Menopause: The Journal of The North American Menopause Society
Vol. 26, No. 1, pp. 32-38
DOI: 10.1097/GME.0000000000001162

A prospective study of inflammatory biomarker levels and risk of early menopause

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Abstract

Objective: Early menopause, the cessation of ovarian function before age 45, has consequences for fertility and cardiovascular health. Evidence from studies of women with autoimmune conditions and genetic studies supports a role for inflammation in early menopause, but the association of inflammatory markers and risk has not been directly evaluated.

Methods: We assessed the relation of the soluble fraction of tumor necrosis factor alpha receptor 2 (sTNFR2), C-reactive protein, interleukin-6 (IL6) levels with incident early menopause among Nurses' Health Study II participants who provided a premenopausal blood sample in 1996 to 1999. Cases ($n = 328$) were women reporting natural menopause between blood collection and age 45.

Controls ($n = 492$) included (1) 328 women with menopause after age 47, matched 1:1 with cases on age at blood collection and other factors; and (2) 164 additional women with menopause after age 45.

Results: In multivariable models comparing cases and $n = 492$ controls, we observed a significant association of sTNFR2 levels and risk of early menopause ($P = 0.002$). Compared with women with the lowest sTNFR2 levels, odds ratios (95% CIs) for quartiles 2 to 4 were 0.60 (0.38-0.95), 0.93 (0.61-1.43), and 1.40 (0.93-2.11). Results further adjusting for antimüllerian hormone levels were similar in magnitude, as were results from sensitivity analyses of matched cases and controls ($n = 328$ pairs), nonsmokers, and leaner women. C-reactive protein and IL6 levels were unrelated to risk.

Conclusions: The observation of lower risk of early menopause among women with moderate sTNFR2 levels compared with women with lower and higher levels warrants further prospective study.

Key Words: Antimüllerian hormone – Inflammation – Menopause – Prospective studies – Tumor necrosis factor-alpha.

The prevalence of early natural menopause in western populations is high, with up to 10% of women experiencing the cessation of ovarian function before the age of 45.[1] The consequences of early menopause for family planning are substantial, especially as women increasingly delay childbearing. Furthermore, early cessation of ovarian function may increase risk of cardiovascular disease and other chronic conditions.[2,3] Although autoimmune conditions and genetic factors have been associated with early menopause and primary ovarian insufficiency (POI), or menopause before age 40, population-based studies indicate that these factors do not explain the majority of cases.[4,5] The ability to predict early menopause before the fertility decline would allow women to make more informed decisions on the timing of childbearing and increase treatment options.

Inflammatory factors are integral in reproduction, and could potentially contribute to ovarian aging and menopause timing. In premenopausal women, tumor necrosis factor alpha (TNFa), C-reactive protein (CRP), and interleukin (IL)-6 levels fluctuate across the menstrual cycle, with levels increasing at ovulation and peaking during menstruation.[6-8] Recent studies of TNFa in particular indicate that this cytokine plays an important role in follicle recruitment, ovulation, follicle atresia, and corpus luteum function.[9,10] Two cross-sectional studies have observed lower TNFa levels in POI cases compared with controls.[11,12]

Received February 12, 2018; revised and accepted May 7, 2018.

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Funding/support: This project was supported by UM1CA176726, R01CA67262, and R01HD078517 from the U.S. Department of Health and Human Services, National Institutes of Health.

Financial disclosure/conflicts of interest: None reported.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Website (www.menopause.org).

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To our knowledge, whether inflammatory marker levels are associated with risk of early menopause or overall timing of menopause has not been evaluated in prospective studies. We have assessed the relation of levels of biomarkers of inflammation and risk of early menopause in a prospective study nested within the Nurses' Health Study II (NHS2).

METHODS

Study population

The NHS2 is a prospective study of 116,429 US female registered nurses who responded to a mailed questionnaire in 1989. Participants were 25 to 42 years old at baseline and provided information on medical history and health-related behaviors such as oral contraceptives (OCs), menstrual and pregnancy history, and smoking status. Cohort members have completed questionnaires every 2 years to update information on risk factors and to identify new diagnoses of disease. The cumulative response rate for blood cohort members is >94%. The study protocol was approved by the Institutional Review Board at Brigham and Women's Hospital.

