

Sex matters in children living with HIV: sex differences in virological, immunological and proteomic outcomes in early-treated pediatric HIV

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Abstract

Sex-related differences in HIV pathogenesis are well documented in adults, yet evidence in children living with HIV (CLHIV) remains limited and inconsistent. This study investigates sex-specific differences in virological, immunological and proteomic outcomes in an African cohort of early-treated CLHIV. We analyzed 159 children with perinatally acquired HIV from the EARTH multicenter cohort (2018–2021) in South Africa, Mozambique, and Mali. All initiated ART within 90 days of diagnosis. Outcomes included HIV-1 RNA viral decay, HIV-1 DNA reservoir size, immunological progression, ART adherence, and plasma proteomics (92 Olink proteins). Bayesian regression and survival models were used. Baseline characteristics were comparable between sexes. Females showed steeper viral decay than males (Δ slope = $-0.01 \log_{10}$ copies/mL/year; 95% CrI: -0.01 to -0.001 ; PP = 0.994), directional support for faster reservoir decline (PP = 0.87), and lower hazard of immunological progression (HR = 0.62; 95% CrI: 0.29–1.24; PP = 0.91), despite a tendency toward higher suboptimal adherence (HR = 1.29; 95% CrI: 0.78–2.13; PP = 0.85). MCP-2 expression was higher in females (BF = 4.7). Sex-specific differences were observed in early-treated CLHIV. Females showed faster viral decay and lower immunological progression, while reservoir and adherence differences were less certain. These findings highlight sex as a relevant determinant of pediatric HIV disease trajectories and responses to treatment.

Introduction

Approximately 39 million people were living with human immunodeficiency virus (HIV) at the end of 2022 including 1.5 million children (CLHIV), and 53% were female¹. Differences in HIV pathogenesis between women and men are well recognized and likely reflect a combination of environmental, social, and genetic factors^{2–5}. While sex differences have a biological basis, including hormonal and genetic factors, gender differences arise from social and behavioral determinants, including inequities in socioeconomic status, access to healthcare, and education.. Including inequities in socio-economic status, access to health care, and education^{3,5,6}. Although gender-related factors may indirectly influence outcomes through caregiving, treatment access, and adherence, this study focuses primarily on biological sex differences. Sexual dimorphism in the mammalian immune system occurs in both innate and adaptive immune responses, including differences in key immune-related signal pathways, number of T cells, B cells, and immunoglobulins, and the activation status of different cell subsets⁷. In the context of HIV, sex can affect different phases of HIV pathogenesis, including infection acquisition, viral replication, immune reconstitution, viral persistence, antiretroviral therapy (ART) efficacy, and cure⁵. Females tend to have lower HIV viral loads during early infection and exhibit better control of viral replication in the absence of ART. However, several studies report higher HIV-related morbidity and mortality among females. These sex-related differences have been attributed to the immunomodulatory effects of the major female sex steroids hormones, such as estrogen and progesterone, and genetic factors associated with X-linked genes including certain toll-like receptors^{5,8}. Increased interferon (IFN)-alpha production by plasmacytoid dendritic cells in response to HIV-derived TLR ligands is reported in

women and correlates with plasma progesterone levels; a potential biological mechanism underlying the observed sex differences in HIV viral loads ^{9,10}.

Although HIV sex-related differences have been well described in adults, studies reporting differences in children are limited and with conflicting results. Some studies reported lower baseline HIV RNA levels in females than males or faster viral decays with no difference in CD4 cell count or percentage ^{11–13}.

Another study reported lower CD4 cell counts in girls ¹⁴ in contrast to another reporting higher CD4 cell percentages among girls ¹⁵. These inconsistencies suggest that sex-related differences observed in adults cannot necessarily be directly extrapolated to pediatric HIV infection.

The sex differences in HIV-1 persistence and reservoir size are also contradictory with some reporting lower HIV-1 DNA levels in females than males ^{16,17} and comparable levels in others ^{18–20}; moreover, more effective immune selection of HIV-1 reservoir cells in females has also been reported ²¹.

In summary, there is a clear knowledge gap on the sex differences of pediatric HIV pathology. These inconsistencies, together with the scarcity of data in early-treated children, highlight a critical gap in understanding sex-specific HIV disease trajectories in pediatric populations. This gap is further compounded by developmental pharmacokinetic and pharmacodynamic differences between children and adults, which may affect antiretroviral drug exposure, safety, and efficacy.

In this study, we analyzed children with perinatally acquired HIV enrolled in the multicenter EARTH cohort to investigate sex differences in longitudinal HIV-1 RNA viral decay, total HIV-1 DNA reservoir dynamics, immunological progression, ART adherence, and immune proteomic profiles. By integrating these complementary outcomes in children initiating ART early after diagnosis, we aimed to characterize whether sex-specific differences in HIV disease trajectories are already detectable during early childhood.

Results

Baseline Characteristics

Of 159 participants included in the analysis, 74 (46.5%) were female and 85 (53.5%) males. The median duration of follow-up was 39.7 months (IQR: 29.3–44.1), higher in males than females (PP ($\Delta > 0$) = 0.150). The median age at HIV diagnosis was 31.0 days of life (IQR: 0.0–47.0), with ART initiation at a median of 33.0 days (IQR: 23.0–75.0). Age at ART initiation was similar between sexes, with a median of 33 days of life overall (IQR: 23–75), 34 days (IQR: 26–63) in females, and 32.5 days (IQR: 18–82.5) in males (Posterior Pr($\Delta > 0$) = 0.716). for sex differences in age at diagnosis approached 0.5, suggesting minimal evidence for systematic differences between females and males.

Baseline viral load at ART initiation was ≥ 5 log₁₀ copies/mL in 44.7% of participants, with a higher proportion among females than males (51.4% vs. 38.8%; PP ($\Delta > 0$) = 0.942). Most children presented

with preserved immune status at baseline, with 93.5% having CD4 percentages $\geq 25\%$. Although the observed proportion was higher among females (98.6% vs. 88.9%), the posterior probability ($PP(\Delta > 0) = 0.992$) indicates that the difference is highly unlikely to favor females and instead strongly supports a higher proportion among males. Median absolute CD4 counts were higher in females than males (2330 [1616;2726] vs. 1864 [1492;2729] cells/mm³), ($PP(\Delta > 0) = 0.970$).

