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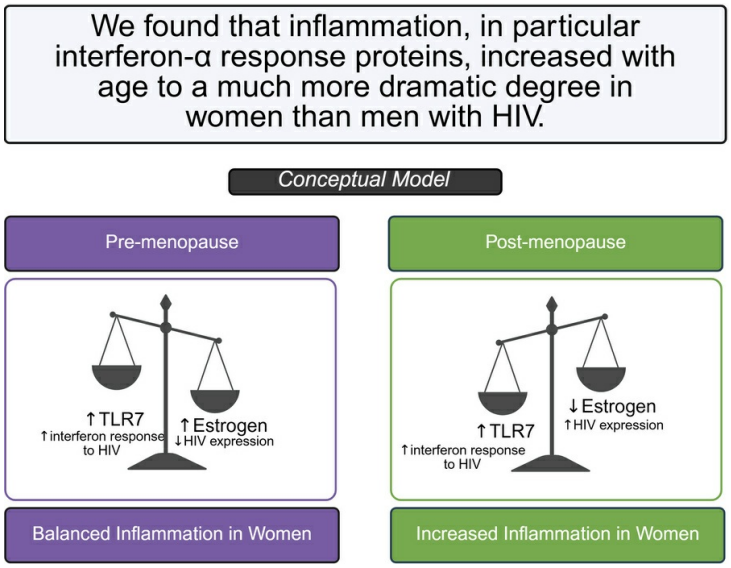
Graphical abstract



Age Modifies the Association Between Sex and the Plasma Inflammatory Proteome in Treated HIV

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Abstract

Background

Among antiretroviral therapy (ART)-suppressed people with HIV (PWH), women have higher levels of some inflammatory markers than men, but the broader effect of sex on the inflammatory proteome, and whether these differences are modified by age, remains unclear.

Methods

363 plasma inflammatory protein levels (Olink Inflammation Explore) were assessed in ART-suppressed PWH sampled from the Center for AIDS Research Network of Integrated Clinical Systems (CNICS). The relationship between sex and 363 plasma proteins – including 22 in the interferon-α response pathway – was assessed with linear regression models adjusting for confounders, assessing interactions by age.

Findings

Of 922 participants, 162 (18%) were female. The median age was 47, above which the majority of women had undetectable plasma anti-Müllerian hormone levels, a biomarker of ovarian reserve. Age impacted the influence of sex on the inflammatory proteome. Older age (>47) was associated with greater increases among women than men in 194 proteins. Interferon-α response proteins were higher in men in those ≤47 ($p=0.024$), but higher in women in those >47 ($p=0.005$, p-interaction <0.001). Among the 131 proteins associated with mortality risk ($q<0.05$), only 5 differed by sex among those ≤47, while 79 differed by sex in those >47, with nearly all being higher in women . Women had decreased mortality than men ≤47 ($p<0.001$) but had similar mortality >47 ($p=0.84$).

Interpretation

The menopausal transition appears to have a dramatic effect on systemic Type I interferon responses and the broader inflammatory proteome in women with HIV. Among older PWH, women have greater inflammation than men, including the majority of proteins linked with mortality risk.

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Introduction

As people with HIV (PWH) age worldwide, the aging-related comorbidity burden for PWH has become an increasing health challenge; despite improvements in life expectancy, discrepant comorbidity-free years of survival remain lower than in people without HIV (1, 2). Furthermore, comorbidity burden is not distributed equally among PWH. Prior epidemiologic studies have found sex differences in PWH across age strata, with women with HIV demonstrating a higher comorbidity burden than men, particularly at older ages (3, 4). The life expectancy gap is also greater among women with HIV than men with HIV (5). The mechanisms underlying these sex and age-associated differences are likely multifactorial, with immune activation playing a contributing role (6).

Several studies have demonstrated women with HIV have higher markers of immune activation and inflammation when compared to men with HIV, even when suppressed on antiretroviral therapy (ART) (7–9). One potential genetic mechanism for this difference lies in the greater expression among women of toll-like receptor 7 (TLR7), which senses viral RNA in plasmacytoid dendritic cells (pDCs) and serves as a potent inducer of interferon response (10, 11). Prior studies have found that females have had a stronger interferon response to HIV RNA stimulation of pDCs than men (10). This may be due to incomplete silencing of the X-chromosome, where the TLR7 gene is encoded (11–14). Estrogen effects also appear to play a role in increased inflammation in women with HIV, with studies finding that estrogen promotes HIV latency (15), and that HIV expression from infected cells increases across the menopausal transition (16). Taken together, these findings suggest that sex assigned at birth and ovarian aging may play key roles in the immune response to HIV infection.

Prior work by our group in the Center for AIDS Research (CFAR) Network of Integrated Clinical Systems (CNICS) cohort assessed sex differences in 13 plasma markers of immune activation and inflammation and their association with the cardiovascular outcomes of acute myocardial infarction, venous thromboembolism, stroke, and death in ART-suppressed PWH. We observed that women had higher immune activation

biomarkers than men and that these biomarkers appeared to predict clinical events more strongly in women than in men (17). Interestingly, age also played an important role, with women above the median cohort age of 47 displaying statistically significant increases in several immune activation biomarkers. These findings support the relationship between immune activation and clinical outcomes in PWH, and that age may modify the impact of sex on inflammation. Whether there are important sex differences in the larger plasma inflammatory proteome in this setting, or relevant effect modifications by age, remains unclear.

To address this question, we leveraged a case-cohort study within the CNICS cohort. Using the Olink Inflammation Explore proximity extension assay (PEA) proteomics platform, we evaluated the effect of sex and its interaction with age on 363 unique plasma proteins in ART-suppressed PWH.

Results

Participant demographics and characteristics

Of the 922 included participants in the sub-cohort (**Figure 1**), 162 (18%) were female sex at birth and a plurality identified as Black (46%) (**Table 1**). The median age was 47 years (IQR 39, 53). Age did not differ by sex assigned at birth, but the racial breakdown differed by sex with the majority of women identifying as Black (77%). Women also had a higher prevalence of obesity (52% versus 19%) and diabetes (19% versus 12%). As expected, women had a higher CD4+ T cell count than men (639 cells/mm^3 versus 566 cells/mm^3), but the nadir CD4+ T cell count did not differ by sex.

Sex differences in the inflammatory proteome are modified by age

Without stratifying by age, after adjustment for potential confounders and false discovery rate (FDR) correction, we identified only 24 (7%) of the 363 proteins differed by sex (**Figure 2**). The proteins assessed with their respective beta coefficients, p-values, and q-values are available in **Supplementary Data**. Several inflammatory (e.g., IL-6) and immune activation proteins (e.g., CD70) were higher in women than men, while

some immunoregulatory proteins (e.g., integrin β6 [ITGB6]) were lower in women. We next sought to determine whether there was evidence of sex-age interaction, dichotomizing age above and below the median age of 47. Importantly, the majority of women older than 47 had undetectable plasma AMH levels, suggesting late perimenopausal or menopausal status, while most women younger than 47 had detectable AMH levels (**Figure 3**). Using a cutoff of q<0.10 for the sex-by-age interaction term, there was evidence of interaction in 194/363 (53%) proteins (**Figures 4, Supplemental Figure 1**). The impact of older age on the inflammatory proteome was far stronger in women than men, with the vast majority of proteins increasing to a greater degree in women than men with older age. Additional adjustment for low-level viremia or restricting to those with an HIV RNA ≤40 copies/mL did not change the primary inferences regarding interaction between age and sex on the plasma proteome (**Supplementary Data**). Gene ontology enrichment analysis revealed that pathways involved in both innate and adaptive immune activation tended to increase to a greater degree with age among women than men (**Supplemental Figure 2**).

