Maturitas 179 (2024) 107872

Contents lists available at ScienceDirect

Maturitas

journal homepage: www.elsevier.com/locate/maturitas

Original article

Utility of iron biomarkers in differentiating menopausal status: Findings from CoLaus and PREVEND

Lum Kastrati [a,b,c], Dion Groothof [d], Hugo G. Quezada-Pinedo [e,f], Hamidreza Raeisi-Dehkordi [g], Lia Bally [c], Martin H. De Borst [d], Stephan J.L. Bakker [d], Pedro-Marques Vidal [h], Michele F. Eisenga [d], Taulant Muka [i,j,*]

[a] Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland
[b] Graduate School for Health Sciences, University of Bern, Bern, Switzerland
[c] Department of Diabetes, Endocrinology, Nutritional Medicine and Metabolism UDEM, Inselspital, Bern University Hospital, University of Bern, Switzerland
[d] Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, 9713, GZ, Groningen, the Netherlands
[e] The Generation R Study Group, University Medical Center Rotterdam, Rotterdam, the Netherlands
[f] Department of Cardiology, Bern University Hospital, University of Bern, Bern, Switzerland
[g] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands
[h] Department of Medicine, internal medicine, Lausanne university hospital (CHUV), University of Lausanne, Lausanne, Switzerland
[i] Epistudia, 3011 Bern, Switzerland
[j] Meta-Research Innovation Center at Stanford (METRICS), Stanford University, Stanford, CA, USA

ARTICLE INFO

Keywords:
Menopause
Transferrin
Ferritin
Iron
Hepcidin

ABSTRACT

Aim: To examine the association of iron biomarkers with menopausal status and assess whether these biomarkers can help differentiate menopausal status beyond age.
Methods: In this cross-sectional study we included 1679 women from the CoLaus and 2133 from the PREVEND cohorts, with CoLaus used as primary cohort and PREVEND for replication. Ferritin, transferrin, iron, and transferrin saturation (TSAT) were used to assess iron status. Hepcidin and soluble transferrin receptor were assessed only in PREVEND. Menopausal status was self-reported and defined as menopausal or non-menopausal. Logistic regressions were used to explore the association of these iron biomarkers with menopause status. Sensitivity, specificity, area under the receiver operating characteristic curves (AUC), positive and negative predictive values as well as cut-off points for the iron biomarkers were calculated. The model with the highest AUC was defined as the best.
Results: In the CoLaus and PREVEND cohorts, respectively, 513 (30.6 %) and 988 (46.3 %) women were post-menopausal. Ferritin (OR, 2.20; 95 % CI 1.72–2.90), transferrin (OR, 0.03; 95 % CI 0.01–0.10), and TSAT (OR, 1.28; 95 % CI 1.06–1.54) were significantly associated with menopausal status in CoLaus, with the findings replicated in PREVEND. AUC of age alone was 0.971. The best model resulted from combining age, ferritin, and transferrin, with an AUC of 0.976, and sensitivity and specificity of 87.1 % and 96.5 %, respectively. Adding transferrin and ferritin to a model with age improved menopause classification by up to 7.5 %. In PREVEND, a model with age and hepcidin outperformed a model with age, ferritin, and transferrin.
Conclusion: Iron biomarkers were consistently associated with menopausal status in both cohorts, and modestly improved a model with age alone for differentiating menopause status. Our findings on hepcidin need replication.

1. Introduction

Menopause is a physiological phenomenon that marks the end of the reproductive lifespan in women, with an impact on their life characterized by increased risk of several chronic diseases and mortality [1]. According to the World Health Organization (WHO), the number of postmenopausal women is projected to exceed one billion over the next decade as a result of population aging, with implications for research

* Corresponding author at: Epistudia, 3011 Bern, Switzerland.
E-mail address: taulant.muka@epistudia.ch (T. Muka).

https://doi.org/10.1016/j.maturitas.2023.107872
Received 23 February 2023; Received in revised form 21 September 2023; Accepted 23 October 2023
Available online 4 November 2023

and population health [2]. Identifying biomarkers that could help better define menopausal status could therefore have several beneficial implications. From a public health perspective, improving the information on menopausal status is essential for investigation of causal relations, describing populations and informing policy decision-making, in continuing the efforts for healthy aging.

Nevertheless, clinical importance of early diagnosis of menopause becomes relevant especially in cases of premature ovarian insufficiency (POI) (menopause before the age of 40) and/or early menopause (menopause before the age of 45), which are characterized with an increased risk of central adiposity, increased bone loss and risk of chronic diseases [3]. Moreover, early diagnosis becomes particularly relevant when discussing the initiation of Hormone Replacement Therapy (HRT) [4].

Because data on menopausal status is not routinely collected in clinical practice, true prevalence of menopause is impossible to determine. Oftentimes, lack of such data force the use of alternative information (e.g., using age as a cut-off), leading to misclassification bias and erroneous conclusions [5]. Contrary to occasions in which menopause is induced through medical interventions (e.g., surgical removal of both ovaries), defining menopausal status is not always straightforward. According to the WHO, physiological menopause is defined as a period of 12 consecutive months of amenorrhea [2]. Thus, menopausal status can only be retrospectively defined, prolonging the time to diagnosis, making it prone to recall bias and increased rates of misclassification. Several biomarkers have been suggested to help define menopausal status such as; frequency of menses, follicle stimulating hormone (FSH), anti-Müllerian hormone (AMH), inhibin B levels, and vasomotor and urogenital signs and symptoms—jointly referred to as the STRAW +10 criteria [6,7]. While the capacity of the aforementioned hormones in predicting time to menopause has been well documented, their ability to define menopausal status has not been conclusively demonstrated [8–10]. Therefore, new methods for defining menopausal status shall be explored.