At baseline, the median HIV reservoir size was $4.7 \text{ copies} \times 10^3$ (IQR: $1.1 - 12.9 \times 10^3$). Median values were somewhat higher in males than females (5.67 [1.51;13.90] vs. 3.65 [1.14;12.22] copies), $PP(\Delta > 0) = 0.283$.

The baseline median nadir CD4 percentage was 24.0% (IQR: 19.0–29.8), with slightly higher values observed in females as compared with males. (26.4% [19.9;33.3] vs. 22.9% [18.6;27.8]), $PP(\Delta > 0) = 0.998$.

Baseline clinical and immunological characteristics of the cohort are shown in Table 1.

Table 1
Baseline clinical and immunological characteristics of the cohort

Characteristics	Total N = 159	Female N = 74	Male N = 85	Posterior Pr($\Delta > 0$)
Enrolling site, n (%)				
South Africa				
Tygerberg Hospital (Cape Town)	43 (27.0%)	25 (33.8%)	18 (21.2%)	0.964
Perinatal Health Research Unit (Johannesburg)	36 (22.6%)	13 (17.6%)	23 (27.1%)	0.080
Africa Health Research Institute (KwaZulu Natal)	24 (15.1%)	8 (10.8%)	16 (18.8%)	0.079
Mozambique				
Centro de Investigaçao en Saúde de Manhica (Maputo)	29 (18.2%)	15 (20.3%)	14 (16.5%)	0.732
Ariel Foundation (Maputo)	23 (14.5%)	11 (14.9%)	12 (14.1%)	0.561
Mali				
Gabriel Touré Hospital (Bamako, Mali)	4 (2.52%)	2 (2.70%)	2 (2.35%)	0.567
Time to follow-up, Months				
Median [IQR]	39.7 [29.3;44.1]	38.6 [28.3;44.1]	41.0 [31.7;44.8]	0.150
Preterm birth, n (%)				
Yes	51 (34.0%)	24 (33.8%)	27 (34.2%)	0.533
Weight-for-age z-score				
WAZ <2SD Zscore	42 (26.4%)	21 (28.4%)	21 (24.7%)	0.699
Age at HIV diagnosis (days of life)				
Median [IQR]	31.0 [0.00;47.0]	30.0 [0.00;44.8]	31.0 [0.00;48.0]	0.753
Age at ART initiation (days of life)				

Characteristics	Total N = 159	Female N = 74	Male N = 85	Posterior Pr($\Delta > 0$)
Median [IQR]	33.0 [23.0;75.0]	34.0 [26.0;63.0]	32.5 [18.0;82.5]	0.716
ART regimen initiation, n (%)				
3TC + ABC/AZT+LPVr	105 (66.0%)	52 (70.3%)	53 (62.4%)	0.864
3TC + ABC+NVP	1 (0.63%)	1 (1.35%)	0 (0.00%)	0.464
3TC + AZT+NVP	27 (17.0%)	10 (13.5%)	17 (20.0%)	0.682
Children adherence, n (%)				
Suboptimal	111 (69.8%)	59 (79.7%)	52 (61.2%)	0.994
Visits with suboptimal adherence – % Median [IQR]		21.0 [10.0–38.0]	14.0 [0.0–33.0]	0.989
Baseline viral load (log10), n (%)				
≥ 5 logs	71 (44.7%)	38 (51.4%)	33 (38.8%)	0.942
Baseline CD4				
Percentage CD4, n (%)				
< 15%	7 (4.55%)	1 (1.37%)	6 (7.41%)	0.043
15–25%	3 (1.95%)	0 (0.00%)	3 (3.70%)	0.072
≥ 25%	144 (93.5%)	72 (98.6%)	72 (88.9%)	0.992
Absolute CD4 count (Cell/mm ³)				
Median [IQR]	2012 [1538;2736]	2330 [1616;2726]	1864 [1492;2729]	0.970
Nadir CD4 (%)				
Median [IQR]	24.0 [19.0;29.8]	26.4 [19.9;33.3]	22.9 [18.6;27.8]	0.998
Time to virological suppression (days)				

Characteristics	Total N = 159	Female N = 74	Male N = 85	Posterior Pr($\Delta > 0$)
Median [IQR]	790 [364;1134]	732 [249;1082]	829 [491;1198]	0.189
Reservoir				
Median [IQR]	4723 [1145;12887]	3654 [1143;12224]	5671 [1507;13903]	0.283

HIV-1 Viral decay

At ART initiation, although females had higher baseline viral load than males, females had a more pronounced plasma HIV-1 RNA decline thereafter. The model estimated that, for each additional year on ART, females experienced a steeper reduction in viral load, with an estimated difference in decay slope of -0.01 log10 copies/mL per year (95% credible interval (CrI): -0.01 to -0.001 , $PP(\Delta < 0) = 0.994$), indicating a strong sex-specific effect on virological response. Estimated marginal viral load trajectories by sex are shown in Fig. 1, illustrating lower predicted viral loads over time in females relative to males.

Median time to suppression was 790 days (IQR: 364–1134) overall, with shorter times in females (732 days, IQR: 249–1082) compared with males (829 days, IQR: 491–1198), $PP(\Delta < 0) = 0.189$.

Figure 1. Longitudinal HIV-1 viral load trajectories by sex following ART initiation. Model-estimated HIV-1 viral load trajectories by sex over time, showing faster decay in females.

HIV reservoir dynamics

During follow-up, reservoir size declined in both sexes. Females showed a steeper estimated decrease than males, with an annual difference in reservoir decay of -0.005 log10 copies per million cells per year (95% credible interval (CrI): -0.012 to 0.003). The posterior probability that reservoir decay was faster in females was 0.870, indicating directional support for a sex-related difference. Estimated marginal reservoir trajectories by sex are shown in Figure 2, illustrating consistently lower predicted reservoir levels over time among females compared with males.

Clinical outcomes

Immunological Progression

Females demonstrated a lower hazard of immunological progression than males (Fig. 3). The estimated hazard ratio was 0.62 (95% credible interval (CrI): 0.29–1.24), corresponding to a 0.910 posterior

probability that females experienced a reduced risk of progression.