NHS2 blood cohort

Between 1996 and 1999, NHS2 members who had not been diagnosed with cancer were invited to provide blood samples. Cohort member were aged 32 to 54 years at blood collection. Women who were premenopausal, not using exogenous hormones, and who had not been pregnant in the past 6 months were asked to collect one sample in the follicular phase of the menstrual cycle (days 3-5) and one sample in the luteal phase (7-9 d before the anticipated start date of next menses). Women with irregular cycles were asked to collect their luteal phase sample 22 days after last menses. Premenopausal women unwilling to collect timed samples and those currently using hormone therapy (HT) or OCs were asked to provide a single untimed sample. The actual start date of next menses was then confirmed by postcard, allowing for confirmation of cycle phase. Upon receipt, samples were centrifuged, separated into blood components, aliquoted, and archived at −130°C or colder in continuously monitored nitrogen freezers. Samples were received from 29,611 women and approximately 23,000 (79%) were premenopausal. Participants providing a blood sample did not differ substantially from the main NHS2 cohort in terms of BMI (26 vs 26 kg/m^2) and parity (1.9 vs 1.9 children), proportion ever smoking (34% vs 36%) and ever using OCs (86% vs 88%), and other factors.[13]

Assessment of menopause timing and case and control identification

Beginning in 1989, NHS members were asked if their menstrual periods had ceased permanently, with response options of (1) No, premenopausal; (2) Yes, no menstrual periods; (3) Yes, had menopause but now have periods induced by hormones; and (4) Not sure (eg, started hormones before cessation of periods). Women reporting that their periods had stopped were then asked: (1) at what age did your periods cease (open response); and (2) for what reason did your periods cease, with response options of surgery, radiation or chemotherapy, or natural. All women were then asked about use of HT including timing and the type used. These questions have been reported on each biennial questionnaire.

In selecting cases and controls for this nested analysis, we excluded women with diagnoses of cardiovascular disease or cancer (other than nonmelanoma skin cancer) before blood collection. From among women who were premenopausal and <age 45 at blood collection, we identified those who reported natural menopause between the date of receipt of their blood sample and age 45, through the end of follow-up in June, 2011 ($n = 328$). Cases reporting hysterectomy or oophorectomy before report of menopause were not eligible. We then defined two sets of controls. First, cases were matched 1:1 with control women who were (1) premenopausal at the time of blood collection; (2) reported menopause at age ≥47; and (3) did not report hysterectomy or oophorectomy before age 47 ($n = 328$ controls). Cases and controls were matched by age at the time of blood collection (±4 mo), as well as by fasting status, time of day, season of blood collection, and sample type (luteal phase or untimed). Second, to maximize the generalizability of our sample, we selected additional controls who experienced menopause at age ≥45, preferentially selecting women with natural menopause at ages 45 and 46 to ensure that the control group included women on the earlier end of the normal range of menopause age ($n = 164$), for a total of 492 controls.

Assessment of inflammatory biomarkers and anti-Müllerian hormone

Blood samples were assayed for inflammatory factors and anti-Müllerian hormone (AMH) at Children's Hospital, Boston, MA, in 2016. The soluble fraction of the TNFa receptor 2 (sTNFR2) was measured as a proxy for TNFa, as sTNFR2 tends to be more stable and better reflects average circulating TNFa than measuring TNFa directly and is used commonly as a proxy for TNFa.[14,15] sTNFR2 was measured by ELISA using immobilized monoclonal antibody to human sTNFR2 (Genzyme, Cambridge, MA). CRP was measured using a high-sensitivity latex-enhanced immunonephelometric assay on a BNII analyzer (Dade Behring, Newark, DE). IL6 was measured by a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay, R&D systems, Minneapolis, MN). AMH was measured by an ultra-sensitive ELISA assay from ANSH Labs (picoAMH; Webster, TX). Samples were labeled by number only, and case and controls were handled identically, shipped in the same batch and assayed in the same analytic run. The coefficient of variations from samples from blinded plasma pools assayed alongside our analytic samples were as follows: sTNFR2 = 5.7%; CRP = 7.3%; IL6 = 10.6%; AMH = 8.6%.

