk of multiple sclerosis. *Ann. Neurol.* **67**, 824–830 (2010).
5. Balfour, H. H. Jr et al. Behavioral, virologic, and Immunologic factors associated with acquisition and severity of primary Epstein-Barr virus infection in university students. *J. Infect. Dis.* **207**, 80–88 (2013).
6. Odumade, O. A., Hogquist, K. A. & Balfour, H. H. Jr. Progress and problems in Understanding and managing primary Epstein-Barr virus infections. *Clin. Microbiol. Rev.* **24**, 193–209 (2011).
7. Hislop, A. D., Taylor, G. S., Sauce, D. & Rickinson, A. B. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. *Annu. Rev. Immunol.* **25**, 587–617 (2007).
8. Murata, T. et al. Molecular Basis of Epstein-Barr Virus Latency Establishment and Lytic Reactivation. *Viruses* 13, (2021).
9. Ascherio, A. & Munger, K. L. Epidemiology of multiple sclerosis: from risk factors to Prevention-An update. *Semin Neurol.* **36**, 103–114 (2016).
10. Läderach, F. & Münz, C. Epstein Barr Virus Exploits Genetic Susceptibility to Increase Multiple Sclerosis Risk. *Microorganisms* 9, (2021).
11. Li, Z. X., Zeng, S., Wu, H. X. & Zhou, Y. The risk of systemic lupus erythematosus associated with Epstein-Barr virus infection: a systematic review and meta-analysis. *Clin. Exp. Med.* **19**, 23–36 (2019).
12. Balandraud, N. & Roudier, J. Epstein-Barr virus and rheumatoid arthritis. *Joint Bone Spine*. **85**, 165–170 (2018).
13. Ebert, A. et al. Risk of inflammatory bowel disease following hospitalisation with infectious mononucleosis: A Danish nationwide cohort study (1977–2021). *Nat. Commun.* **15**, 8383 (2024).
14. Vindegaard, N., Petersen, L. V., Lyng-Rasmussen, B. I., Dalsgaard, S. & Benros, M. E. Infectious mononucleosis as a risk factor for depression: A nationwide cohort study. *Brain Behav. Immun.* **94**, 259–265 (2021).
15. Houen, G. & Trier, N. H. Epstein-Barr virus and systemic autoimmune diseases. *Front. Immunol.* **11**, 587380 (2020).
16. Murata, T. Epstein-Barr virus: the molecular virology and the associated diseases. *Fujita Med. J.* **9**, 65–72 (2023).
17. Björkegren, E. & Svaleryd, H. Birth order and health disparities throughout the life course. *Soc. Sci. Med.* **318**, 115605 (2023).
18. Reini, K. & Saarela, J. Birth order and sickness absence: Register-based evidence from Finland. *PLoS One*. **18**, e0280532 (2023).
19. Ponsonby, A. L. et al. Exposure to infant siblings during early life and risk of multiple sclerosis. *JAMA* **293**, 463–469 (2005).
20. Kikkawa, T. et al. Birth order and paediatric allergic disease: A nationwide longitudinal survey. *Clin. Exp. Allergy*. **48**, 577–585 (2018).
21. Rostgaard, K. et al. Sibship structure and risk of infectious mononucleosis: a population-based cohort study. *Int. J. Epidemiol.* **43**, 1607–1614 (2014).
22. Rostgaard, K., Nielsen, N. M., Melbye, M., Frisch, M. & Hjalgrim, H. Siblings reduce multiple sclerosis risk by preventing delayed primary Epstein-Barr virus infection. *Brain* **146**, 1993–2002 (2023).
23. Van Gool, A. et al. Analytical techniques for multiplex analysis of protein biomarkers. *Expert Rev. Proteom.* **17**, 257–273 (2020).
24. Stefura, W. P., Graham, C., Lotoski, L. & HayGlass, K. T. Improved Methods for Quantifying Human Chemokine and Cytokine Biomarker Responses: Ultrasensitive ELISA and Meso Scale Electrochemiluminescence Assays. *Methods Mol. Biol.* 91–114 (2019). (2020).