Treatment Adherence

In contrast, females showed a higher risk of suboptimal treatment adherence. The estimated hazard ratio for suboptimal adherence was 1.29 (95% CrI: 0.78–2.13), with a 0.850 posterior probability that suboptimal adherence was more frequent among females (Fig. 4).

Suboptimal treatment adherence at any time during follow-up was reported in 69.8% of participants. A higher proportion of females experienced this suboptimal adherence than males (79.7% vs. 61.2%) (PP ($\Delta > 0$) = 0.994).

Proteomics

Among the proteins analyzed, MCP-2, a chemokine involved in monocyte recruitment and inflammatory signaling, showed the strongest evidence of sex-related differential expression, with higher levels observed in females (9.86, IQR: 9.12–10.68) than males (9.54, IQR: 8.94–10.05) (Bayes factor BF10 = 4.7; posterior probability (PP) = 0.991). This finding substantially supports a sex-specific difference.

CCL20, a chemokine implicated in mucosal immunity and lymphocyte trafficking, showed anecdotal evidence of differential expression (BF10 = 1.16), with posterior directional probabilities indicating higher expression in females (0.964). Similarly, IFN- γ , a key cytokine involved in antiviral immune responses and macrophage activation, exhibited anecdotal evidence (BF10 = 1.00), with posterior probabilities also suggesting higher levels in females (0.955). IL-18R1, the receptor for IL-18 and an important mediator of inflammatory signaling and Th1 immune responses, presented anecdotal evidence of differential expression (BF10 = 1.14), again with posterior directional probabilities consistent with higher expression in females (0.959). IL-8, a chemokine primarily involved in neutrophil recruitment and acute inflammatory responses, likewise showed anecdotal evidence (BF10 = 1.32), with posterior probabilities indicating higher expression in females (0.966). Across these proteins, effect sizes were modest, with most log₂ fold changes indicating higher expression in females.

This pattern was observed across multiple inflammatory and immune-related proteins, including chemokines, cytokines, growth factors, and markers of immune activation. Differential protein expression results are summarized in Fig. 5.

Discussion

In this multicenter pediatric cohort of children with perinatally acquired HIV receiving early ART, we identified evidence of sex-specific differences, most consistently in viral decay, with more limited and suggestive findings for reservoir and clinical outcomes, and modest proteomic differences. Despite broadly comparable baseline clinical characteristics, females exhibited faster viral decay, a tendency

toward greater long-term HIV reservoir reduction and a lower hazard of immunological progression, despite less favorable treatment adherence. Collectively, these findings support biological sex having a meaningful role in shaping early HIV disease trajectories and treatment responses in childhood.

A central finding of this study is the more pronounced decline in plasma HIV-1 RNA observed in females over time following ART initiation. Although females presented with higher baseline viral load compared with males, longitudinal modelling indicated a steeper reduction in viral load during follow-up among females, with a high posterior probability supporting faster viral decay. The observation of faster decay despite a less favorable baseline virological profile suggests that the longitudinal difference cannot be explained simply by lower viral burden among females at ART initiation. These findings are consistent with prior observations from pediatric cohorts, including the European CARMA cohort, where female children exhibited faster early viral decay after treatment initiation, independent of baseline viral load ¹³. In addition, strong sex biases in pediatric HIV control have been previously described, with females over-represented among children exhibiting spontaneous or elite viral control, as shown by a multicohort study of pediatric HIV infection where most elite controllers were female, suggesting a biological advantage in immune-mediated viral control among girls ^{22,23}.

In our study, reservoir size declined in both sexes during follow-up, with females showing a steeper estimated decrease than males. However, the magnitude and certainty of this difference were more limited than for viral decay: the credible interval included the null, while the posterior probability provided directional support for faster reservoir decline in females. These results should therefore be interpreted as suggestive rather than conclusive evidence of sex-specific reservoir dynamics. Emerging evidence in adults further demonstrates sex-specific differences in HIV-1 reservoir biology. In a recent study using near-full-length proviral sequencing in long-term ART-suppressed adults, females exhibit a distinct reservoir landscape, characterized by lower proviral complexity and a higher proportion of intact proviruses integrated into transcriptionally repressive chromatin, supporting sex-dependent immune selection pressures shaping reservoir persistence ²¹.

Females in our cohort also demonstrated a lower hazard of immunological progression than males, despite female children having a higher probability of suboptimal adherence as measured by caregiver-reported indicators. Together, these findings suggest a potential contribution of intrinsic biological factors that may confer partial protection against immune deterioration in females. At baseline, females also showed higher absolute and nadir CD4 measurements, suggesting that some sex-related differences in immune status may already be present early in the disease course. Nevertheless, the uncertainty around the longitudinal estimate means that the observed difference in immunological progression should be interpreted cautiously.

Sex-specific differences were also observed in inflammatory and immune signaling proteins. Among the 92 proteins evaluated, MCP-2 showed the strongest evidence of differential expression, with higher levels in females. MCP-2 is involved in monocyte recruitment and inflammatory signaling and may therefore reflect sex-related variation in innate immune activity. Several additional inflammatory and

chemotactic mediators, including IL-8, IL-18R1, CCL20 and IFN- γ , showed directionally higher expression in females. While heightened inflammatory signaling in adults is often associated with immunopathology, in early-treated pediatric HIV infection this profile may reflect a more effective antiviral immune response. Whether such immune activation confers long-term benefit or risk remains uncertain and warrants further longitudinal investigation.

Overall, these proteomic patterns suggest enhanced innate immune activation and pro-inflammatory signaling in females, which may contribute to the observed differences in virological decay and reservoir dynamics. Potential mechanisms may include sex-related differences in immune maturation, hormonal influences on immune regulation, or differential innate immune sensing of viral antigens^{24–27}.