Covariate assessment

At blood collection, women provided current information regarding weight, menstrual cycle regularity, menstrual cycle characteristics, exogenous hormone use, physical activity,

alcohol use, smoking status, and medication use, including nonsteroid anti-inflammatory drugs (NSAIDs). At baseline (1989), women provided information on race, ethnicity, height, age at menarche, and the number of years until their menstrual cycles became regular after menarche. Smoking status, number of cigarettes per day, pregnancy history, and OC use were measured every 2 years. Cumulative breast feeding history was measured in 1993 and 1997. For time varying factors, we used data measured closest in time to blood collection. In addition, we identified women reporting clinician-made diagnoses of rheumatoid arthritis, ulcerative colitis, Graves' disease, hypothyroidism, multiple sclerosis, and systemic lupus erythematosus before blood collection.

Statistical analysis

We compared characteristics of cases and matched control at blood collection using *t* tests for continuous factors and chi-square or Fisher's exact tests for categorical factors. Because inflammatory factor levels were not normally distributed, and to allow for evaluation of nonlinear associations, we divided women into quartiles based on levels in controls. Our primary analyses compared cases ($n = 328$) with all controls ($n = 492$) to maximize power and generalizability, using unconditional logistic regression to estimate odds ratios (ORs) and 95% CIs. In addition to age-adjusted models, we controlled for risk factors for early menopause chosen a priori, and factors improving model fit based on likelihood ratio tests. Our main multivariable (MV) model included age in months, fasting status at blood collection, ethnicity, body mass index, pack-years of cigarette smoking, years until menstrual cycle regularity, duration of OC use, parity, duration of exclusive breast feeding, alcohol intake, and exogenous hormone use. Factors tested but not meeting selection criteria included race, NSAIDs use, age at menarche, and physical activity. In a second MV model, we further adjusted for AMH levels, the strongest predictor of early menopause in our population, modeling AMH with both linear and quadratic terms.

To further evaluate potential associations of AMH with inflammatory markers, we compared geometric means of AMH levels across quartiles of inflammatory factors using all cases and controls. To assess whether inflammatory biomarker levels were related to overall menopause timing, we compared number of years until menopause across quartiles of inflammatory factors; these analyses included all cases ($n = 328$) and controls experiencing natural menopause ($n = 449$).

We conducted several sensitivity analyses. First, we used survival methods to model the relation of inflammatory factors and years until menopause. Second, we used conditional logistic regression to compare risk in matched cases and controls ($n = 328$ pairs). MV models for these analyses included the same factors listed above, with the exception of matching factors (age and fasting status). Finally, we evaluated relations in subpopulations of women, including never smokers; women with BMI $\leq 25\,kg/m^2$; women without autoimmune conditions; women providing luteal-phase samples; and women not using NSAIDs or exogenous hormones, to confirm that associations were not driven by residual confounding by these factors. Statistical analyses were conducted with SASv9.4 (Cary, NC).

RESULTS

Characteristics comparing early menopause cases ($n = 328$) and controls ($n = 492$) are shown in Table 1. Cases and controls differed significantly by ethnicity, smoking status, pack-years of smoking, duration of exclusive breast feeding, and AMH levels. Cases were similar to controls in terms of mean age, BMI, use of OCs and NSAIDs, and physical activity and other factors.

Early menopause cases had marginally significantly higher geometric mean sTNFR2 levels than controls (2,250 vs 2,182 pg/mL; $P = 0.05$), but mean CRP and IL6 did not vary by case status (Table 2). Levels of sTNFR2, CRP, and IL6 were significantly correlated; pair-wise Spearman correlations were as follows: sTNFR2 versus CRP = 0.28; sTNFR2 versus IL6 = 0.32; CRP versus IL6 = 0.51 (all $P < 0.0001$).

Risk of early menopause by quartile of inflammatory marker levels is shown in Table 3. sTNFR2 levels were associated with early menopause risk in a nonmonotonic fashion. In multivariable models, ORs (95% CI) for early menopause for women in quartiles 2 to 4 of sTNFR2 were 0.59 (95% CI = 0.38-0.93), 0.93 (95% CI = 0.61-1.42), and 1.40 (95% CI = 0.93-2.12) compared with women in quartile 1 ($P = 0.002$). Additional adjustment for AMH levels had minimal effect on ORs and sTNFR2 levels remained associated with risk. In contrast, CRP and IL6 levels were unrelated to early menopause. Results from analyses limited to 328 matched cases and controls were highly similar, but less precise due to lower sample size (see Table, Supplemental Digital Content 1, http://links.lww.com/MENO/A340, which presents results from analyses of inflammatory factors and risk of early menopause limited to matched cases and controls.)