After menopause, ferritin and hepcidin levels can increase up to threefold in comparison to levels before menopause [11–13]. Contrarily, there is a slight decrease in soluble transferrin receptor (sTfR), and transferrin levels after menopause. The changes in iron biomarker levels after menopause are not understood completely, but lack of menstruation has been suggested as the main factor, as well as change on the inflammatory and hormonal status [12,14,15]. While as we have shown in a recent publication, levels of iron biomarkers are age-dependent, the relationship between iron biomarkers and their combined influence with age in classifying menopause status is yet to be explored [13]. To our knowledge, no study has explored the role of iron biomarkers in defining menopausal status. This study therefore aimed to identify the utility of iron biomarkers in differentiating menopausal status.

2. Materials and methods

2.1. Study population

In this study, we used cross-sectional data from the baseline assessment (2003–2006) of CoLaus study as a primary cohort to examine the association between ferritin, transferrin, iron and transferrin saturation (TSAT) levels and menopausal status. Prevention of Renal and Vascular End Stage Disease (PREVEND) cohort data from the second screening (2001 to 2003), was used to replicate the results. Levels of two other iron biomarkers; hepcidin and soluble transferrin receptor (sTfR) were only available in the PREVEND study, thus, due to the inability of replicating finding on these biomarkes, they were included in a sensitivity analysis.

Briefly, the CoLaus cohort is a single-centre population-based cohort of people living in Lausanne, Switzerland. The baseline assessment was conducted from June 2003 to May 2006 and participants were followed up from 2003 to 2017. Inclusion criteria consisted of written informed consent and age 35–75 years. There were 6733 participants at the baseline and all of them provided informed consent. The study was approved by the Institutional Ethic's Committee of the University of Lausanne [16].

The PREVEND study investigates the risk factors for and the prevalence and consequences of microalbuminuria in otherwise healthy adults (≥18 years) in the city of Groningen, Netherlands [17]. Briefly, all inhabitants of the city of Groningen aged 28–75 years were invited, from 1997 to 1998, to participate in the study and were asked to complete a brief questionnaire and provide morning urine. The urinary albumin concentration (UAC) was determined in 40,856 responders. Pregnant women and participants with insulin-dependent diabetes mellitus were excluded. 6000 Participants with a UAC $\geq$ 10 mg/L were enrolled. Additionally, a randomly chosen control group with a UAC of <10 mg/L (n = 2592) were enrolled. These 8592 participants constitute the PREVEND cohort. A second screening round took place from 2001 to 2003, encompassing 6894 participants. The PREVEND study has been approved by the local medical ethics committee (MEC 96/01/022) and was undertaken in accordance with the Declaration of Helsinki. All participants provided written informed consent.

2.2. Exclusion criteria

Participants were excluded from this analysis in case of (i) contradictory or no information on menopausal status; (ii) hysterectomy or ovariotomy; (iii) Hormone Replacement Therapy (HRT); (iv) C-Reactive Protein (CRP) > 10 mg/L; (v) missing data on independent variables.

2.3. Ascertainment of menopausal status and age at menopause

Physiological menopause was defined as self-reported spontaneous cessation of menstruations.

In the baseline of CoLaus cohort, menopausal status was assessed by asking the participants the following question "*are you menopaused?*". For assessment of age at menopause, participants were questioned "*age at last menses*".

In PREVEND only "*Age at time of menopause*" by type of menopause (natural or non-natural based on their reported HRT status or surgical menopause) was asked, and no data was available regarding menopausal status. Therefore, when the age of menopause onset was reported, we classified women as postmenopausal, otherwise, as non-menopausal.

2.4. Assessment of iron biomarker levels

2.4.1. CoLaus

Ferritin was assessed by immunoturbidimetric method (Tina-quant 4th generation, Roche Diagnostics, Switzerland). Transferrin was assessed by immunoassay. We converted transferrin units from mg/dL to g/L to match the data on PREVEND. Iron was released from transferrin by acetic acid and was reduced to the ferrous state by hydroxylamine and thioglycolate. The ferrous ion was immediately complexed with the FerroZine Iron Reagent (SYNCHRON LX® System(s), UniCel®). We also converted iron units from microg/dL to umol/L to match the data on PREVEND.

Transferrin saturation (TSAT) was calculated as 100 × serum iron (μmol/L) ÷ 25 × transferrin (g/L) [18]. No information on fasting status of participants was available.

2.4.2. PREVEND

Fasting blood samples were drawn in the morning from all subjects from April 24, 2001, to December 3, 2003. Serum iron was measured using a colorimetric assay, ferritin using immunoassay, and transferrin using an immunoturbidimetric assay (all Roche Diagnostics). TSAT was calculated as in CoLaus. Serum hepcidin was calculated with a competitive enzyme-linked immunosorvent assay. An automated homogenous immunoturbidimetric assay with intra and interassay

coefficients of variations <2 % and 5 % quantified sTfR [18].