Given this striking evidence for interaction between sex and age on the inflammatory proteome, we performed stratified analyses above and below the median age of 47. Among those younger than 47, more proteins were higher in men than in women at the q<0.05 threshold (**Figure 5A**), many of which tended to be immunoregulatory, including ITGB6. In those above age 47, however, women tended to have higher levels of far more proteins than men at the FDR-corrected q<0.05 level, of which the majority were pro-inflammatory (**Figure 5B**).

Qualitative interaction between age and sex in the interferon-α pathway

Since HIV expression from cells is known to increase in women as they cross the menopausal transition (16), and women are known to have a more robust Type I interferon response to HIV than men (14, 18, 19), all 22

interferon-α induced proteins in the panel were also specifically assessed. Strikingly, levels of most proteins in the Hallmark interferon- α response pathway increased to a greater degree with age in women than men.

In the stratified analysis, in those ≤47, the levels of most interferon-α induced protein levels were higher in men (**Figure 6A**), while in those >47, nearly all proteins were higher in women (**Figure 6B**). To provide a quantitative assessment of interaction across all 22 proteins, we created an interferon-α response score as the mean of all z-scored proteins in the pathway and compared adjusted mean values across the age and sex strata (**Figure 6C**). While there was little evidence for a difference by age in the interferon-α response score in men, older age was associated with a dramatic increase in interferon-α score among women. Indeed, the interferon-α response score was higher in men in those ≤47 (p=0.024), but higher in women in those >47 (p=0.005, p for interaction <0.001).

Given these findings, we created similar scores for Hallmark IL-6 and coagulation pathways. Both of these pathways had evidence of interaction by sex and age (**Figures 7A and 7B**). Due to many of the proteins overlapping between these pathways, we found high correlation between the interferon-α response composite score with IL-6 and coagulation response scores (rho: 0.87, P<0.0001 and rho: 0.83, P<0.0001, respectively), and thus could not separate these pathways analytically.

Clinical significance of age-sex interactions in inflammatory proteins

In the overall cohort of 922 participants, 103 died of a non-accidental cause over a median 9 years of follow-up, of which 16 were female. In the mortality analysis, 127 (35%) of all proteins assessed were associated with increased hazard of mortality and 4 (1.1%) with decreased hazard of mortality after adjustment and FDR correction. When restricting the analysis to these 131 mortality-associated proteins, among those younger than 47, only five differed by sex, and all were higher in men (**Figure 8A**). Strikingly, among those older than 47, 79 (60%) differed by sex at the q<0.05 level, with almost all of them higher in

women. Furthermore, nearly all the proteins that were higher among women in the older age group were associated with increased mortality (**Figure 8B**). Only three proteins were higher in men in the older age stratum, and two of them (LRNN1 and ENPP5) were associated with decreased mortality. These findings were mirrored in Kaplan-Meier curves stratified by sex above and below the age of 47. In those under the age of 47, women have lower mortality than men ($P<0.0001$), whereas among those older than 47, women have a similar mortality as men ($P=0.84$, **Figure 9**). Collectively, these data suggest that among older PWH, women have plasma proteomic profiles associated with greater mortality risk than men, which coincides with a loss of "female advantage" in mortality that is evident in younger participants.

The relationship between inflammatory proteins and mortality risk is not statistically significantly modified by sex

Since the higher levels of mortality-associated proteins in women than men in the older age stratum might have less clinical importance if the relationship between these proteins and mortality was weaker among women than men, we explored whether sex might modify the relationship between inflammatory proteins and mortality. We found no evidence that sex modified the association between any protein and mortality at the FDR-corrected $q<0.10$ level. In fact, the interaction terms tended to favor stronger relationships between biomarkers and mortality in women than in men (**Supplemental Figure 3A**), including pathways involved in innate and adaptive immune activation (**Supplemental Figure 3B**).

Discussion

We found that among ART-suppressed PWH, age dramatically modifies the relationship between sex at birth and inflammation. While men tended to have more inflammation than women among younger PWH, among those older than 47, women tended to have much higher levels of inflammation than men. The interaction by age was particularly striking for interferon-α response proteins, which increased with age to a

much more dramatic degree among women than men. Furthermore, putatively post-menopausal women with HIV had consistently higher levels of mortality-associated proteins than similarly aged men with HIV. These findings may suggest a mechanism to explain the loss of "female advantage" in life expectancy in PWH as well as a potential menopausal effect that may be responsible for exacerbating these sex differences.

Several studies have previously demonstrated sex differences in inflammatory biomarkers in PWH. Among PWH, women with HIV have higher immune activation biomarkers than men (7–9, 17), as well as a more robust Type I interferon response to HIV, likely due to higher expression of the X-linked TLR7, which often escapes X inactivation in female cells (10, 12, 19, 20). Estrogen-related factors also appear to play a role, with evidence that estrogen may suppress HIV transcription (21). Indeed, Gianella *et al* found that HIV transcription from infected cells increased in women with HIV across the menopausal transition, an effect that was not seen in similarly-aged men (16). Collectively, these prior observations suggest that while women are predisposed to respond to HIV expression with a more robust Type I interferon response, they have less HIV expression from cells during premenopause given the latency-promoting effects of estrogen. Conversely, as systemic estrogen levels decline with menopause, HIV expression from the reservoir increases, and since women are predisposed to respond with a more robust Type I interferon response, Type I interferon responses and inflammation would be expected to increase dramatically. That is what was observed here. We found that women tended to have lower inflammation than men at younger ages, but consistently higher inflammation than men at older, putatively post-menopausal ages. We also found evidence of interaction by age and sex with IL-6 and coagulation pathways, which are biologically linked to the interferon pathway. Interferon-α may induce IL-6 production (22, 23) and markers of coagulation (24, 25), further corroborating our findings. We should note that not all prior studies of younger PWH have found lower Type I interferon response proteins in putatively pre-menopausal women than men. For example, in a study of much younger PWH in sub-Saharan Africa, Streeck *et al* found higher levels of CXCL10 in likely predominantly pre or perimenopausal women (median age

39) than men (8). This may reflect differences in host genetics or other environmental exposures including other prevalent infections that may tip the balance of Type I interferon responses, overwhelming the protective effects of estrogen on suppressing HIV expression.

The sex differences in inflammatory proteins we observed at older ages are also likely to be clinically significant. When restricting to proteins that were associated with increased mortality, the vast majority were higher among women than men in the older age stratum. Higher levels of mortality-associated proteins in women might not have a clinical consequence if the relationship between these proteins and mortality was weaker in women than men, but that is not what was seen here. Indeed, while we found no statistical evidence that sex modified the association between inflammatory proteins and mortality, the relationships tended to be, if anything, stronger in women.

While most epidemiologic studies conducted in the general population find that women have a longer life expectancy than men (26), in several studies evaluating the survival trends in PWH, women have similar life expectancies to men and a greater "life expectancy gap" than men when compared to the general population (5, 27). No data currently exists suggesting that among PWH, post-menopausal women are at a greater mortality risk than men of a similar age. However, in our analysis, we found that women under the age of 47 demonstrated lower mortality than men whereas putatively post-menopausal women above the age of 47 had a similar mortality to men. Taken together, these findings support that a potential mechanism for the loss of "female advantage" in life expectancy among PWH may be through increased inflammation, perhaps mitigated by estrogen's HIV latency-promoting effects at pre-menopausal ages and unmasked with menopause-associated estrogen depletion.

Prior studies have found a menopausal effect on immune activation in women with HIV. In a study evaluating the contribution of self-reported menopause to immune activation biomarkers in women with HIV,

Peters *et al* found that menopausal women with HIV had higher sCD14 and sCD163 levels, markers of monocyte and M2 macrophage activation, respectively, than their premenopausal counterparts (28). Prior studies in PWH have also found that sCD163 is an independent marker of all-cause mortality with women with HIV demonstrating higher levels of sCD163 than men with HIV and that sCD163 levels had a stronger relationship with mortality (29). In our analysis, the vast majority of women older than our median age of 47 had undetectable plasma AMH levels, consistent with late perimenopause or menopausal status, while most younger women had detectable levels. While a single AMH level without self-reported menstrual status data cannot be used to determine menopausal status, this suggests that the median age of the cohort coincided with entry into perimenopause. These findings suggest that menopausal status may have important effects on the plasma inflammatory proteome even when accounting for age, although this merits further study.