Several limitations should be acknowledged. Likely due to our limited sample size, some estimates, particularly those related to reservoir dynamics, were characterized by wide credible intervals. Adherence was assessed using caregiver-reported measures and may be subject to reporting bias. Finally, despite the multicenter design, residual confounding factors due to unmeasured biological or social factors cannot be excluded. In addition, given the modest effect sizes and the exploratory nature of the proteomic analyses, these findings should be interpreted cautiously and validated in larger pediatric cohorts. Further longitudinal studies integrating virological, immunological, and proteomic data will be essential to clarify whether these sex-related immune differences translate into clinically meaningful long-term outcomes.

In conclusion, our findings reinforce the importance of integrating sex considerations into pediatric HIV research from the earliest stages. Sex-specific differences in virological control, immune outcomes, and inflammatory signaling are already evident in early-treated children with perinatally acquired HIV. Sex shapes not only social experiences but also biological susceptibility to disease, and inattention to sex differences in infancy may contribute to persistent and potentially irreversible health disparities.

Data on sex-specific outcomes in early life remain limited, and this study contributes important evidence to inform both research and policy. Our findings have important implications for pediatric HIV management, cure research, and clinical trial design, highlighting the need to treat sex not merely as a demographic variable but as a fundamental biological determinant of treatment response.

Methods

Ethical approval

The study was approved by Mozambique National Bioethics Committee, 53/CNBS/2018; Stellenbosch University Bioethics Committee, 1832; University of Witwatersrand and AHRI Bioethics Committee, 171114, and Bamako Faculté de Médecine Bioethics Committee, 2019/53. Mothers signed an informed consent for their infants as well as their participation in the study.

Study design and participants

A total of 215 children enrolled in the EARTH prospective, multicenter cohort between May 1st, 2018, and August 1st, 2021. Recruitment occurred across six sites, comprising four urban and two rural settings: Johannesburg and Cape Town (South Africa); uMkhanyakude District in KwaZulu-Natal (South Africa); Maputo and Manhiça (Mozambique); and Bamako (Mali).

Of the 215 enrolled children, 56 were excluded, primarily due to having fewer than five viral load measurements, resulting in 159 participants included in the present analysis. Eligible participants included infants with perinatally acquired HIV who initiated ART within 90 days after diagnosis, as well as breastfed children diagnosed with HIV at ≤ 90 days of age who commenced ART within 90 days after diagnosis. Exclusion criteria included: a second or subsequent negative HIV RNA PCR test, diagnosis of malignancy, current use of immunosuppressive therapy (defined as >15 days of treatment with >2 mg/kg/day of prednisone-equivalent), and withdrawal of informed consent. The characteristics of the EARTH Cohort are described elsewhere²⁸.

Procedures

The independent Ethics and Institutional Review Boards/Committees of participating institutions and countries approved the study. Participant management and biological sampling were performed in accordance with the Declaration of Helsinki, the International Ethical Guidelines for Health-related Research Involving Humans CIOMS-WHO (2016), the Additional Protocol to the Oviedo Convention on biomedical research (2005) and the European Commission Ethical considerations for clinical trials on medicinal products conducted with minors (2017). Personal data was processed according to the European General Data Protection Regulation (GDPR) and the local legislation.

Written informed consent was obtained from parents or legal guardians of all enrolled participants, with assent obtained from minor participants or minor caregivers where required by local regulations. A dedicated Expert Working Group provided support to ensure compliance with the applicable rules and provide effective practical solutions for addressing ethical and regulatory hurdles related to sharing data and transferring samples²⁹. The screening procedures and laboratory sample collection and analysis are described elsewhere²⁸.

Plasma isolation and targeted proteomics

EDTA whole blood samples were processed according to EARTH study Standard Operating Procedures (SOP) at local laboratories. Details of these procedures were previously described³⁰. Baseline plasma samples were randomized across 96-well plates, and protein quantification was performed using the Multiplex Proximity Extension Assay (PEA) using the Olink Target 96 Inflammation Olink panel. A total of 1 μ L of plasma per sample was used to assess the presence of inflammatory proteins. Protein expression levels were reported as normalized protein expression (NPX), an arbitrary unit provided by Olink and expressed on a log₂ scale, enabling comparison across samples; higher NPX values indicate higher protein expression³⁰.

Study definitions

Virological suppression: defined as ≥ 2 consecutive viral load (VL) measurements below the site-specific limit of detection (< 20 cp/mL at five sites and < 150 cp/mL at one site). Time to virological suppression defined as the time elapsed from ART initiation to the first occurrence of two consecutive viral load measurements meeting the definition of virological suppression.

Viral decay: defined as the trajectory of VL measurements from enrollment to the end of follow-up (death, loss to follow-up, or study completion). CD4 trajectories were described as changes in CD4 percentage over follow-up.

HIV reservoir size: defined as total HIV-1 DNA measured in purified Peripheral Blood Mononuclear Cells (PBMCs) by real time quantitative PCR (qPCR).

Nadir CD4 %: defined as the lowest CD4 percentage record for each participant.

Immunological progression: defined as loss of preserved immune status, operationalized as a decrease in CD4 percentage below 25% during follow-up.

Sub-optimal adherence was defined as the presence of any of the following events: a) amount of medication more than foreseen in the container, b) medication stock-out, c) ART interruption exceeding 10% of the days between visits or d) missing more than 10% of planned ART doses between visits, or e) delay in collecting ART greater than 10% in the number of days between visits.

Statistical analysis

General approaches

Primary inference was conducted within a Bayesian framework using R version 4.5.1. Missing data were handled using multiple imputation by chained equations (MICE) implemented in mice R package³¹. Figures and graphical summaries, including graphical abstracts, were developed using ggplot2 R package and BioRender^{32,33}.

Models were fitted using Hamiltonian Monte Carlo as implemented in the rstanarm R package³⁴. Weakly informative normal priors centered at zero were used for regression coefficients (standard deviation = 5 for Gaussian models and 2.5 for binomial models). Four Markov chains were run with sufficient iterations to ensure convergence, which was assessed using standard diagnostics.

Sex-based comparisons (females vs males) were performed using posterior distributions of group contrasts. Δ defined as the estimated value in females minus that in males. For all parameters, results are reported as posterior probabilities (PP), defined as the probability that the estimated effect is in the prespecified direction (e.g., $\Delta > 0$), with 95% credible intervals for longitudinal analysis results.