2.5. Statistical analysis

Descriptive statistics for continuous variables were presented as means and Standard Deviations (SD), or median (interquartile range) when the distribution was not normal. Categorical variables were presented as percentages. To achieve normal distributions, skewed variables were natural log-transformed (Ferritin, Transferrin, sTfR and Hepcidin) or square root transformed (TSAT and Iron). Age-adjusted logistic regression models were used to cross-sectionally explore whether transferrin, ferritin, and TSAT levels were associated with menopausal status (postmenopausal vs. non-menopausal), separately for CoLaus and PREVEND cohorts. Odds Ratios (OR) of one (log or square root) transformed unit and 95 % confidence intervals (CI) were calculated. To explore the capacity of iron biomarkers to differentiate postmenopausal women from non-menopausal women, we used data from CoLaus and a model having only age as predictor variable (referred to as the base model). Then, we built additional logistic models containing information on the participants' levels of ferritin, transferrin, TSAT and iron, adjusted for age. We thereafter evaluated a final model that consisted of age and combination of iron biomarkers. The Area Under the Receiver Operating Characteristic Curves (AUCs) were estimated from the logistic regressions to evaluate the utility of transferrin, ferritin, TSAT and iron as diagnostic criterion for menopausal status. In addition, we evaluated the performance of univariable and multivariable models for discriminating menopausal status by calculating the sensitivity, specificity, percentages of correctly classified percentages, and positive and negative predictive values (PPV and NPV) of the final model. Liu's method was used to identify optimal cut-offs for age, transferrin, and ferritin to define menopausal status. The transformed variables were back-transformed [19]. The difference in AUC of the multivariable models with age as base model was used to identify the best model. We then replicated the best model in the PREVEND cohort. All analyses were performed using Statistical Package STATA version 17 (Station College, Texas, USA).

2.6. Sensitivity analysis

Because menopause is primarily known to occur between the age of 40 and 60, we restricted our analysis to this age group [12]. Second to this, to explore the utility of iron biomarkers in distinguishing women who have recently transitioned to menopause, we excluded all women older than 60 years old and those who had menopause for longer than 5 years. As menopause occurs predominantly between the ages of 40 and 55 years old, we further restricted the population to this age-range to further investigate the utility of iron biomarkers at classifying menopause status at younger populations. As menopause is not only related with a change in iron levels, but with a change in cardiovascular biomarkers levels as well as with a chronic state of inflammation, we explored the utility of cardiovascular risk factors, Body Mass Index (BMI) and CRP in differentiating menopausal status [20]. Finally, because information on hepcidin and sTrF was only available in PREVEND we evaluated the capacity of these biomarkers on defining menopausal status. We also calculated cut-off points using the Youden's index. We also calculated ORs per SD increase.

3. Results

3.1. General characteristics

A total of 3812 (1679 from CoLaus and 2133 from PREVEND) women with information on menopausal status and iron biomarkers were included in this study (Fig. 1 & Fig. S1). Included participants were younger and had a better cardiovascular profile (Table S1). Table 1 summarizes the baseline characteristics of all women included in the analysis. In the CoLaus cohort, 513 (30.5 %) women were postmenopausal, compared to 988 (46.3 %) in the PREVEND cohort.

3.2. Association of iron biomarkers with menopausal status

The association of ferritin, transferrin, and TSAT with menopausal status was positively consistent in both cohorts, albeit TSAT was marginally significant in PREVEND. On the contrary, iron levels were not related to menopausal status in either of the cohorts (Table 2).

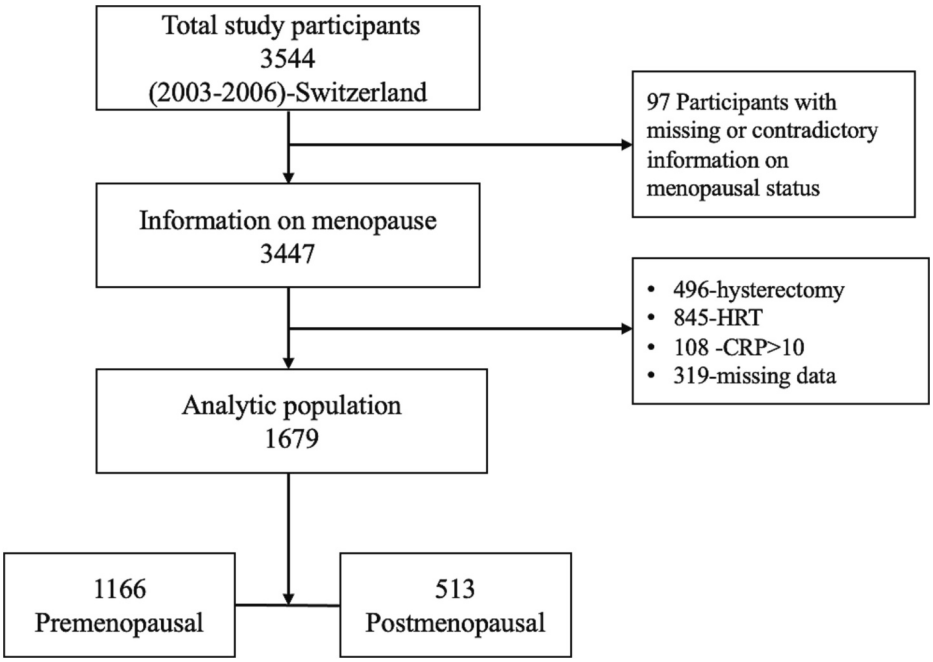

Fig. 1. Flowchart of participants from the CoLaus cohort .