This study has several limitations. While we were able to see a strong sex effect, women only represented 18% of the cohort, which may have underpowered our ability to detect an association between sex and clinical outcomes. It is possible that residual confounding or unmeasured confounders also contributed to the findings seen here given the observational nature of our study, but we cannot think of an obvious alternative explanation for the interactions between age and sex on the inflammatory proteome observed here. While we had single measurements of AMH, we did not have serial measurements or self-reported menstrual status data to allow accurate determination of menopausal status. We also did not measure HIV expression in this cohort. Strengths of this analysis include the diverse nature of the cohort, which includes women and men with HIV across the United States. This study is unique in its proteomic data on a large cohort of PWH with adjudicated clinical events and the inclusion of sex hormone data to assist with the determination of menopausal status, and highlights the needs of an understudied population, women with HIV.

Conclusion

The impact of sex on the plasma inflammatory proteome is highly dependent on age among ART-suppressed PWH, with women exhibiting more inflammation than men primarily at older ages. Whether menopause contributes to unmasking these sex differences requires further study and is of high clinical importance as many of these pathways are associated with increased mortality. These findings might also suggest a protective role of estrogen replacement therapy among post-menopausal women with HIV and a clinical trial to address this question is in development in the AIDS Clinical Trial Group (NCT06856174).

Methods

Sex as a biological variable

As the objective of this study was to evaluate sex differences, sex was included as a biological variable. This study enrolled both cis-gender and transgender participants. Due to the small number of transgender participants resulting in low power, we only evaluated the cis-gender women in the analyses stratified by sex.

Study design and population

This study leveraged a case-cohort study within CNICS, a national cohort study integrating clinical data, laboratory data, and serial biospecimen collection from PWH across eight Center for AIDS Research (CFAR) sites (30). All enrolled adult PWH who had viral suppression from ART (HIV RNA <400 copies/mL) for at least six months and had an available plasma aliquot after January 1, 2010 were eligible for inclusion. Study participants who had an acute infection, hospitalization, or receipt of immunomodulatory or immunosuppressive therapy within three months of plasma sampling were excluded.

From 9430 eligible ART-suppressed participants, we randomly sampled 1000 participants, of whom 922 had sufficient plasma remaining for analysis, comprising the “sub-cohort”. Participants in the sub-cohort were also followed for incident mortality, informed by the national death index.

Given the objective of the parent study to evaluate the association of inflammatory proteins with the long-term risk of clinical outcomes in ART-suppressed PWH, the first available plasma specimen after six months of ART-mediated viral suppression was selected as "time zero" for the study. For the mortality analysis, participants were followed from the date of plasma sampling to the date of censoring, i.e., the date of death or the last laboratory or clinic visit date. Accidental deaths were also censored.

Proteomic measurements

Inflammatory proteome measurements were obtained for 363 unique plasma proteins in singlicate on cryopreserved plasma using the Olink Inflammation Explore Panel (Olink, Upsalla, Sweden), a PEA platform with high sensitivity and specificity (31). Relative protein levels are expressed in standardized Npx units for each protein, with each unit reflecting a 2-fold increase in protein level.

AMH levels

In order to evaluate whether age 47 could be used to approximate the age of menopause, anti-Müllerian hormone (AMH) levels, a biomarker of ovarian reserve, were tested in cis-gender female participants. Serum samples stored at −80°C were run in singlicate using the pico-AMH assay (Ansh Labs, TX, US). Values were plotted by age to evaluate when AMH values became undetectable, signifying entry into perimenopause.

Statistical analysis

The relationship between sex assigned at birth and plasma proteins was assessed with linear regression models adjusted for age, site, race, nadir CD4, men who have sex with men (MSM) status, smoking (ever smoker, yes/no), injection drug use (IDU) history (ever IDU, yes/no), hepatitis C virus (HCV) history, obesity status (body mass index >30 kg/m^2) and ASCVD risk score. Covariates were selected *a priori* based on covariates that might plausibly be in the causal pathway between sex and inflammation among PWH. Given

effect modification by age in our prior work in the cohort (17), age-sex interaction terms were generated, dichotomizing age above versus below the median age of 47, which also approximates the age of menopause for women with HIV (32).

To assess the clinical relevance of sex differences in inflammatory proteins, we restricted to proteins that predicted a shorter time to non-accidental death (i.e., censoring deaths known to be due to accidents or trauma) in Cox proportional hazards models adjusted for the VACS Index (33) and CNICS site at the FDR-corrected $q<0.05$ level. To explore whether sex modified the relationship between inflammatory proteins and mortality, we used sex-by-protein interaction terms. Hazard ratios for the interaction term were reported as a direct measure of the degree to which sex modifies the association between biomarkers and mortality. To further evaluate whether age modified the impact of sex on mortality risk, we performed Kaplan-Meier analysis of time to non-accidental death, incorporating sampling weights, testing differences by sex with a long-rank test, and stratifying by age.

Statistical tests (both p and q-values) with an alpha level of 0.05 were considered significant for all analyses except the interaction terms, which used an alpha level of 0.10. All p-values were adjusted for multiple comparisons by controlling the FDR using the Storey Q method (34). Known biologic relationships between statistically significant proteins ($q<0.05$) were plotted using STRING network maps (https://string-db.org/) (35) with Cytoscape software (v3.10.3). Pathway enrichment analysis was performed using the C5: Gene Ontology sets from MSigDB (https://www.gsea-msigdb.org/gsea/msigdb) restricting to annotated pathways including the proteins represented in the Olink panel and further restricting to Biologic Process and immunologic pathways given the scope of our hypothesis (36). Given known sex differences in Type I interferon pathways, as well as hypothesized changes in this pathway during menopause, all 22 Olink panel proteins in the Hallmark interferon alpha pathway (M5911) were also assessed (13, 18), and a composite interferon-α response score was calculated as the mean of all z-scored Npx values. To assess additional pathways known to

be induced by Type I interferons, composite scores for Hallmark IL-6 response pathway proteins (n=22) and coagulation pathway proteins (n=7) were also assessed. Sex-by-age interactions were also explored for these composite variables as above. All data analyses were conducted using R (37).

Ethical approvals

This CNICS study received approval from the University of California San Francisco Institutional Review Board as well as those from the participating sites. All study participants were informed about the study and provided written informed consent prior to study participation.

Role of the funding source and data availability

The funding sources played no role in the study design, data collection, analysis, interpretation of results, the writing of the manuscript, or the decision to submit for publication. The proteins assessed with their respective beta coefficients, p-values, and q-values are available in **Supplementary Data**. Additional supporting data are available in the BioStudies database (http://www.ebi.ac.uk/biostudies) under accession number- S-BSST2240 (38).

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Author contributions

Rebecca A. Abelman: Dr. Abelman helped develop the research question, interpreted the data, and wrote the manuscript.

Samuel R. Schnittman: Dr. Schnittman helped with interpretation of the data and the writing of the manuscript.

Natalia Murad: Dr. Murad performed the statistical analysis and guided the interpretation of the results for the manuscript.

Adam Olshen: Dr. Olshen performed the statistical analysis and guided the interpretation of the results for the manuscript.

Gabriele B. Beck-Engeser: Ms. Beck-Engeser was the full research specialist for Dr. Hunt's lab. She helped with the Olink analysis for this project.

Noah Aquino: Mr. Aquino also works in the lab with Dr. Hunt. He assisted with the Olink analysis for this project.

Gabrielle C. Ambayec: Ms. Ambayec was a research specialist for Dr. Hunt's lab. She helped perform the Olink analysis for the project.

Edward R. Cachay: Dr. Cachay is a Co-Investigator in the CNICS cohort. He contributed to the interpretation of data.

