**SUPPLEMENTAL MATERIAL**

**Table S1.** General characteristics of included and excluded participants of both cohorts

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Characteristics** | **CoLaus**  |  | **PREVEND** |  |
|  | **Included**(n=1679) | **Excluded**(n=1865) | **Included**(n=2133) | **Excluded**(n=1317) |
| **Age, years** | 48.4 ± 9.89 | 57.13 ± 9.75 | 51.3 ± 11.46 | 55.3 ± 11.45 |
| **Cholesterol, mmol/L** | 5.3 (4.7 - 6.1) | 5.7 (5,1- 6.4) | 5.3(4.6 - 6.1) | 5.5 (4.8 - 6.2) |
| **Systolic BP, mm Hg** | 118 (109 - 130) | 126 (115 - 139) | 116 (107 - 129) | 122 (110 - 138) |
| **Diastolic BP, mm Hg** | 76 (69 - 83) | 78 (71-85) | - | - |
| **BMI, kg/m2** | 23.4 (21.20 - 26.91) | 24.7 (22.03 - 28.08) | 25.4 (22.92 - 28.54) | 26.5 (23.8 – 30.08) |
| **Glucose, mmol/L** | 5.1(4.8 - 5.5) | 5.3 (4.9 - 5.6) | 4.7 (4.3 - 5.1) | 4.7 (4.4 - 5.3) |
| **CRP** | 1.1(0.5-2.4) | 1.6(0.7-3.6) | 1.2(0.6-2.8) | 2.0(0.9-5.1) |

Data are means ±SD or median (interquartile range), or *n (%)* where indicated; BP- Blood Pressure; BMI-Body Mass Index; CRP- C Reactive Protein Data on diastolic blood pressure are missing in PREVEND cohort

**Table S2.** Menopause differentiation utility of iron biomarkers, not adjusted for age

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **CoLaus** |  |  | **PREVEND** |  |
|  | **Sensitivity** | **Specificity** | **AUC** | **Sensitivity** | **Specificity** | **AUC** |
| **Ferritin** | 34.7 (30.6-39.0) | 90.9 (89.1-92.5) | 0.7454 | 70.8 (67.8-73.6) | 75.8 (73.2-78.3) | 0.8062 |
| **Transferrin** | 0.78 (0.2-2.0) | 99.7 (99.2-99.9) | 0.5872 | 40.3 (37.2-43.3) | 73.6 (71.0-76.2) | 0.6326 |
| **TSAT** | 0.0 (0.0-0.07) | 100 (99.7-100) | 0.5316 | 18.5 (16.2-21.1) | 79.3 (76.8-81-6) | 0.5464 |
| **Iron** | 0.0 (0.0-0.07) | 100 (99.7-100) | 0.5316 | 0.0 (0.0-0.04) | 100 (99.7-100) | 0.4984 |
| **Hepcidin** | - | - | - | 74.1(71.1-76.8) | 76.2(73.6-78.7) | 0.8127 |

TSAT-Transferrin Saturation; AUC-Area Under the Curve

**Table S3**. Association of iron biomarkers with menopausal status, participants younger than 60 years

|  |  |  |
| --- | --- | --- |
| **Cohort** | **CoLaus**(n=1435) | **PREVEND**(n=1651) |
| **Exposure** | **OR** | **95% CI** | **P-value** | **OR** | **95% CI** | **P-value** |
| **Age** | 1.60 | 1.52-1.70 | <0.001 | 1.81 | 1.69-1.93 | <0.001 |
| **Ferritin\*** | 2.25 | 1.72-2.95 | <0.001 | 2.21 | 1.74-2.80 | <0.001 |
| **Transferrin\*** | 0.02 | 0.01-0.10 | <0.001 | 0.09 | 0.02-0.30 | <0.001 |
| **Iron\*** | 1.08 | 0.84-1.40 | 0.523 | 1.07 | 0.83-1.40 | 0.564 |
| **TSAT\*** | 1.28 | 1.06-1.54 | 0.007 | 1.21 | 0.99-1.48 | 0.056 |
| **Hepcidin\*** |  |  |  | 2.67 | 2.06-3.47 | <0.001 |

OR-Odds Ratio; 95% CI- 95% Confidence intervals; TSAT-Transferrin Saturation

- Variables denoted with \* have been transformed and adjusted for age.