Table 1
General characteristics of all eligible participants from CoLaus and PREVEND cohorts.

Characteristics	CoLaus ($n = 1679$) (2003–2006) Switzerland	PREVEND ($n = 2133$) (2001−2003) Netherlands
Postmenopausal n (%)	513 (30.6)	988(46)
Age, years	48.4 ± 9.89	51.3 ± 11.46
Cholesterol, mmol/L	5.4 (4.7–6.11)	5.3 (4.6–6.0)
Systolic BP, mmHg	118 (109–130)	116 (107–129)
Diastolic BP, mmHg	76 (69–83)	–
BMI, kg/m^2	23.42 (21.20–26.91)	25.39 (22.92–28.54)
Glucose, mmol/L	5.1 (4.8–5.5)	4.7 (4.3–5.1)
Transferrin, g/L	235.9 (212.7–268.1)	245 (215.8–270.1)
Ferritin, µg/L	65 (37–113)	55 (28–106)
Iron µmol/L	17(13.07–21.48)	15(11–18)

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

Table 2
Association of age and iron biomarkers with menopausal status.

Cohort	CoLaus (n = 1967)			PREVEND (n = 2133)		
Exposure	OR	95 % CI	P-value	OR	95 % CI	P-value
Age	1.62	1.53–1.71	<0.001	1.80	1.70–1.93	<0.001
Ferritin*	2.20	1.72–2.90	<0.001	2.21	1.74–2.80	<0.001
Transferrin*	0.03	0.01–0.10	<0.001	0.08	0.03–0.30	<0.001
Iron*	1.09	0.84–1.40	0.515	1.07	0.83–1.40	0.595
TSAT*	1.28	1.06–1.54	0.007	1.21	0.99–1.48	0.056

OR-Odds Ratio; 95 % CI- 95 % Confidence intervals; TSAT-Transferrin Saturation. Variables denoted with * have been transformed and adjusted for age.

3.2.1. *Utility of iron biomarkers to differentiate menopause; findings from primary cohort (CoLaus)*

Menopausal status was strongly related to age (Table 3A). Ferritin, transferrin, TSAT and iron alone showed lower performance than age, with ferritin performing better than transferrin, TSAT and iron (Table S2). Both transferrin and ferritin improved the base model significantly (p-value < 0.001), with a marginal improvement in the AUC, while TSAT and iron did not ($p > 0.05$) (Table 3A & Table 3B). The best model resulted from combining age, ferritin, and transferrin (Table 4). Age alone correctly classified 92.3 % ($n = 1550$) of the whole sample. Including transferrin and ferritin contributed to correctly classifying 1.4 % ($n = 23$) more participants.

Using Liu's method, the most optimal cut-off levels for age, ferritin, and transferrin in distinguishing women's menopausal status were 50.75 years, 79.05 µg/L, 2.34 g/L respectively, in the CoLaus cohort,

Table 3A
Menopause differentiation utility of iron biomarkers, CoLaus cohort.

	Sensitivity (95 % CI)	Specificity (95 % CI)	AUC	*AUC Δ	P-value
Age	83.80 (80.3–86.9)	96.10 (94.8–97.1)	0.9716	–	–
Age + Ferritin	86.70 (83.5–89.6)	96.10 (94.8–97.1)	0.9751	0.0035	<0.001
Age + Transferrin	85.00 (81.6–88.0)	96.10 (94.9–97.2)	0.9747	0.0021	<0.001
Age + Iron	84.21 (80.8–87.3)	96.04 (94.8–97.1)	0.9717	0.0001	0.715
Age + TSAT	84.4 (81.0–87.4)	96.00 (94.8–97.1)	0.9724	0.0008	0.150

TSAT-Transferrin Saturation; AUC-Area Under the Curve; Δ-Difference; *Difference in AUC between the base model and other models.

Table 3B
Menopause differentiation utility of iron biomarkers, PREVEND cohort.

	Sensitivity (95 % CI)	Specificity (95 % CI)	AUC	AUC Δ*	*P*-value
Age	92.4 (90.6–94.0)	93.9 (92.3–95.2)	0.9845	–	–
Age + Ferritin	93.0 (91.2–94.5)	94.7 (93.2–95.9)	0.9866	0.0021	>0.001
Age + Transferrin	92.8 (91.0–94.3)	94.2 (92.6–95.4)	0.9852	0.0007	0.051
Age + Iron	92.4 (90.6–94.0)	93.7 (92.1–95.0)	0.9845	0.0000	0.582
Age + TSAT	92.7 (90.9–94.2)	93.8 (92.2–95.1)	0.9846	0.0001	0.056

TSAT-Transferrin Saturation; AUC-Area Under the Curve; Δ Difference.
*-Difference in AUC between the base model and other models.