Joseph J. Eron: Dr. Eron is a Co-Investigator in the CNICS cohort. He contributed to the interpretation of data.

Michael Saag: Dr. Saag is a Co-Investigator in the CNICS cohort. He contributed to the interpretation of data.

Robin M. Nance: Ms. Nance is a research specialist that works with the CNICS cohort. She provided the causes of death data and contributed to the interpretation of data.

Joseph A. Delaney: Dr. Delaney contributed to the interpretation of data, particularly the causes of death data.

Stephanie A. Ruderman: Dr. Ruderman contributed to the interpretation of data.

Richard D. Moore: Dr. Moore is a Co-Investigator in the CNICS cohort. He contributed to the interpretation of data.

Kenneth H. Mayer: Dr. Mayer is a Co-Investigator in the CNICS cohort. He contributed to the interpretation of data.

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Heidi M. Crane: Dr. Crane is a Co-Investigator in the CNICS cohort. She contributed to the interpretation of data.

Peter W. Hunt: Dr. Hunt is a Co-Investigator in the CNICS cohort. He assisted with refining the research question, data management, statistical analysis, and interpretation of the study results in addition to providing input on the preparation of this manuscript.

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Graphical abstract and Figure 1 were created in BioRender. Abelman, R. (2025) https://BioRender.com/hmlntzh

Table 1. Demographics and Clinical Characteristics by Sex Assigned at Birth

Median [IQR] or N (%)	Overall	Women	Men
N	922	162	760
Age (years)	47 [39, 53]	47 [40, 53]	47 [38, 53]
Specimen Year			
2010-2012	513 (56)	89 (55)	424 (56)
2013-2015	227 (25)	51 (31)	176 (23)
2016-2019	182 (20)	22 (14)	160 (21)
Study Site			
CWRU	111 (12)	16 (10)	95 (13)
Fenway	60 (7)	3 (2)	57 (8)
JHU	88 (10)	32 (20)	56 (7)
UAB	215 (23)	42 (26)	173 (23)
UCSD	208 (23)	15 (9)	193 (25)
UCSF	22 (2)	4 (2)	18 (2)
UNC	157 (17)	40 (25)	117 (15)
UW	61 (7)	10 (6)	51 (7)
Race/Ethnicity			
White	362 (39)	27 (17)	335 (44)
Black	424 (46)	125 (77)	299 (39)
Hispanic	104 (11)	7 (4)	97 (13)
Other	32 (3)	3 (2)	29 (4)
Obese (BMI >30 kg/m^2)	232 (25)	84 (52)	148 (19)
ASCVD (%)	4.5 [1.7, 9.1]	3.0 [0.7, 7.2]	4.5 [2.0, 9.5]
VACS Index 1.0	12 [6, 24]	22 [10, 34]	12 [0, 23]
eGFR (mL/min/1.73 m^2)	95 [78, 109]	93 [74, 110]	95 [78, 109]
Hepatitis C Ab+	154 (17)	32 (20)	122 (16)
Diabetes	119 (13)	31 (19)	88 (12)
Statin Use	218 (24)	43 (27)	175 (23)
Ever Smoker	420 (46)	80 (49)	340 (45)
Ever IDU	160 (17)	28 (17)	132 (17)
CD4 Nadir (cells/mL^3)	245 [81, 396]	241 [72, 356]	250 [84, 412]
CD4 Count (cells/mL^3)	579 [405, 804]	639 [449, 873]	566 [397, 783]
HIV Viral Load	<40	<400	
<40 copies/ml	829 (90)	146 (90)	683 (90)
41-400 copies/ml	93 (10)	16 (10)	77 (10)
On integrase strand transfer inhibitor	286 (31)	50 (31)	236 (31)
On protease inhibitor	368 (40)	72 (44)	296 (39)
On NNRTI	388 (42)	58 (36)	330 (43)

Figure 1: CONSORT diagram of included participants.

Figure 2: Plasma Inflammatory Proteome by Sex at Birth. Plasma levels of inflammatory and immunoregulatory proteins, assessed using the Olink Inflammation Explore panel, are compared between women and men after adjustment for CNICS site, age, race, nadir CD4, MSM status, smoking, IDU history, HCV history, obesity, and ASCVD risk score. Adjusted beta coefficients (per 2-fold relative increase in protein levels) for women vs. men are plotted on the X axis and nominal significance level on the Y axis, with proteins higher in women in red and those higher in men in blue.

Figure 3: Plasma Anti-Müllerian Hormone Levels by Age in ART-suppressed Women with HIV. In panel **A**, plasma Anti-Müllerian Hormone (AMH) levels are plotted by age among 193 women in the sub-cohort, and correlation assessed with a spearman's rho. The vertical dotted line denotes the median age of the cohort of 47. In panel **B**, plasma AMH levels are plotted in those above and below the median age of 47, with the colored boxes denoting median value and error bars denoting the interquartile range (P for Wilcoxon rank-sum text

Figure 4: Plasma Inflammatory Proteome Associations with Age are Modified by Sex at Birth. The degree to which sex at birth modified the association between age (≤47 vs. >47) and plasma inflammatory proteins was assessed with an interaction term, adjusting for CNICS site, race, nadir CD4, MSM status, smoking, IDU history, HCV history, obesity, and ASCVD risk score. Adjusted beta coefficients (per 2-fold relative increase in protein levels) for the sex-by-age interaction term are plotted on the X axis and nominal significance level on the Y axis, with positive numbers (and red color) indicating a greater increase with age among women and negative numbers (and blue color) indicating a greater increase with age among men. .

Figure 5: Sex Differences in Inflammatory Proteome Stratified by Age. Plasma levels of inflammatory and immunoregulatory proteins are compared between women and men after adjustment for CNICS site, age, race, nadir CD4, MSM status, smoking, IDU history, HCV history, obesity, and ASCVD risk score. Adjusted beta coefficients (per 2-fold relative increase in protein levels) for women vs. men are plotted on the X axis and nominal significance level on the Y axis, with proteins higher in women in red and those higher in men in blue among those younger **(A)** vs. older than age 47 **(B)**.

Figure 6: Qualitative Interaction in the Association between Sex and Age on Interferon-α Response Protein Levels. All 22 Hallmark Interferon-α Response Pathway proteins represented in the Olink Inflammation Explore panel were z-scored and assessed for interaction by sex and age (≤47 vs. >47) after adjustment for CNICS site, race, nadir CD4, MSM status, smoking, IDU history, HCV history, obesity, and ASCVD risk score. The beta coefficients for female vs. male sex (red indicating a greater increase among women vs blue for men) in the age ≤47 **(A)** and >47 **(B)** strata are depicted in STRING protein network map for all 22 proteins in the pathway. A composite mean Interferon-α response protein level score was calculated among all 22 z-scored proteins and adjusted means were calculated for each age-sex stratum and compared, highlighting minimal changes with age among men, but statistically significant changes with age among women **(C)**.

C. Interferon-α Response Protein Composite Score by Sex and Age

Figure 7: Qualitative Interaction in the Association between Sex and Age on IL-6 and Coagulation Pathway Protein Levels. Composite mean IL-6 and coagulation response protein level scores were calculated among all z-scored proteins and adjusted means were calculated for each age-sex stratum and compared. For the IL-6 pathway, similar to the interferon-α pathway, there were minimal changes with age among men, but statistically significant changes with age among women **(A)**. There were similar findings in the coagulation pathway **(B)**. Both models were adjusted for race, smoking status, injection drug use history, hepatitis C serostatus, nadir CD4 count, ASCVD risk score, men who have sex with men status, obesity, and CNICS site.