25. Kjerulff, B. et al. Lifestyle and demographic associations with 47 inflammatory and vascular stress biomarkers in 9876 blood donors. *Commun. Med.* **4**, 50 (2024).
26. Califf, R. M. Biomarker definitions and their applications. *Exp. Biol. Med.* **243**, 213–221 (2018).
27. Joyner, M. J. & Paneth, N. Promises, promises, and precision medicine. *J. Clin. Invest.* **129**, 946–948 (2019).
28. König, I. R., Fuchs, O., Hansen, G., von Mutius, E. & Kopp, M. V. What is precision medicine? *Eur. Respir J.* **50**(4), 1700391 (2017).
29. Schüssler-Fiorenza Rose, S. M. et al. A longitudinal big data approach for precision health. *Nat. Med.* **25**, 792–804 (2019).
30. Erikstrup, C. et al. Cohort profile: the Danish blood donor study. *Int. J. Epidemiol.* **52**, e162–e171 (2023).
31. Hansen, T. F. et al. DBDS genomic cohort, a prospective and comprehensive resource for integrative and Temporal analysis of genetic, environmental and lifestyle factors affecting health of blood donors. *BMJ Open.* **9**, e028401 (2019).
32. Leung, A. K. C., Lam, J. M. & Barankin, B. Infectious mononucleosis: an updated review. *Curr. Pediatr. Rev.* **20**, 305–322 (2024).
33. Divani, A. A., Luo, X., Datta, Y. H., Flaherty, J. D. & Panoskaltsis-Mortari, A. Effect of oral and vaginal hormonal contraceptives on inflammatory blood biomarkers. *Mediators Inflamm.* 379501 (2015). (2015).
34. Park, H. Association between oral contraceptive use and the High-Sensitivity C-Reactive protein level in premenopausal Korean women. *Healthc. (Basel)* **10**(2), 361 (2022).
35. Sørensen, C. J. et al. Combined oral contraception and obesity are strong predictors of low-grade inflammation in healthy individuals: results from the Danish blood donor study (DBDS). *PLoS One*. **9**, e88196 (2014).
36. Gaskins, A. J. et al. Endogenous reproductive hormones and C-reactive protein across the menstrual cycle: the biocycle study. *Am. J. Epidemiol.* **175**, 423–431 (2012).
37. Ballantyne, C. M. & Nambi, V. Markers of inflammation and their clinical significance. *Atheroscler Suppl.* **6**, 21–29 (2005).

38. Ansar, W. & Ghosh, S. Inflammation and inflammatory diseases, markers, and mediators: role of CRP in some inflammatory diseases. in Biology of C Reactive Protein in Health and Disease (eds Ansar, W. & Ghosh, S.) 67–107 (Springer India, New Delhi, (2016).
39. Ngo, S. T., Steyn, F. J. & McCombe, P. A. Gender differences in autoimmune disease. *Front. Neuroendocrinol.* **35**, 347–369 (2014).
40. Gleicher, N. & Barad, D. H. Gender as risk factor for autoimmune diseases. *J. Autoimmun.* **28**, 1–6 (2007).
41. Schmidt, L. S. et al. Infectious exposure in the first years of life and risk of central nervous system tumours in children: analysis of birth order, childcare attendance and seasonality of birth. *Br. J. Cancer*. **102**, 1670–1675 (2010).
42. Perrillat, F. et al. Day-care, early common infections and childhood acute leukaemia: a multicentre French case-control study. *Br. J. Cancer*. **86**, 1064–1069 (2002).
43. Kamper-Jørgensen, M., Wohlfahrt, J., Simonsen, J. & Benn, C. S. The childcare database: a valuable register linkage. *Scand. J. Public. Health*. **35**, 323–329 (2007).
44. Ansar, W. & Ghosh, S. C-reactive protein and the biology of disease. *Immunol. Res.* **56**, 131–142 (2013).
45. Pope, J. E. & Choy, E. H. C-reactive protein and implications in rheumatoid arthritis and associated comorbidities. *Semin. Arthritis Rheum.* **51**, 219–229 (2021).