**Table S4**. Menopause differentiation utility of iron biomarkers, younger than 60 years, CoLaus cohort

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sensitivity (95% CI)** | **Specificity (95% CI)** | **AUC** | **AUC** 𝚫 | **P-value** |
| **Age** | 69.1 (63.3-74.6) | 96.1 (94.8-97.1) | 0.9459 | - | - |
| **Age + Ferritin** | 74.7 (69.1-79.8) | 96.1 (94.9-97.2) | 0.9526 | 0.0067 | 0.001 |
| **Age + Transferrin** | 71.0 (65.2-76.4) | 96.1 (94.9-97.2) | 0.9518 | 0.0059 | >0.001 |
| **Age + TSAT** | 69.1 (63.3-74.6) | 96.2 (94.9-97.2) | 0.9474 | 0.0015 | 0.1440 |
| **Age + Iron**  | 69.5 (63.6-75.0) | 96.1 (94.8-97.2) | 0.9461 | 0.0002 | 0.6843 |
| **Age+Ferritin+Transferrin** | 74.7 (69.1-79.8) | 96.7 (95.6-97.7) | 0.9552 | 0.0093 | >0.001 |

TSAT- Transferrin Saturation; AUC𝚫- Difference of Area Under the Curve

**Table S5.** Association of iron biomarkers with menopausal status, participants younger than 60 years, premenopausal, or menopaused in the last 5 years.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Cohort** |  | **CoLaus**(n=1321) |  |  | **PREVEND**(n=1548) |  |
| **Exposure** | **OR** | **95% CI** | **P-value** | **OR** | **95% CI** | **P-value** |
| **Age** | 1.54 | 1.45-1.64 | <0.001 | 1.78 | 1.66-1.90 | <0.001 |
| **Ferritin\*** | 1.98 | 1.48-2.64 | <0.001 | 2.00 | 1.62-2.62 | <0.001 |
| **Transferrin\*** | 0.04 | 0.01-0.18 | <0.001 | 0.09 | 0.02-0.30 | <0.001 |
| **Iron\*** | 1.15 | 0.87-1.52 | 0.316 | 1.07 | 0.82-1.40 | 0.592 |
| **TSAT\*** | 1.29 | 1.06-1.58 | 0.011 | 1.20 | 0.98-1.47 | 0.069 |
| **Hepcidin\*** | - | - | - | 2.56 | 1.98-3.33 | <0.001 |

OR-Odds Ratio; 95% CI- 95% Confidence intervals; TSAT-Transferrin Saturation

- Variables denoted with \* have been transformed and adjusted for age

**Table S6**. Menopause differentiation utility of iron biomarkers, including only participants younger than 60 years, premenopausal, or menopaused in the last 5 years, CoLaus cohort

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sensitivity** | **Specificity** | **AUC** | **AUC** 𝚫 | **p-value** |
| **Age** | 52.9 (47.7-61.0) | 97.7 (96.6-98.5) | 0.9273 | - | - |
| **Age + Ferritin** | 54.2 (46.6-62.2) | 97.8 (96.7-98.5) | 0.9345 | 0.0072 | 0.006 |
| **Age + Transferrin** | 50.3 (42.2-58.4) | 98.0 (97.1-98.7) | 0.9334 | 0.0061 | 0.009 |
| **Age + TSAT** | 49.7 (41.6-57.8) | 97.9 (96.9-98.7) | 0.9300 | 0.0027 | 0.125 |
| **Age + Iron** | 52.9(44.7-61.0) | 97.8(96.7-98.5) | 0.9282 | 0.0009 | 0.284 |
| **Age+Ferritin+Transferrin** | 54.8 (46.7-62.8) | 98.2 (97.3-98.9) | 0.9368 | 0.0095 | 0.002 |

TSAT-Transferrin Saturation; 𝚫 Difference of Area Under the Curve

**Table S7.** Association of iron biomarkers with menopausal status, participants aged 40 to 55 years

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Cohort** |  | **CoLaus**(n=971) |  |  | **PREVEND**(n=1013) |  |
| **Exposure** | **OR** | **95% CI** | **P-value** | **OR** | **95% CI** | **P-value** |
| **Age** | 1.61 | 1.5-1.74 | <0.001 | 1.77 | 1.65-1.92 | <0.001 |
| **Ferritin\*** | 2.58 | 1.92-3.48 | <0.001 | 2.17 | 1.70-2.76 | <0.001 |
| **Transferrin\*** | 0.01 | 0.01-0.06 | <0.001 | 0.07 | 0.02-0.26 | <0.001 |
| **Iron\*** | 1.01 | 0.98-1.04 | 0.476 | 1.14 | 0.88-1.49 | 0.309 |
| **TSAT\*** | 1.33 | 1.09-1.61 | 0.004 | 1.29 | 1.05-1.58 | 0.014 |
| **Hepcidin** | - | - | - | 2.67 | 2.04-3.49 | <0.001 |

OR-Odds Ratio; 95% CI- 95% Confidence intervals; TSAT-Transferrin Saturation

- Variables denoted with \* have been transformed and adjusted for age

**Table S8**. Menopause differentiation utility of iron biomarkers, including only participants aged 40 to 55 years CoLaus cohort