Table 4
Final model (Age, ferritin, and transferrin) performance in CoLaus and PREVEND cohorts.

	Sensitivity (95 % CI)	Specificity (95 % CI)	AUC	PPV	NPV
CoLaus	87.1 (83.9–89.9)	96.5 (95.4–97.5)	0.9764	91.7 %	94.4 %
PREVEND	93.0 (91.2–94.5)	94.4 (92.9–95.7)	0.9868	93.4 %	94.0 %
PREVEND*	93.6 (91.8–95.1)	94.8 (93.4–96.0)	0.9872	93.8 %	94.6 %

AUC -Area Under the Curve; PPV-Positive Predictive Value; NPV-Negative Predictive Value.
PREVEND*- Model with age and hepcidin.

with slight differences in the PREVEND cohort (Table 5).

3.2.2. *Utility of iron biomarkers to differentiate menopause; findings from replication cohort (PREVEND)*

Results in PREVEND showed similar results of iron biomarkers alone in differentiating menopausal status, although both ferritin and transferrin performed better compared to CoLaus cohort (Table S2). Similarly, the results of the final model including age, ferritin and transferrin were replicated in PREVEND (Table 4).

3.3. *Sensitivity analysis*

Results were consistent across all sensitivity analyses. Restricting the analysis to populations aged between 40 and 60 years old, as well as excluding women that were menopaused for >5 years in a second sensitivity analysis, did not affect the association between transferrin and ferritin and menopausal status, with TSAT and iron again not significantly improving the model with age. Similar findings were obtained after restricting the population to 40 to 55 years. Restricting the population to this age range increased the magnitude of improvement iron biomarkers had on the base model (Tables S3 to S8). In this age range, iron biomarkers improved classification by 7.5 % ($n = 73$) as opposed to a model only with age. Menopause-related cardiometabolic biomarkers as well as CRP and BMI adjusted for age did not show a better performance in differentiating menopausal status compared to a model containing only age (Table S9).

Finally, sTfR, did not significantly improve a model with age in the PREVEND cohort. On the other hand, hepcidin not only improved a model with age, but it outperformed the model containing age, transferrin, and ferritin (Table S10).

The cut-off points estimated using Youden's Index were very similar to the ones estimated using Liu's method (Tables S11 and S12). OR per SD increase are shown in Table S13.

Table 5
Cut off points derived from Liu's method using univariate models.

	CoLaus			PREVEND		
	Cutoff point	Sensitivity	Specificity	Cutoff point	Sensitivity	Specificity
Age	51.00	90 %	93 %	50.00	95 %	92 %
Ferritin(ug/L)	79.05	65 %	71 %	59.5	72 %	75 %
Transferrin(g/L)	2.34	44 %	45 %	2.55	47 %	38 %
TSAT%	27.79	59 %	48 %	16.36	73 %	75 %

TSAT-Transferrin Saturation.

4. Discussion

4.1. Interpretation of main findings

In this population-based cross-sectional study of the Swiss and Dutch cohorts, we showed that ferritin and transferrin levels are associated with menopausal status and can help determining menopausal status. Ferritin values of ≥79.1 μg/L, showed a sensitivity of 65 % and specificity of 71 %. When added to a model with age, transferrin and ferritin significantly improved the model, with an improvement of the model performance. Their utility was better compared to cardiometabolic biomarkers, despite the changes on cardiometabolic profile after menopause. It is worth mentioning that the magnitude of improvement of a base model by iron biomarkers increased by restricting the age range of our population. For example, without age restrictions, adding iron biomarkers to a model with age consisted of an increase on AUC of only 0.0048, in contrast to an improvement of 0.024 when restricting age between 40 and 55. Moreover, reclassification was improved by 7.5 % as opposed to an improvement of 1.4 %.

4.2. Our findings in context with previous research

Although there are studies on the change of levels of iron biomarkers after menopausal transition, none has evaluated their ability in determining menopausal status. In line with our findings, a Danish study among 1359 nonpregnant women found that postmenopausal women had higher ferritin levels than premenopausal women [21]. Another study in the USA found a higher ferritin and lower sTfR among postmenopausal women [22]. The NHANES III study found that ferritin levels stayed relatively low before menopause, after which they increased [23]. These changes come as a result of first and foremost the cessation of blood loss through the cessation of menses [14]. Moreover, menopausal transition and menopause have been characterized with an increase in inflammation as well as obesity and a change in body composition, conditions associated with changes in iron storages [15,24,25].

The better performance of hepcidin could be explained by the hypothesized correlation between hepcidin and oestradiol levels in the blood, with oestradiol suppressing hepcidin in order to increase iron absorption [12,26].

Previous studies on the relation between hormonal levels and menopausal status have had inconsistent findings. An FSH level of >40 IU/L has been proposed as indicative of postmenopausal status or late postmenopausal transition [27,28]. When using this cut-off to differentiate between perimenopausal and postmenopausal women, Stellato et al. reported a sensitivity of 55 %, a specificity of 66 %, and a PPV of 38 % [29]. Several other studies reported impaired ability of FSH, AMH, and inhibin B on differentiating menopausal status [9,30–34]. On the other hand, our findings show a better performance of iron biomarkers when defining postmenopausal status. However, these studies have differentiated between menopausal transition stages. We had no information on the different stages of menopausal transition (pre and perimenopause).