A) IL-6 Response Protein Composite Score by Sex and Age

B) Coagulation Response Protein Composite Score by Sex and Age

Figure 8: Sex Differences in Mortality-associated Plasma Proteins Stratified by Age. STRING protein network maps are shown for all 131 mortality-associated proteins in the panel, coloring each protein that was significantly different by sex at the FDR-corrected q<0.05 level (blue higher in men, red higher in women) in those with age ≤47 **(A)** and in those with age >47 **(B)**. Proteins associated with increased mortality in the cohort are denoted with upward pointing triangles and proteins associated with decreased mortality are denoted by downward pointing arrows.

Figure 9: Sex-Age Interaction in Mortality. Kaplan-Meier analysis plots of time to non-accidental death, incorporating sample weights and testing differences by age with a long-rank test in participants below **(A)** and above the median age of 47 **(B)** are shown below, with red denoting females and blue denoting males. In the table below each plot, the number of participants at risk using sampling weights is reported.

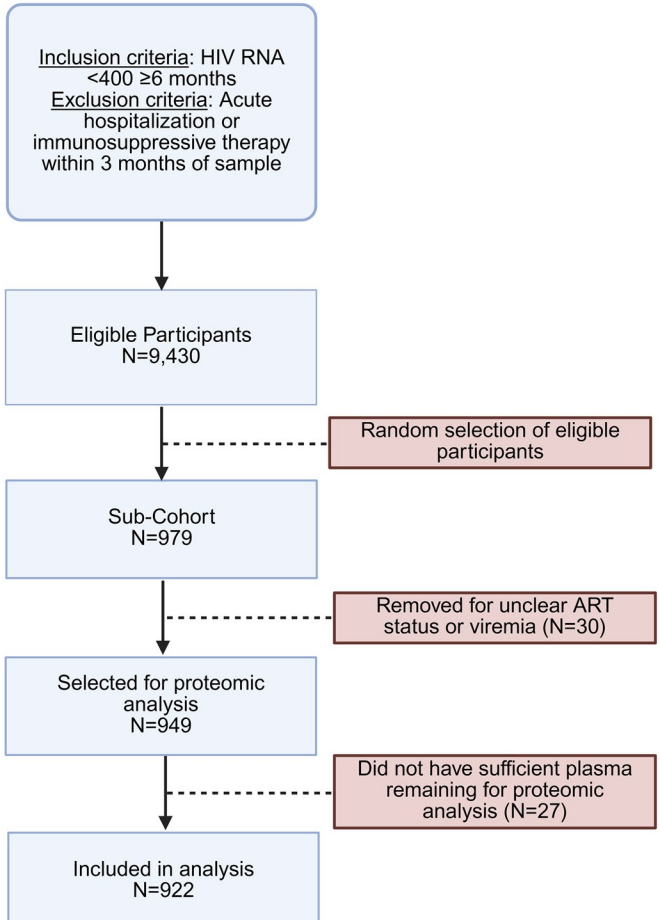
Supplemental Figure 1: A STRING protein network map is shown for all proteins in the panel, coloring each protein for which the sex-by-age interaction term was significant at the FDR-corrected q<0.05 level according to its beta coefficient (red denoting greater increases with age among women and blue denoting greater increases with age among men).

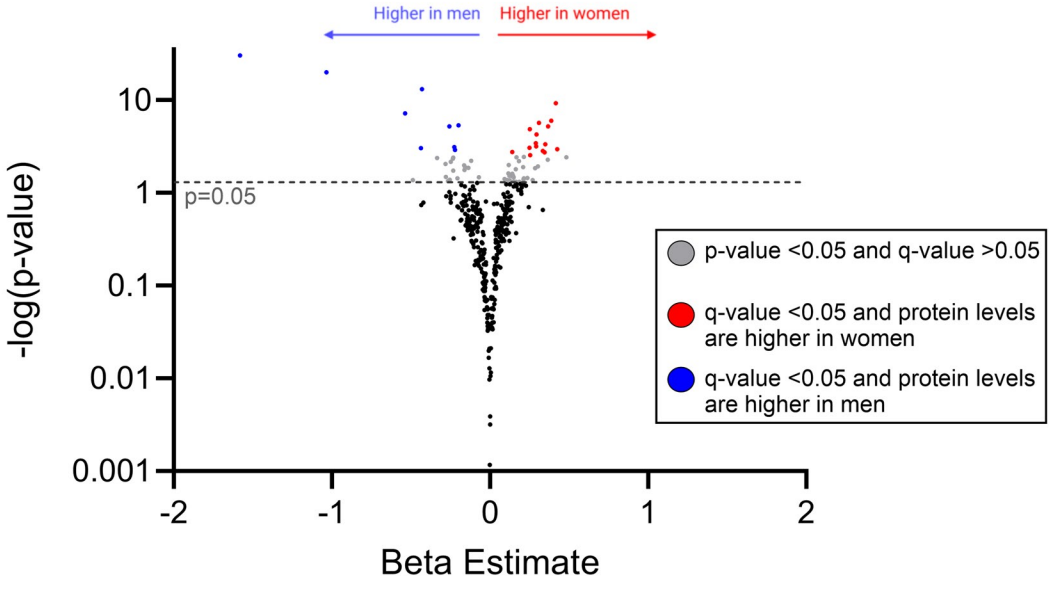
Network Map for Protein-Age Associations Modified by Sex

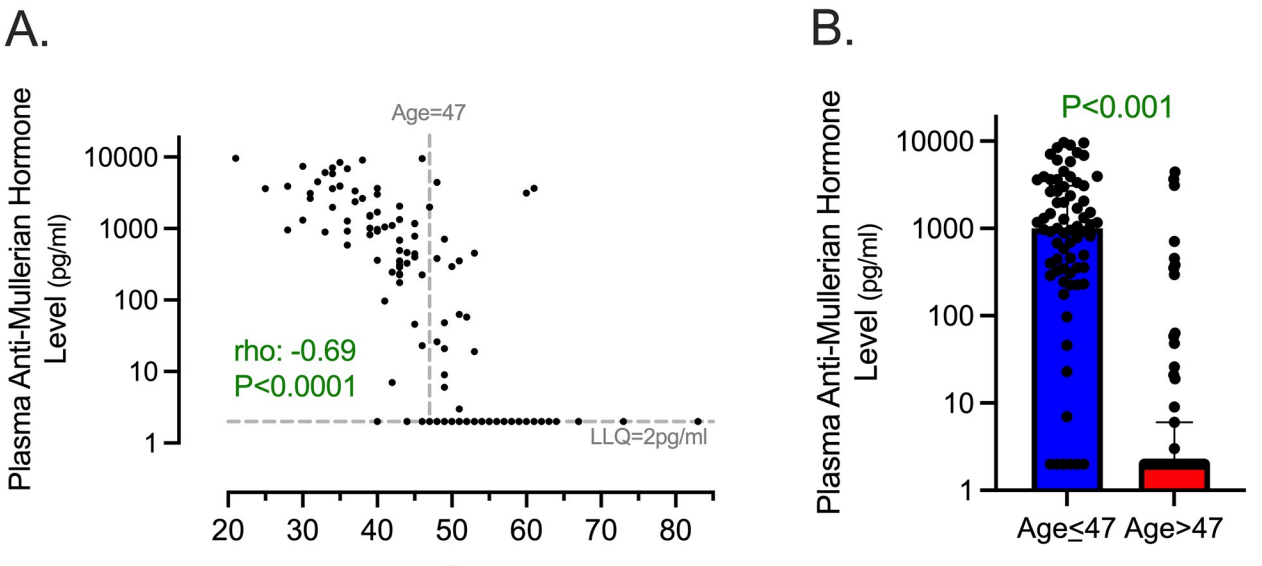
Supplemental Figure 2: Pathways Changing to a Greater Degree with Age in Women. A gene ontology enrichment analysis (among all pathways containing proteins in the panel) was performed on the sex-by-age interaction term; pathways associated with greater increases with age among women with positive normalized enrichment scores and those with greater increases among men with negative enrichment scores; and nominal p values are depicted by the legend.