When data were multiply imputed, models were fitted separately within each imputed dataset and posterior draws were pooled to obtain a combined posterior distribution for inference. Sensitivity analyses using non-informative priors were conducted and are presented in the Supplementary Material.

Baseline Characteristics

Descriptive statistics were used to characterize the study population at baseline, both for the overall cohort and stratified by sex assigned at birth. Continuous variables were summarized using medians with interquartile ranges (IQR). Normality was assessed using the Shapiro-Wilk test. Categorical variables were described using frequencies and percentages, calculated based on the number of individuals with available data for each variable, excluding missing values unless otherwise specified.

Continuous variables were analysed using Bayesian Gaussian linear regression models with sex as the sole predictor, estimating mean differences between groups. For categorical variables, Bayesian binomial regression models with a logit link were fitted for each category level, and sex contrasts were expressed as the probability of absolute differences in predicted proportions between females and males.

HIV-1 Viral decay and HIV reservoir dynamics

For analyses of virological dynamics, only participants with at least five longitudinal plasma HIV-1 RNA measurements were included. Longitudinal trajectories of log₁₀-transformed viral load were modelled using a mixed-effects linear Bayesian Gaussian regression model, adjusted for clinically relevant covariates.

This model included fixed effects for sex, time on ART, age at ART initiation, and their association, as well as a random intercept to account for within-subject correlations. Sex-time interaction was assigned a weakly informative prior centered at zero, with variance constrained to reflect plausible biological effect sizes (with a mean of -0.01 and a standard deviation of 0.005), reflecting prior evidence suggesting faster viral decay in females reported in previous pediatric HIV studies¹³.

Similarly, the association between HIV reservoir size decay (measured as total HIV-1 DNA) and sex was assessed using a linear, mixed effects Bayesian Gaussian regression model adjusted for sex, age at ART initiation, and baseline CD4 percentage.

Clinical outcomes

Time-to-event analyses were primarily conducted using Bayesian survival models implemented in the brms R package³⁵. Survival Kaplan–Meier curves were used for descriptive purposes. Endpoints included overall time to CD4 immunological progression and sub-optimal adherence. Participants who did not experience the event of interest were censored at their last known follow-up; for virological suppression, individuals who never achieved suppression were censored at their last available viral load measurement.

Differences between survival KM curves by sex were assessed using the log-rank test as an exploratory, descriptive comparison. The effect of sex on survival was quantified using hazard ratios (HR) with corresponding 95% credible intervals derived from the posterior distribution. Survival probabilities and cumulative incidence functions are presented graphically with their corresponding 95% uncertainty intervals.

Proteomics

Sex differences in protein expression were assessed using Bayesian two-sample t-tests implemented in bayesfactor R package, with results reported as posterior log2 fold changes together with Bayes factors (BF10) to quantify the strength of evidence in favor of a sex effect ³⁶.

To facilitate interpretation and visualization of Bayesian results, Bayes factors (BF10) were categorized according to conventional evidence thresholds: $BF \geq 100$ (decisive evidence), $BF \geq 10$ (strong evidence), $BF \geq 3$ (substantial evidence), $BF \geq 1$ (anecdotal evidence supporting H1), and $BF < 1$ (evidence supporting the null hypothesis). These categories were used solely for graphical representation using a predefined color palette ³⁷.

Declarations

Data availability

The datasets generated and/or analyzed during the current study are not publicly available due to the sensitive nature of individual-level data from pediatric participants living with HIV and to protect participant confidentiality. De-identified data may be made available from the corresponding author upon reasonable request, subject to applicable ethical and institutional approvals, data-sharing agreements, and the conditions of the informed consent obtained from participants' parents or legal guardians.

Competing interests

The authors declare no conflict of interest directly related to the topic of this work. This work was supported by the EPIICAL project, funded through an independent grant by ViiV Healthcare UK.

Authors' contributions

All authors critically revised the manuscript and approved the final version. SDR conceptualized and designed the study and contributed to methodology development. NG contributed to proteomics and immunological assays. MC, AV, ML, TN, AI, SB, PV, KO, SF, DN, AJ, AL, NR, OB, TS, and NI coordinated study implementation across sites and oversaw data collection. MS, ML, EN, TN, and KR contributed to virologic assays. AO and AB contributed to data management.

Formal analysis was performed by IG and SDR. IG drafted the original manuscript. AB, MSP, and SDR contributed to manuscript review and editing. PP, CG, PR, SP, AT, and PR contributed to cohort design and

coordination.