There have also been studies that have estimated menopausal status of participants in cases where data on menopausal status was missing or was invalid. When dealing with such scenarios, some studies have used age as a cut-off for defining menopausal status [5,35,36]. Another study assigned probabilities of menopausal status based on the age and the prevalence of menopause in their sample [37]. However, the variability in the age of menopause can lead to misclassification and misleading results [38]. If such an approach is to be implemented, our results suggest that in addition to age, iron biomarkers can help improving the rate of correctly classified participants, especially for women aged 40 to 55 years old. In addition, iron biomarkers levels could be used as priors when using Bayesian statistics on estimating the true prevalence of menopause in a given population.

4.3. Implications of our findings

Menopause has often been associated with societal stigma and only lately movements to overcome it have started to arise. Awareness campaigns in some countries have been followed by amendments in policies, exemplified by the UK government's decision on reducing the costs of HRT, a key preventive measure of the menopausal symptoms and the heightened risk for various diseases [39]. WHO advocates the inclusion of diagnosis, treatment, and management of menopause as part of universal health coverage [40]. However, regardless the proven fact that this is a high-risk community, menopause is poorly managed even in developed countries [41]. One of the reasons is also the difficulty of diagnosing menopausal status. A survey of 4014 women in the UK reports that 30 % of women declared a delay in diagnosis of menopause, and multiple visits were needed to define their menopausal status. In addition, this report states the health economic impact of menopause and the benefits of such preventive strategies [39]. Timely diagnosis is crucial for management of menopause and the prevention of chronic diseases, whose risk increases after this transition. Therefore, an objective method is urgently needed. Ferritin and transferrin are attractive candidates for menopausal identification due to their cost-effectiveness, widespread accessibility, and well-known uses and interpretation [42]. Thus, new methods that could aid in the diagnosis of menopausal status and shortening the time to diagnosis, avoiding the recall bias of self-identification, would lead to a more accurate designation of policies with timely screening and more successful prevention programs, especially in cases of POI [41]. This gains more relevancy considering ferritin and other iron biomarkers have been implicated in contributing to menopause and sex-specific-related cardiometabolic conditions, thus their significance may extend beyond the menopausal transition itself [43].

4.4. Strengths and limitations

The strengths of this study are the population-based study design, the large sample size and the detailed data available that included different iron biomarkers measured. Furthermore, our results were replicated in PREVEND cohort study, showing consistency through most of our main and sensitivity analyses.

Some limitations that need to be addressed are self-reported menopausal status where recall bias cannot be ruled out; however, we expect this to be independent of iron assessment. Most of our sample was of Caucasian origin; this might reduce some confounding bias but also the

generalizability of our results to other ethnic backgrounds. The two cohorts also differed on assessments of menopausal status. While in CoLaus both menopausal status and age of menopause were used, in PREVEND only age of reported natural menopause was used as indicator of postmenopausal status. Moreover, the two cohorts had different sociodemographic characteristics and used different methods for iron biomarkers quantification, which could have contributed to differences in cut-off values of iron biomarkers to define menopause status. However, we believe these factors did not produce substantial changes in our findings as the direction and the effect sizes were similar, and no substantial differences were identified between cohorts. Moreover, in the CoLaus cohort, over 20 % of the participants were using HRT, versus only 7 % in PREVEND. Lastly, no information on different stages of the menopausal transition was available, which precludes the assessment of the capacity of iron biomarkers on differentiating between different stages of menopause. However, our sensitivity analyses suggest that iron biomarkers can help improving classification of menopausal status of women in early post menopause (less than five years since last menstrual period). This is particularly important when discussing HRT initiation [4]. Nevertheless, more studies exploring the changes in iron status from perimenopause to menopause are needed to corroborate our findings.

5. Conclusions

Ferritin and transferrin levels were consistently related to menopausal status and modestly improved a model relying on age on defining the menopausal status, while our findings on hepcidin need further replication. This improvement was higher when restricting the range of age to the age in which menopause is more common to occur. Further research is needed to explore whether iron biomarkers can have clinical and public health utility in menopause staging and management.

Contributors

Lum Kastrati participated in all the statistical analyses and drafting the manuscript.

Dion Groothof proposed the idea and helped in drafting the article.

Hugo G. Quezada-Pinedo revised and wrote part of the article.

Hamidreza Raeisi-Dehkordi contributed to revision of the paper for important intellectual content.

Lia Bally contributed to revision of the paper for important intellectual content.

Martin H. De Borst contributed to revision of the paper for important intellectual content.

Stephan J.L. Bakker contributed to revision of the paper for important intellectual content.

Pedro-Marques Vidal drafted, reviewed and supervised the study.

Michele F. Eisenga revised the manuscript and helped on results interpretation.

Taulant Muka drafted, reviewed and supervised the study.

Funding

This work was funded by the SNSF project, grant IZLIZ3_200256.

Ethical approval

The CoLaus study was approved by the Institutional Ethics Committee of the University of Lausanne.

The PREVEND study has been approved by the local medical ethics committee (MEC 96/01/022).

Provenance and peer review

This article was not commissioned and was externally peer reviewed.