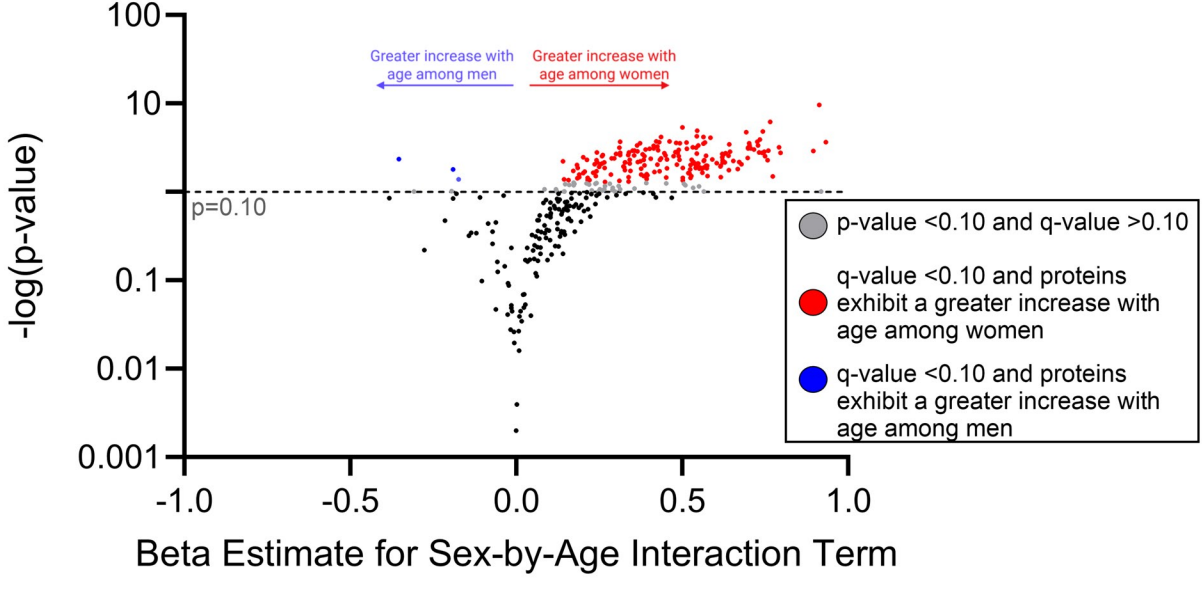
Supplemental Figure 3: Degree to which Sex Modifies the Association between Inflammatory Proteome and Mortality. The mortality hazard ratio for the sex-by-biomarker interaction term (X axis) is plotted by significance level (Y axis). No proteins met statistical significance at the FDR-corrected q<0.10 level and thus are all in grey **(A)**. Pathway enrichment by gene ontology is performed on the sex-by-biomarker interaction terms for mortality to identify pathways that are more strongly associated with increased mortality among women (positive normalized enrichment score) or men (negative normalized enrichment score), with nominal p-values denoted according to the color scale **(B)**.

A. Sex-Biomarker Interaction with Mortality

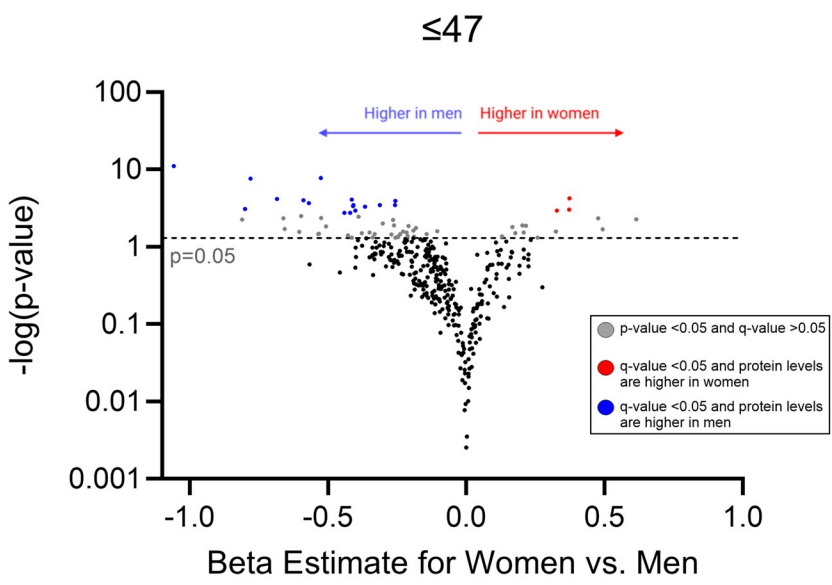

Inclusion criteria: HIV RNA <400 ≥6 months
Exclusion criteria: Acute hospitalization or immunosuppressive therapy within 3 months of sample
Eligible Participants N=9,430
Random selection of eligible participants
Sub-Cohort N=979
Removed for unclear ART status or viremia (N=30)
Selected for proteomic analysis N=949
Did not have sufficient plasma remaining for proteomic analysis (N=27)
Included in analysis N=922


Higher in men
Higher in women
10
1
0.1
0.01
0.001
p=0.05
-log(p-value)
-2
-1
0
1
2
Beta Estimate
p-value <0.05 and q-value >0.05
q-value <0.05 and protein levels are higher in women
q-value <0.05 and protein levels are higher in men

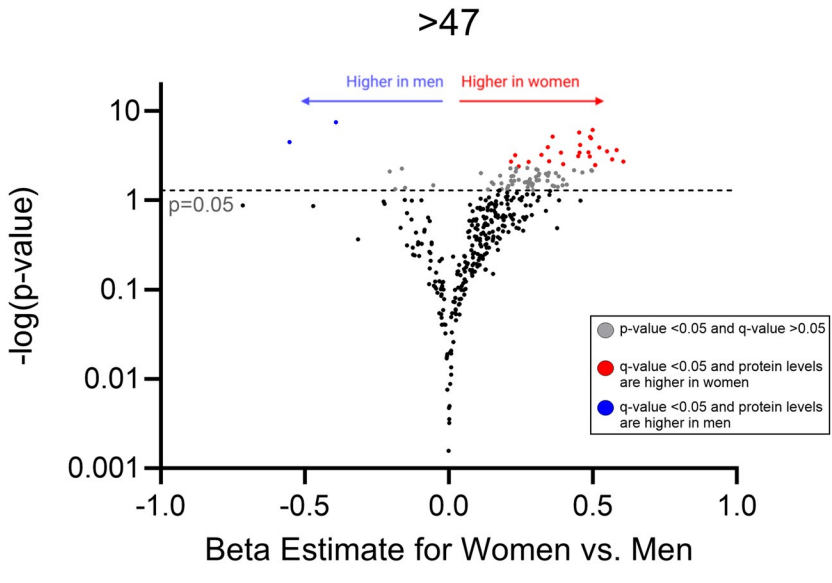

A.
Plasma Anti-Mullerian Hormone Level (pg/ml)
10000
1000
100
10
1
Age=47
rho: -0.69
P<0.0001
LLQ=2pg/ml
20
30
40
50
60
70
80
Age
B.
Plasma Anti-Mullerian Hormone Level (pg/ml)
P<0.001
10000
1000
100
10
1
Age≤47
Age>47