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Figures

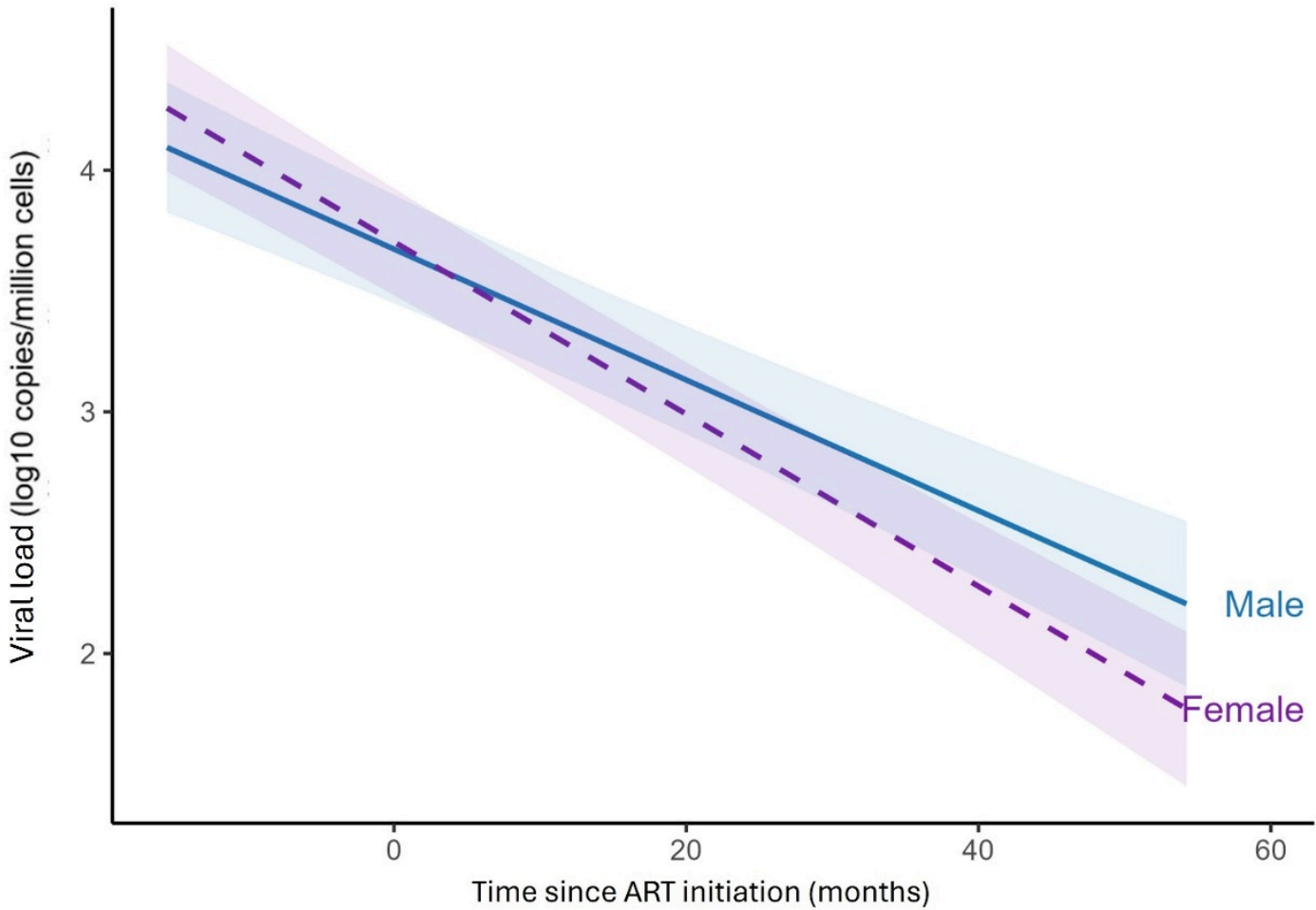


Figure 1

Longitudinal HIV-1 viral load trajectories by sex following ART initiation. Model-estimated HIV-1 viral load trajectories by sex over time, showing faster decay in females.

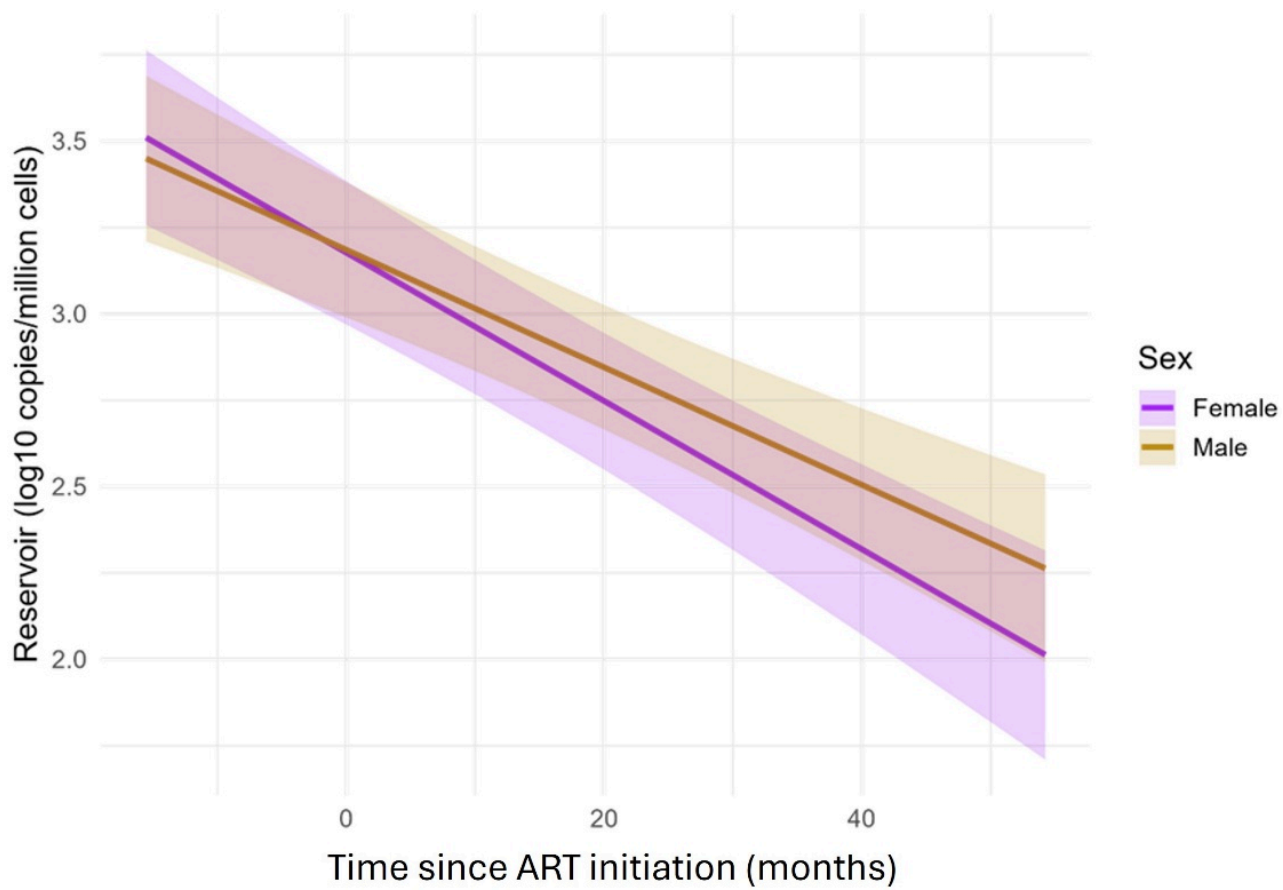


Figure 2

Longitudinal trajectories of HIV-1 reservoir size by sex following ART initiation. Model-estimated HIV-1 reservoir trajectories by sex, showing lower levels over time in females.