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Sensitivity** | **Specificity** | **AUC** | **AUC** 𝚫 | **p-value** |
| **Age** | 45.8 (37.7-54.0) | 95.7 (94.1-97.0) | 0.8905 | - | - |
| **Age + Ferritin** | 53.6 (45.4-61.7) | 95.6 (94.0-96.9) | 0.9082 | .0177 | 0.0017 |
| **Age + Transferrin** | 50.3 (42.1-58.5) | 95.7(94.1-97.0) | 0.9063 | .0158 | 0.001 |
| **Age + TSAT** | 46.4 (38.3-54.6) | 95.6 (93.9-96.9) | 0.8950 | .0045 | 0.1636 |
| **Age + Iron** | 45.8 (37.7-54.0) | 95.8 (94.2-97.1) | 0.8950 | .0045 | 0.7546 |
| **Age+Ferritin+Transferrin** | 58.2 (49.9-66.1) | 96.2 (94.7-97.4) | 0.9149 | .0244 | 0.0002 |

TSAT-Transferrin Saturation; 𝚫 Difference of Area Under the Curve

**Table S9.** Menopause differentiation utility of routinely measured biomarkers ,CoLaus

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Sensitivity** | **Specificity** | **AUC** | **\*AUC** 𝚫 | **\*\*AUC** 𝚫 | **P-value** |
| **Age** | 83.8% | 96.1% | 0.9716 | - | 0.0048 | - |
| **Age + Ferritin** | 86.7% | 96.1% | 0.9751 | 0.0035 | 0.0007 | <0.001 |
| **Age + Transferrin** | 85.0% | 96.1% | 0.9747 | 0.0021 | 0.0017 | <0.001 |
| **Age + TSAT** | 86.4% | 96.1% | 0.9758 | 0.0042 | 0.0006 | <0.001 |
| **Age + Iron** | 84.2% | 96.0% | 0.9717 | 0.0001 | 0.0047 | 0.71 |
| **Age +BMI** | 85.4% | 95.5% | 0.9720 | 0.0004 | 0.0044 | 0.31 |
| **Age + Ferritin + Transferrin** | 87.1% | 96.6% | 0.9764 | 0.0048 | - | <0.001 |
| **Age + CRP** | 84.1% | 95.8% | 0.9716 | 0 | 0.0048 | 0.91 |
| **Age + SBP + AntiHTA** | 84.0% | 95.0% | 0.9716 | 0 | 0.0048 | 0.81 |
| **Age + DBP + AntiHTA** | 83.0% | 95.0% | 0.9715 | -0.0001 | 0.0049 | 0.92 |
| **Age + Cholesterol + Statin** | 84.2% | 95.8% | 0.9720 | 0.0004 | 0.0044 | 0.37 |
| **Age + HDL+ Statins** | 84.6% | 96.0% | 0.9717 | 0.0001 | 0.0043 | 0.72 |
| **Age + LDL + Statins** | 84.8% | 96.2% | 0.9720 | 0.0004 | 0.0040 | 0.35 |
| **Age + Glucose + Antidiabetics** | 84.0% | 96.0% | 0.9717 | 0.0001 | 0.0043 | 0.54 |

TSAT-Transferrin Saturation; AUC-Area Under the Curve; SBP-Systolic Blood Pressure; DBP-Diastolic Blood Pressure; AntiHTA-Antihypertensive medication; HDL-High Density Lipoproteins; LDL-Low Density Lipoproteins; Antidiabetics-anti diabetic drugs; \* Difference in AUC between the base model and other models; \*\* Difference in AUC between the final model and age-adjusted models

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **OR (95% CI)** | **P-Value** | **AUC** | **Sensitivity (95% CI)** | **Specificity (95% CI)** |
| **STRF\*** | 0.76(0.47-1.22) | 0.268 | 0.9844 | 92.3(90.4-93.9) | 94.1(92.6-95.4) |
| **Hepcidin\*** | 2.68(2.06-3.47) | <0.001 | 0.9872 | 93.6(91.8-95.1) | 94.8(93.3-96.0) |

**Table S10. Association and** Menopause differentiation utility of iron biomarkers, PREVEND cohort

- Variables denoted with \* have been transformed and adjusted for age.

**Table S11.** Cut off points using Youden’s Index

|  |  |  |
| --- | --- | --- |
|  | **CoLaus** | **PREVEND** |
| **Age** | 51 | 51 |
| **Ferritin (µg/L)** | 73 | 59.0 |
| **Transferrin (g/L)** | 2.5 | 2.57 |

**Table S12.** Performance of cut off points on CoLaus cohort

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Sensitivity** | **Specificity** | **Youden index** |
| **Ferritin (µg/L)** | 75.0% | 66.0% | 0.84 |
| **Transferrin (g/L)** | 74.0% | 40.0% | 0.12 |

**Table S13.** Odds Ratio per Standard Deviation increase

|  |  |  |
| --- | --- | --- |
|  | **CoLaus** | **PREVEND** |
| **Transferrin\*** | 0.54 | 0.68 |
| **Ferritin\*** | 2.04 | 2.22 |
| **TSAT\*** | 1.33 | 1.21 |
| **Hepcidin\*** | - | 2.61 |

Variables denoted with \* have been adjusted for age

Fig S1. Flowchart of participants from the PREVEND cohort.