Research data (data sharing and collaboration)

There are no linked research data sets for this paper. The datasets analysed in this study are not publicly available on the level of individual records, since this was excluded in the informed consents signed by the participants. Requests to access the datasets should be directed via the CoLaus| website (www.colaus-psycolaus.ch/professionals/how-to-collaborate) and to h.hillege@umcg.nl for the PREVEND cohort data.

Declaration of competing interest

The authors declare that they have no competing interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.maturitas.2023.107872.

References

[1] G.A. Greendale, N.P. Lee, E.R. Arriola, The menopause, Lancet 353 (9152) (1999) 571–580, https://doi.org/10.1016/s0140-6736(98)05352-5.
[2] Research on the menopause in the 1990s. Report of a WHO Scientific Group, World Health Organ. Tech. Rep. Ser. 866 (1996) 1–107.
[3] L.T. Shuster, et al., Premature menopause or early menopause: long-term health consequences, Maturitas 65 (2) (2010) 161–166, https://doi.org/10.1016/j.maturitas.2009.08.003.
[4] J.M. Mehta, R.C. Chester, J.M. Kling, The timing hypothesis: hormone therapy for treating symptomatic women during menopause and its relationship to cardiovascular disease, J Womens Health (Larchmt) 28 (5) (2019) 705–711, https://doi.org/10.1089/jwh.2018.7201.
[5] A. Morabia, P. Flandre, Misclassification bias related to definition of menopausal status in case-control studies of breast cancer, Int. J. Epidemiol. 21 (2) (1992) 222–228, https://doi.org/10.1093/ije/21.2.222.
[6] S.D. Harlow, et al., Executive summary of the stages of reproductive aging workshop + 10: addressing the unfinished agenda of staging reproductive aging, J. Clin. Endocrinol. Metab. 97 (4) (2012) 1159–1168, https://doi.org/10.1210/jc.2011-3362.
[7] L.A. Bastian, C.M. Smith, K. Nanda, Is this woman perimenopausal? JAMA 289 (7) (2003) 895–902, https://doi.org/10.1001/jama.289.7.895.
[8] H. Raeisi-Dehkordi, et al., Risk prediction models of natural menopause onset: a systematic review, J. Clin. Endocrinol. Metab. 107 (10) (2022) 2934–2944, https://doi.org/10.1210/clinem/dgac461.
[9] E.A. Lenton, et al., Progressive changes in LH and FSH and LH: FSH ratio in women throughout reproductive life, Maturitas 10 (1) (1988) 35–43, https://doi.org/10.1016/0378-5122(88)90129-6.
[10] H.G. Burger, et al., The endocrinology of the menopausal transition: a cross-sectional study of a population-based sample, J. Clin. Endocrinol. Metab. 80 (12) (1995) 3537–3545, https://doi.org/10.1210/jcem.80.12.8530596.
[11] N. Milman, M. Kirchhoff, Iron stores in 1359, 30- to 60-year-old Danish women: evaluation by serum ferritin and hemoglobin, Ann. Hematol. 64 (1) (1992) 22–27, https://doi.org/10.1007/bf01811467.
[12] R.A. Matta, et al., Interplay of serum hepcidin with female sex hormones, metabolic syndrome, and abdominal fat distribution among premenopausal and postmenopausal women, Egypt. J. Intern. Med. 34 (1) (2022) 8, https://doi.org/10.1186/s43162-022-00098-9.
[13] F. Merlo, et al., Changes in Iron status biomarkers with advancing age according to sex and menopause: a population-based study, J. Clin. Med. 12 (16) (2023), https://doi.org/10.3390/jcm12165338.
[14] C. Kim, et al., Changes in iron measures over menopause and associations with insulin resistance, J Womens Health (Larchmt) 21 (8) (2012) 872–877, https://doi.org/10.1089/jwh.2012.3549.
[15] M. McCarthy, A.P. Raval, The peri-menopause in a woman's life: a systemic inflammatory phase that enables later neurodegenerative disease, J. Neuroinflammation 17 (1) (2020) 317, https://doi.org/10.1186/s12974-020-01998-9.
[16] M. Firmann, et al., The CoLaus study: a population-based study to investigate the epidemiology and genetic determinants of cardiovascular risk factors and metabolic syndrome, BMC Cardiovasc. Disord. 8 (2008) 6, https://doi.org/10.1186/1471-2261-8-6.
[17] S.J. Pinto-Sietsma, et al., Urinary albumin excretion is associated with renal functional abnormalities in a nondiabetic population, J. Am. Soc. Nephrol. 11 (10) (2000) 1882–1888, https://doi.org/10.1681/asn.V11101882.
[18] L. Mercadal, et al., A 3-marker index improves the identification of iron disorders in CKD anaemia, PloS One 9 (2) (2014), e84144, https://doi.org/10.1371/journal.pone.0084144.
[19] X. Liu, Classification accuracy and cut point selection, Stat. Med. 31 (23) (2012) 2676–2686, https://doi.org/10.1002/sim.4509.