46. Vermeire, S., Van Assche, G. & Rutgeerts, P. C-reactive protein as a marker for inflammatory bowel disease. *Inflamm. Bowel Dis.* **10**, 661–665 (2004).
47. Sproston, N. R. & Ashworth, J. J. Role of C-Reactive protein at sites of inflammation and infection. *Front. Immunol.* **9**, 754 (2018).
48. Schoeler, T., Pingault, J. B. & Kutalik, Z. The impact of self-report inaccuracy in the UK biobank and its interplay with selective participation. *Nat. Hum. Behav.* **9**, 584–594 (2024).
49. Rostgaard, K. et al. Primary Epstein-Barr virus infection with and without infectious mononucleosis. *PLOS ONE*. **14**, e0226436 (2019).
50. Rostgaard, K., Wohlfahrt, J. & Hjalgrim, H. A genetic basis for infectious mononucleosis: evidence from a family study of hospitalized cases in Denmark. *Clin. Infect. Dis.* **58**, 1684–1689 (2014).
51. Mosbech, J. et al. The National patient registry. Evaluation of data quality. *Ugeskr Laeger*. **157**, 3741–3745 (1995).
52. Rigas, A. S. et al. The healthy donor effect impacts self-reported physical and mental health – results from the Danish blood donor study (DBDS). *Transfus. Med.* **29**, 65–69 (2017).
53. Chastain, E. M. L. & Miller, S. D. Molecular mimicry as an inducing trigger for CNS autoimmune demyelinating disease. *Immunol. Rev.* **245**, 227–238 (2012).
54. Rostgaard, K., Søegaard, S. H., Stensballe, L. G. & Hjalgrim, H. Antimicrobials use and infection hospital contacts as proxies of infection exposure at ages 0–2 years and risk of infectious mononucleosis. *Sci. Rep.* **13**, 21251 (2023).
55. Winter, J. R. et al. Predictors of Epstein-Barr virus serostatus and implications for vaccine policy: A systematic review of the literature. *J. Glob Health*. **10**, 010404 (2020).
56. Kuri, A. et al. Epidemiology of Epstein-Barr virus infection and infectious mononucleosis in the united Kingdom. *BMC Public. Health*. **20**, 912 (2020).
57. Kenney, A. D. et al. Human genetic determinants of viral diseases. *Annu. Rev. Genet.* **51**, 241–263 (2017).
58. Burgdorf, K. S. et al. Digital questionnaire platform in the Danish blood donor study. *Comput. Methods Programs Biomed.* **135**, 101–104 (2016).
59. Schmidt, M., Pedersen, L. & Sørensen, H. T. The Danish civil registration system as a tool in epidemiology. *Eur. J. Epidemiol.* **29**, 541–549 (2014).
60. Mainz, J., Hess, M. H. & Johnsen, S. P. The Danish unique personal identifier and the Danish civil registration system as a tool for research and quality improvement. *Int. J. Qual. Health Care*. **31**, 717–720 (2019).
61. Lynge, E., Sandegaard, J. L. & Rebolj, M. The Danish National patient register. *Scand. J. Public. Health*. **39**, 30–33 (2011).
62. Bellis, M. A., Hughes, K., Hughes, S. & Ashton, J. R. Measuring paternal discrepancy and its public health consequences. *J. Epidemiol. Community Health*. **59**, 749–754 (2005).
63. Dinh, K. M. et al. Low-grade inflammation is negatively associated with physical Health-Related quality of life in healthy individuals: results from the Danish blood donor study (DBDS). *PLoS One* **14**(3), e0214468 (2019).

Author contributions

RPK, JBD, OBD, drafted the original manuscript. RPK, JBD, OBD, BK, KR, JD, SHS, ECR, MS, KSB, JTB, CM, HU, SB, ES, BAJ, MTB, MN, SRO, OBP, CE, TFH, HH advised on study design and participant selection. MS coordinated sample identification. BK, supervised, coordinated, and performed biomarker measurements. BK, JD, RPK, OBD, JBD implemented quality control measures. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-95276-4.

Correspondence and requests for materials should be addressed to R.P.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.