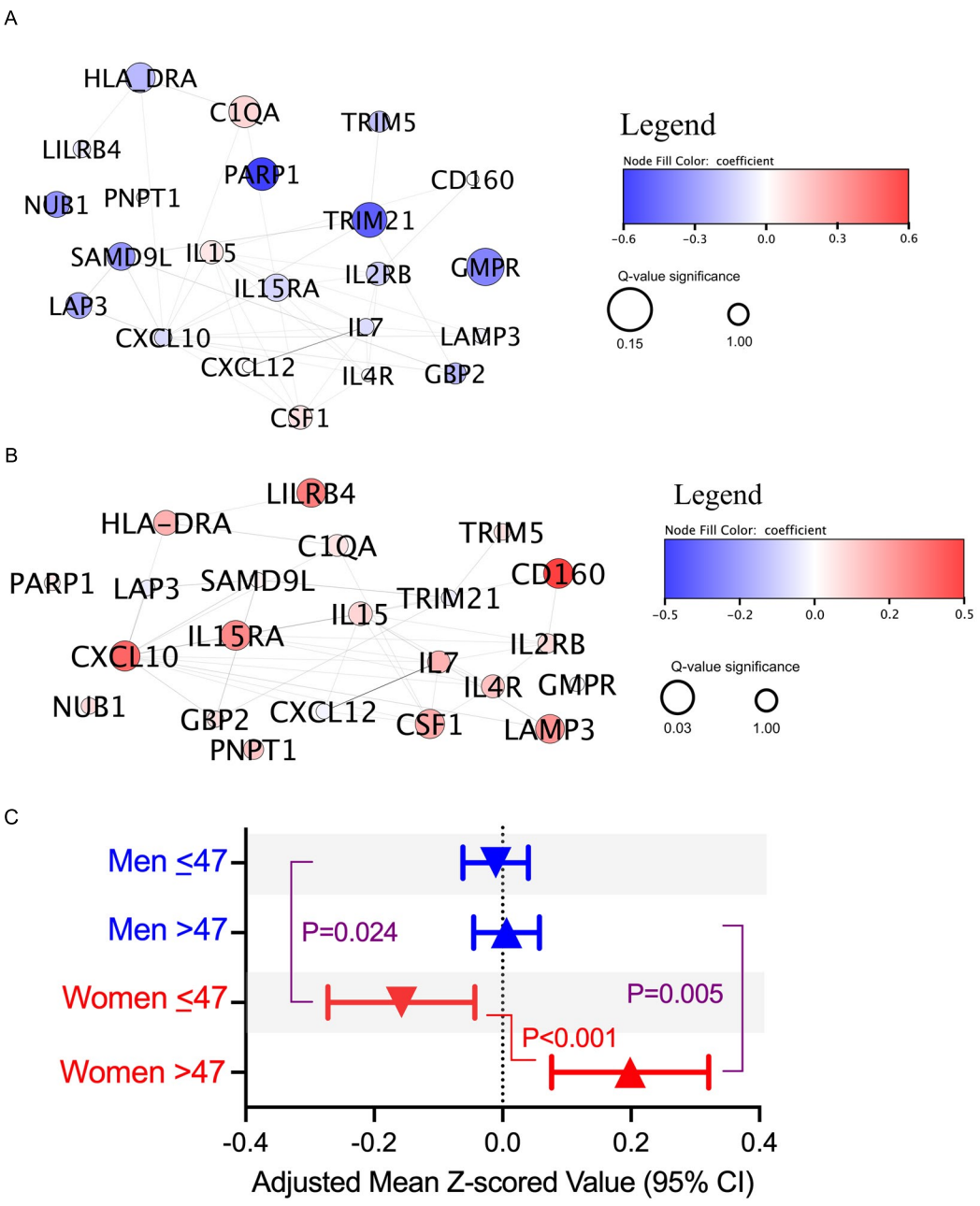

100
10
0.1
0.01
0.001
-log(p-value)
Greater increase with age among men
Greater increase with age among women
p=0.10
-1.0
-0.5
0.0
0.5
1.0
Beta Estimate for Sex-by-Age Interaction Term
p-value <0.10 and q-value >0.10
q-value <0.10 and proteins exhibit a greater increase with age among women
q-value <0.10 and proteins exhibit a greater increase with age among men

A

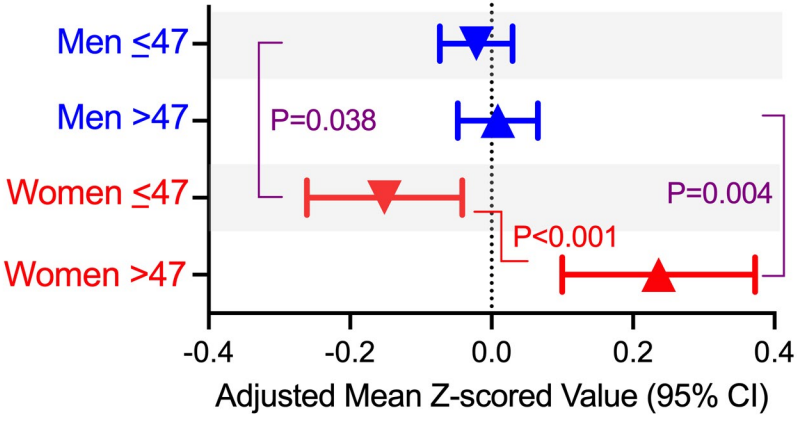

≤47
Higher in men
Higher in women
100
10
1
0.1
0.01
0.001
p=0.05
-log(p-value)
p-value <0.05 and q-value >0.05
q-value <0.05 and protein levels are higher in women
q-value <0.05 and protein levels are higher in men
-1.0
-0.5
0.0
0.5
1.0
Beta Estimate for Women vs. Men

B

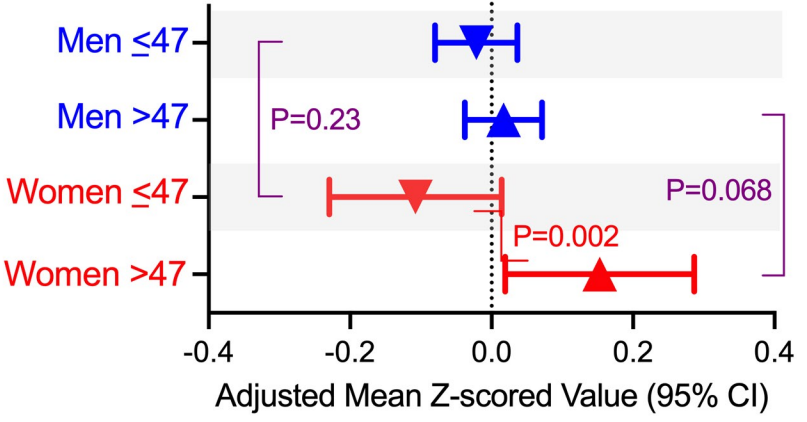

>47
Higher in men
Higher in women
10
1
0.1
0.01
0.001
p=0.05
-log(p-value)
p-value <0.05 and q-value >0.05
q-value <0.05 and protein levels are higher in women
q-value <0.05 and protein levels are higher in men
-1.0
-0.5
0.0
0.5
1.0
Beta Estimate for Women vs. Men


A
HLA_DRA
C1QA
TRIM5
LILRB4
PARP1
CD160
NUB1
PNPT1
TRIM21
SAMD9L
IL15
IL2RB
GMPR
IL15RA
LAP3
IL7
LAMP3
CXCL10
CXCL12
IL4R
GBP2
CSF1
Legend
Node Fill Color: coefficient
−0.6
−0.3
0.0
0.3
0.6
Q-value significance
0.15
1.00
B
LILRB4
HLA-DRA
C1QA
TRIM5
CD160
PARP1
LAP3
SAMD9L
TRIM21
IL15
IL15RA
IL2RB
CXCL10
IL7
IL4R
GMPR
NUB1
GBP2
CXCL12
CSF1
LAMP3
PNPT1
Legend
Node Fill Color: coefficient
−0.5
−0.2
0.0
0.2
0.5
Q-value significance
0.03
1.00
C
Men ≤47
Men >47
Women ≤47
Women >47
P=0.024
P=0.005
P<0.001
-0.4
-0.2
0.0
0.2
0.4
Adjusted Mean Z-scored Value (95% CI)

A


Men ≤47
Men >47
Women ≤47
Women >47
P=0.038
P=0.004
P<0.001
-0.4
-0.2
0.0
0.2
0.4
Adjusted Mean Z-scored Value (95% CI)