[20] R.E. Nappi, et al., Menopause: a cardiometabolic transition, Lancet Diabetes Endocrinol. 10 (6) (2022) 442–456, https://doi.org/10.1016/S2213-8587(22)00076-6.
[21] N. Milman, M. Kirchhoff, T. JØrgensen, Iron status markers, serum ferritin and hemoglobin in 1359 Danish women in relation to menstruation, hormonal contraception, parity, and postmenopausal hormone treatment, Ann. Hematol. 65 (2) (1992) 96–102, https://doi.org/10.1007/BF01698138.
[22] Changes in iron measures over menopause and associations with insulin resistance, J. Womens Health 21 (8) (2012) 872–877, https://doi.org/10.1089/jwh.2012.3549.
[23] L.R. Zacharski, et al., Association of age, sex, and race with body iron stores in adults: analysis of NHANES III data, Am. Heart J. 140 (1) (2000) 98–104, https://doi.org/10.1067/mhj.2000.106646.
[24] S. Lynch, et al., Biomarkers of nutrition for development (BOND)-Iron review, J. Nutr. 148 (suppl_1) (2018) 1001s–1067s, https://doi.org/10.1093/jn/nxx036.
[25] J.X. Yan, et al., Serum ferritin is correlated with non-alcoholic fatty liver disease in middle-aged and older patients with type 2 diabetes, Endocr. Connect. 10 (12) (2021) 1560–1569, https://doi.org/10.1530/ec-21-0367.
[26] M. Lehtihet, et al., Circulating Hepcidin-25 is reduced by endogenous estrogen in humans, PloS One 11 (2) (2016), e0148802, https://doi.org/10.1371/journal.pone.0148802.
[27] R.L. Goldenberg, et al., Gonadotropins in women with amenorrhea. The use of plasma follicle-stimulating hormone to differentiate women with and without ovarian follicles, Am. J. Obstet. Gynecol. 116 (7) (1973) 1003–1012, https://doi.org/10.1016/s0002-9378(16)33850-9.
[28] S.D. Harlow, et al., Recommendations from a multi-study evaluation of proposed criteria for staging reproductive aging, Climacteric 10 (2) (2007) 112–119, https://doi.org/10.1080/13697130701258838.
[29] R.K. Stellato, et al., Can follicle-stimulating hormone be used to define menopausal status? Endocr. Pract. 4 (3) (1998) 137–141, https://doi.org/10.4158/ep.4.3.137.
[30] M.R. Sowers, et al., Follicle stimulating hormone and its rate of change in defining menopause transition stages, J. Clin. Endocrinol. Metab. 93 (10) (2008) 3958–3964, https://doi.org/10.1210/jc.2008-0482.
[31] J. MacNaughton, et al., Age related changes in follicle stimulating hormone, luteinizing hormone, oestradiol and immunoreactive inhibin in women of reproductive age, Clin. Endocrinol. (Oxf) 36 (4) (1992) 339–345, https://doi.org/10.1111/j.1365-2265.1992.tb01457.x.
[32] H.G. Burger, et al., The endocrinology of the menopausal transition: a cross-sectional study of a population-based sample, J. Clin. Endocrinol. Metabol. 80 (12) (1995) 3537–3545, https://doi.org/10.1210/jcem.80.12.8530596.
[33] M.F.R. Sowers, et al., Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition, J. Clin. Endocrinol. Metabol. 93 (9) (2008) 3478–3483, https://doi.org/10.1210/jc.2008-0567.
[34] H.G. Burger, Diagnostic role of follicle-stimulating hormone (FSH) measurements during the menopausal transition–an analysis of FSH, oestradiol and inhibin, Eur. J. Endocrinol. 130 (1) (1994) 38–42, https://doi.org/10.1530/eje.0.1300038.
[35] E. Ron, et al., Cancer incidence in a cohort of infertile women, Am. J. Epidemiol. 125 (5) (1987) 780–790, https://doi.org/10.1093/oxfordjournals.aje.a114595.
[36] V. Siskind, et al., Breast cancer and breastfeeding: results from an Australian case-control study, Am. J. Epidemiol. 130 (2) (1989) 229–236, https://doi.org/10.1093/oxfordjournals.aje.a115329.
[37] A. Kroke, et al., Assignment to menopausal status and estimation of age at menopause for women with missing or invalid data–a probabilistic approach with weighting factors in a large-scale epidemiological study, Maturitas 40 (1) (2001) 39–46, https://doi.org/10.1016/s0378-5122(01)00228-6.
[38] E.B. Gold, et al., Factors associated with age at natural menopause in a multiethnic sample of midlife women, Am. J. Epidemiol. 153 (9) (2001) 865–874, https://doi.org/10.1093/aje/153.9.865.
[39] https://www.fawcettsociety.org.uk/menopauseandtheworkplace.
[40] https://www.who.int/news-room/fact-sheets/detail/menopause.
[41] R.A. Lobo, A. Gompel, Management of menopause: a view towards prevention, Lancet Diabetes Endocrinol. 10 (6) (2022) 457–470, https://doi.org/10.1016/s2213-8587(21)00269-2.
[42] T. Shoji, et al., Both low and high serum ferritin levels predict mortality risk in hemodialysis patients without inflammation, Clin. Exp. Nephrol. 21 (4) (2017) 685–693, https://doi.org/10.1007/s10157-016-1317-1.
[43] F. Khatami, et al., Sex and N-terminal pro B-type natriuretic peptide: the potential mediating role of iron biomarkers, Front. Cardiovasc. Med. 9 (2022), 897148, https://doi.org/10.3389/fcvm.2022.897148.