Table 4 presents adjusted geometric mean AMH levels across quartiles of inflammatory markers. Results suggested a modest nonmonotonic relation of sTNFR2 and AMH ($P = 0.05$), with levels highest in Q2 and Q3 and lowest in Q4. sTNFR2 levels were significantly associated with the timing of menopause. Adjusted geometric mean years until menopause in women in quartiles 1 to 4 were 5.2, 6.0, 5.3, and 4.7 years, respectively ($P = 0.008$). Associations persisted with additional adjustment for AMH ($P = 0.007$). In contrast, CRP and IL6 level were unrelated to AMH levels and time to menopause. Results using survival analysis methods were nearly identical to the main analysis (results not shown).

We conducted a series of sensitivity analyses. Among women with BMI $< 25\,kg/m^2$ ($n = 204$ cases and 307 controls), findings for sTNFR2 were slightly stronger than in the main analysis; ORs for women in quartiles 2 to 4 of sTNFR2 were 0.50 (95% CI = 0.29-0.88), 0.91 (95% CI = 0.54-1.53), and 1.62 (95% CI = 0.96-2.73) compared with women in quartile 1 ($P = 0.001$). Among never smokers ($n = 223$ cases and 323 controls), ORs in quartiles 2 to 4 of sTNFR2 were

TABLE 1. *Characteristics of early menopause cases and controls at the time of blood collection, Nurses' Health Study II, 1996-1999*

Characteristic	Cases ($n = 328$) Mean (SD)	Controls ($n = 492$) Mean (SD)	*P*
Age at blood collection, y	40.2 (2.8)	40.3 (2.7)	0.55
Body mass index, kg/m^2	25.4 (5.8)	25.0 (5.3)	0.32
Age at menarche, y	12.4 (1.3)	12.4 (1.4)	0.96
Years until cycle became regular, y[a]	1.4 (1.4)	1.6 (1.5)	0.06
Pack years of cigarette smoking[b]	13.0 (9.8)	10.7 (8.0)	0.03
Parity (number of pregnancies ≥6 mo)[c]	2.3 (1.0)	2.4 (0.9)	0.28
Duration of exclusive breast feeding, mo[c]	5.1 (6.0)	6.7 (6.7)	0.002
Duration of oral contraceptive use, mo[d]	49.6 (51.1)	52.9 (55.2)	0.41
Antimüllerian hormone, ng/mL[e]	0.14 (0.12)	0.87 (0.78)	<0.0001
	n (%)	*n* (%)	*P*
White race	313 (95.4)	480 (97.6)	0.09
Non-Hispanic ethnicity	318 (97.0)	488 (99.2)	0.02[5]
Former smoker	59 (18.0)	122 (24.8)	0.02
Current smoker	46 (14.0)	47 (9.6)	
Physical activity >1 time/wk	133 (40.6)	217 (44.1)	0.31
Alcohol intake ≥1 drink/d	18 (5.5)	45 (9.2)	0.05
NSAID use	143 (43.6)	211 (42.9)	0.84
Exogenous hormone use	14 (4.3)	11 (2.2)	0.10
History of infertility	87 (26.5)	120 (24.4)	0.49
Autoimmune disorders[f]	54 (16.5)	56 (11.4)	0.04

[a]Among women whose cycles ever became regular.
[b]Limited to ever smokers.
[c]Limited to parous women.
[d]Lifetime duration of oral contraceptive use.
[e]AMH was ln-transformed for analysis and then exponentiated.
[f]Includes self-report of clinician-made diagnosis of rheumatoid arthritis, multiple sclerosis, Graves' disease, hypothyroidism, ulcerative colitis, or systemic lupus erythematosus.

0.47 (95% CI = 0.27-0.82), 0.83 (95% CI = 0.50-1.38), and 1.05 (95% CI = 0.63-1.76) compared with quartile 1 ($P = 0.02$). Results from additional analyses limited to (1) women without autoimmune conditions; (2) women not using exogenous hormones at blood collection; (3) women providing timed luteal phase samples; and (4) women not using NSAIDs were also highly similar to the main analysis (P for sTNFR2 for all analyses ≤0.05; see Table, Supplemental Digital Content 2, http://links.lww.com/MENO/A340, which presents results of the sensitivity analyses of the association of sTNFR2 and risk of early menopause).