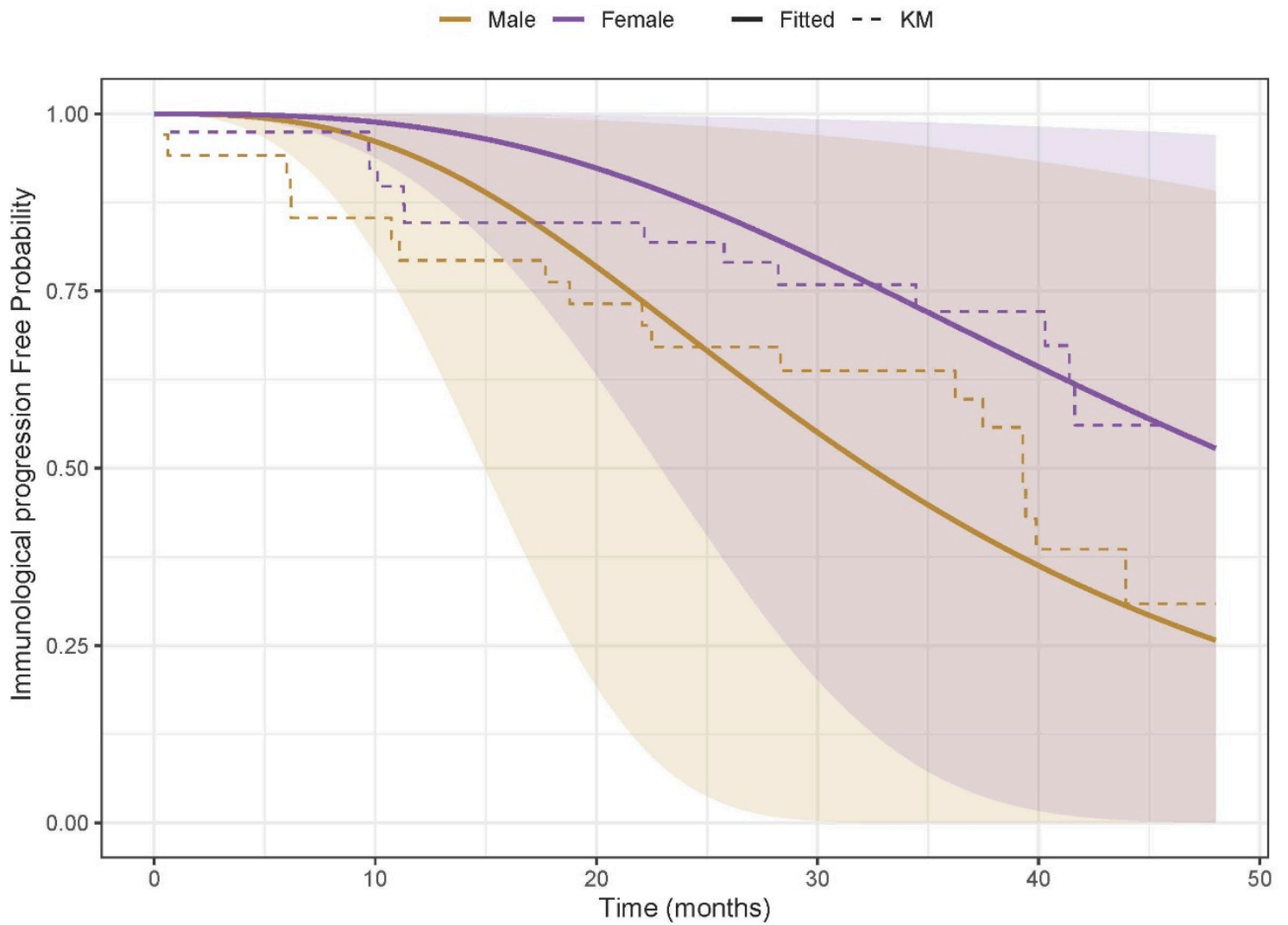


Figure 3

Time to immunological progression by sex: Weibull survival model and Kaplan–Meier estimates.
 Weibull model and Kaplan–Meier estimates of immunological progression by sex, showing lower hazard in females.