B


Men ≤47
Men >47
Women ≤47
Women >47
P=0.23
P=0.068
P=0.002
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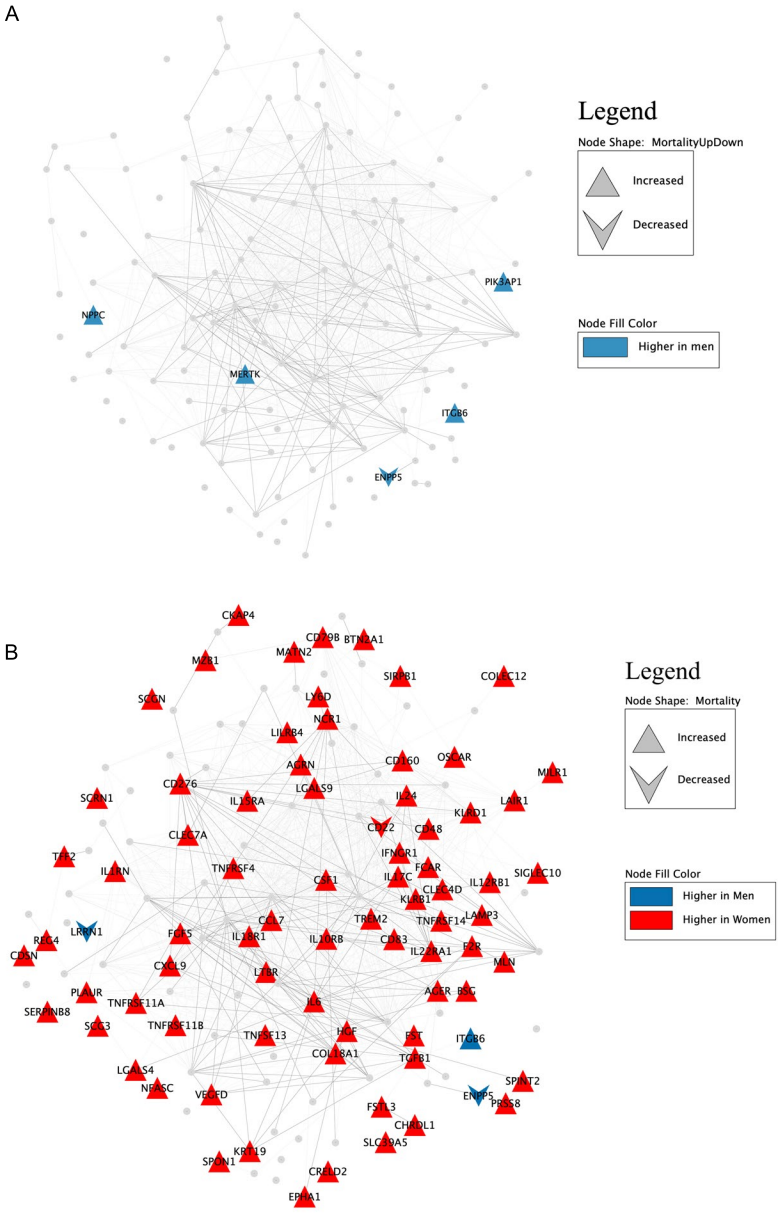
-0.2
0.0
0.2
0.4
Adjusted Mean Z-scored Value (95% CI)


A
Legend
Node Shape: MortalityUpDown
Increased
Decreased
Node Fill Color
Higher in men
PIK3AP1
NPPC
MERTK
ITGB6
ENPP5
B
Legend
Node Shape: Mortality
Increased
Decreased
Node Fill Color
Higher in Men
Higher in Women
CKAP4
CD209
BTN1A1
MZB1
MAN2
SIRPB1
COL6A3
SCGN
LY9D
NCR1
LILRB4
ADGRG
CD160
OSCAR
MLR1
SCGN1
CD276
LGALS9
IL13RA
IL34
LAIR1
KLRD1
CLEC7A
CD22
CD48
TFF2
IFNGR1
IL4R
FCAR
IL17C
IL17RB1
SIGLEC10
IL1RN
TNFRSF4
CA11
CLEC4D
KLRG1
TREM2
TNFRSF14
LAMP3
LRRN1
CCL7
IL18R1
IL10RB
CD83
IL2RA1
FAP
REG4
FCAS
CD5N
CXCL9
MAN
LTBR
PLAUR
TNFRSF11A
ADGRB3
SERPINB8
SCG3
TNFRSF11B
HAS
TNFSF13
HAS
FST
ITGB6
COL18A1
TGFB1
LGALS4
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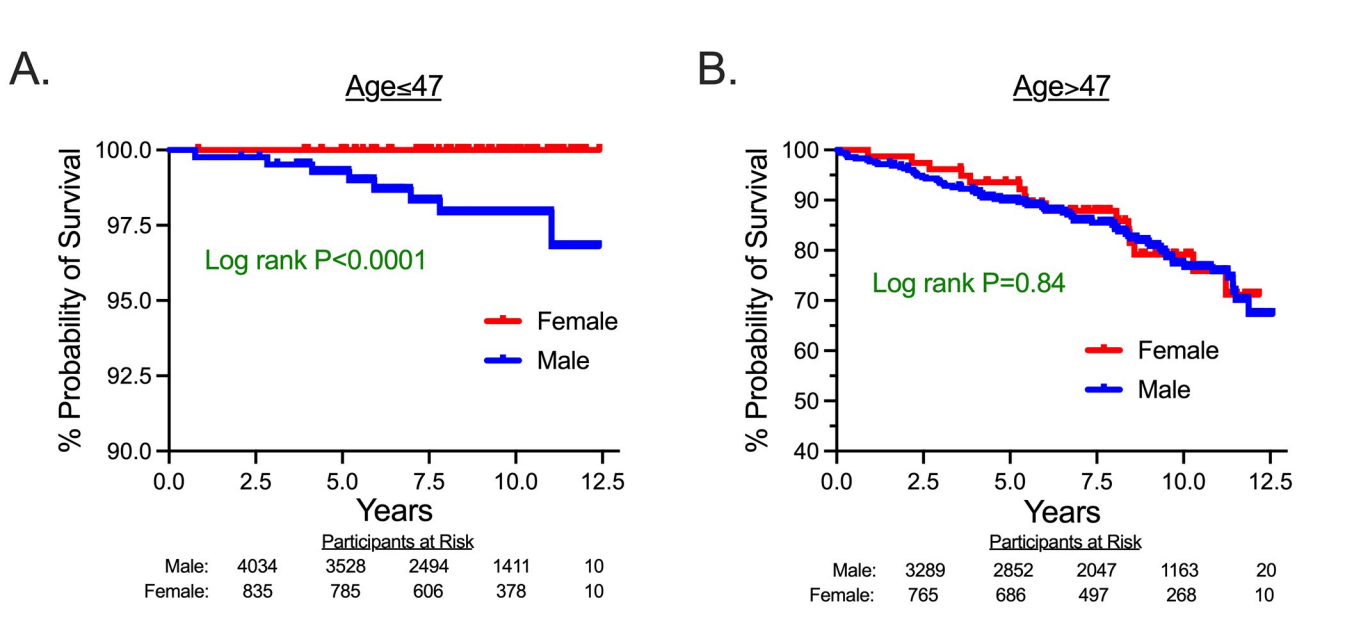
SLC39A5
KRT19
SPON1
CRELD2
EPHA1


A.
Age≤47
100.0
97.5
95.0
92.5
90.0
% Probability of Survival
Log rank P<0.0001
Female
Male
0.0
2.5
5.0
7.5
10.0
12.5
Years
Participants at Risk
Male: 4034 3528 2494 1411 10
Female: 835 785 606 378 10
B.
Age>47
100
90
80
70
60
50
40
% Probability of Survival
Log rank P=0.84
Female
Male
0.0
2.5
5.0
7.5
10.0
12.5
Years
Participants at Risk
Male: 3289 2852 2047 1163 20
Female: 765 686 497 268 10