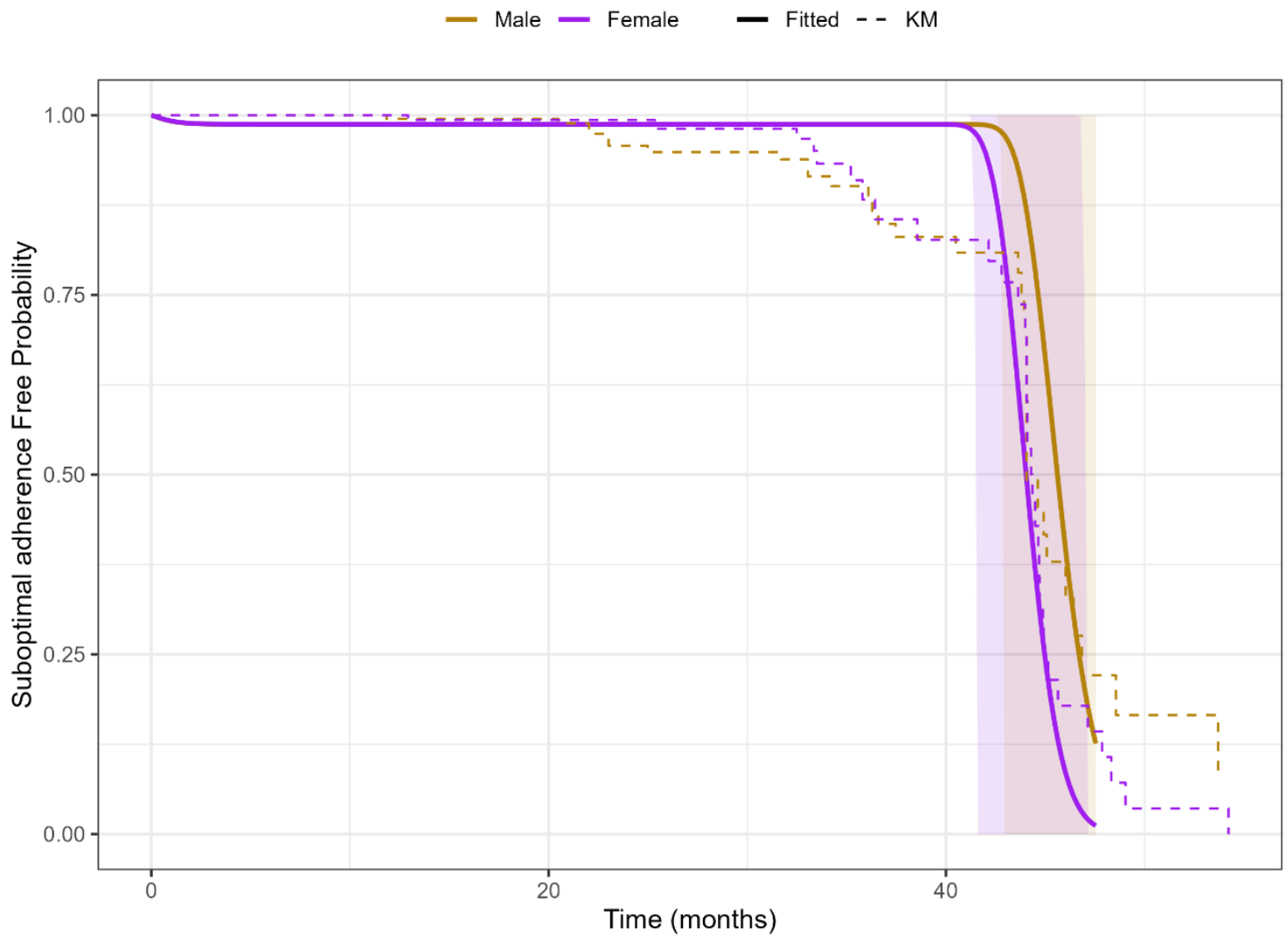


Figure 4

Time to suboptimal treatment adherence by sex: Weibull survival model and Kaplan–Meier estimates.
Time to suboptimal adherence by sex, showing higher risk in females.

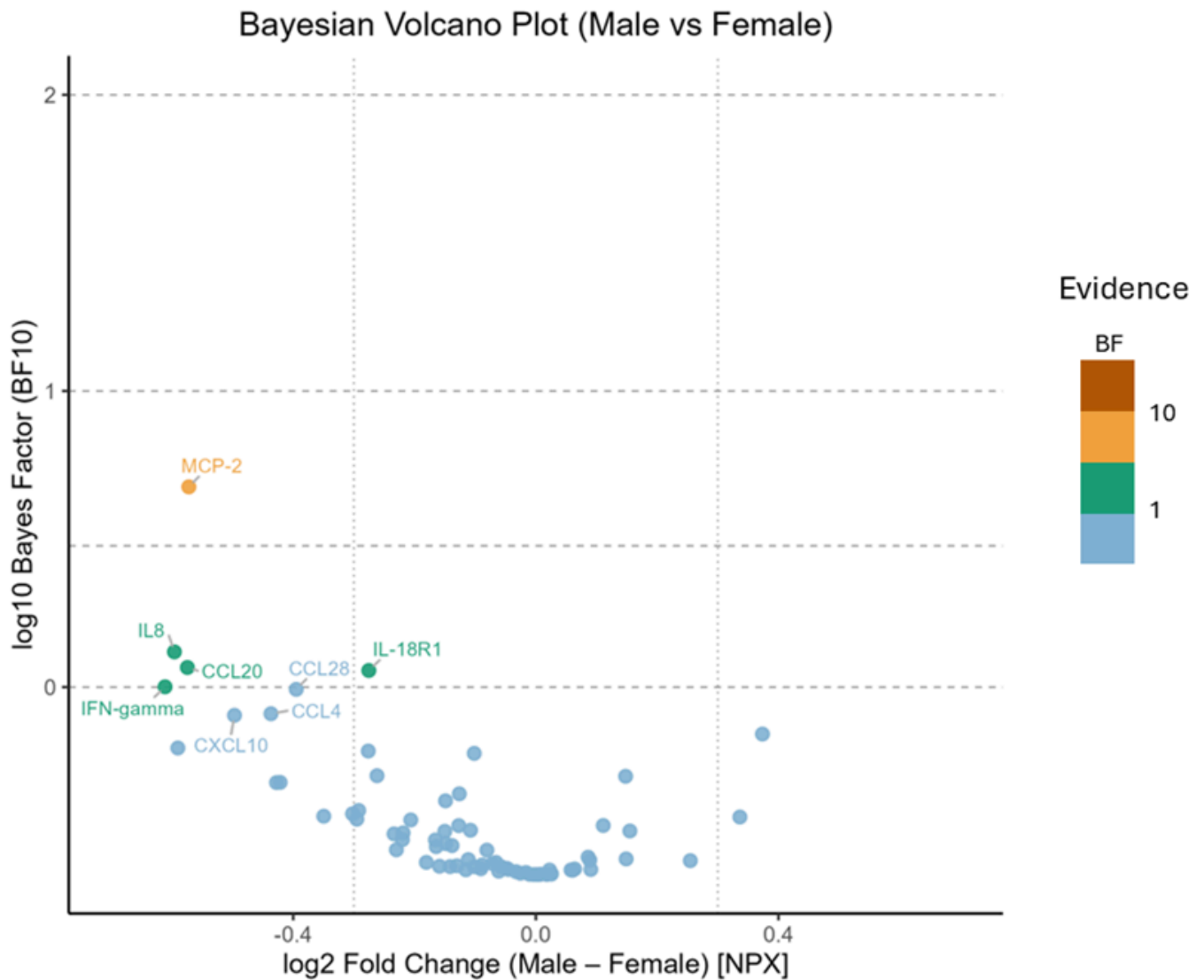


Figure 5

Bayesian volcano plot of differential protein expression by sex. The plot shows log₂ fold changes (male – female) on the x-axis and the strength of evidence (log₁₀ Bayes factor, BF₁₀) on the y-axis. Negative values indicate higher expression in females, whereas positive values indicate higher expression in males. Colors represent categories of evidence based on BF₁₀ thresholds.