DISCUSSION

In our prospective analysis, we observed nonlinear associations of sTNFR2 with early menopause, such that risk was lower in women with moderate sTNFR2 levels compared with women with lower and higher levels. sTNFR2 levels were similarly associated with time until menopause. Adjustment for AMH levels minimally affected results, suggesting that this association is not explained by AMH-related pathways. Furthermore, results were similar in analyses limited to never smokers, lean–normal women, and women without autoimmune disorders.

Few studies have evaluated how inflammatory factors are related to menopause timing. Data from clinical studies and animal models, however, provide some support for a nonlinear relation of sTNFR2 and ovarian function. Concentrations of sTNFR2 are observed to correlate strongly with TNFa and seem to have the primary function of regulating TNFa activity by binding to TNFa and preventing the ligand from binding to cell surface TNFR2.[14,15] TNFa is present in granulosa cells of the ovary in many species, including humans, rats, and mice.[9] Laboratory data suggest that TNFa regulates the balance of follicle development and atresia via apoptosis, as well as estrogen and progesterone production.[9,10] Compared with wild-type mice, TNF knockout mice have higher fertility, larger follicle pools, and a greater number of cycles per 21-day period.[9] The rate of atresia is also significantly lower in TNFa knockouts. Interestingly, TNFa knockout does not seem to lead to earlier depletion of the follicle pool, though previous studies have observed early reproductive decline and

TABLE 2. *Geometric means[a] of inflammatory biomarker levels in early menopause cases and controls, Nurses' Health Study II, 1996-2011*

	sTNFR2, pg/mL Mean (95% CI)	CRP, mg/L Mean (95% CI)	IL-6, pg/mL Mean (95% CI)
Early menopause ($n = 328$)	2,250 (2,198-2,303)	0.86 (0.75-0.98)	1.11 (1.03-1.21)
Controls ($n = 492$)	2,182 (2,141-2,224)	0.81 (0.72-0.91)	1.11 (1.04-1.19)
	$P = 0.05$	$P = 0.54$	$P = 0.97$

CRP, C-reactive protein; IL6, interleukin 6; sTNFR2, soluble fraction of tumor necrosis factor alpha receptor 2.
[a]Ln-transformed for analysis and then exponentiated.

TABLE 3. *Odds ratios and 95% CIs for early menopause by levels of inflammatory biomarkers, Nurses' Health Study II, 1996-2011*

	Cases	Controls	Age-Adjusted[a] OR (95% CI)	MV model[b] OR (95% CI)	MV model + AMH[c] OR (95% CI)
sTNFR2, pg/mL					
<1,908	86	123	Reference	Reference	Reference
1,908 to <2,156	52	123	0.61 (0.40-0.93)	0.59 (0.38-0.93)	0.60 (0.36-1.02)
2,156 to <2,466	70	122	0.82 (0.55-1.23)	0.93 (0.61-1.42)	0.96 (0.58-1.57)
≥2,466	120	124	1.39 (0.96-2.01)	1.40 (0.93-2.12)	1.36 (0.84-2.22)
			$P < 0.001$[d]	$P = 0.002$	$P = 0.02$
CRP, mg/L					
<0.35	81	124	Reference	Reference	Reference
0.35 to <0.75	90	122	1.14 (0.77-1.68)	1.07 (0.71-1.62)	1.28 (0.78-2.09)
0.75 to <1.77	67	122	0.85 (0.56-1.28)	0.85 (0.55-1.32)	0.81 (0.49-1.36)
≥1.77	90	124	1.12 (0.76-1.65)	1.02 (0.64-1.62)	0.95 (0.55-1.63)
			$P = 0.48$	$P = 0.73$	$P = 0.35$
IL6, pg/mL					
<0.66	77	123	Reference	Reference	Reference
0.66 to <0.916	65	122	0.85 (0.56-1.29)	0.83 (0.54-1.29)	0.82 (0.49-1.37)
0.916 to <1.586	99	123	1.29 (0.87-1.90)	1.18 (0.78-1.80)	1.22 (0.74-2.00)
≥1.586	87	124	1.13 (0.76-1.67)	0.94 (0.60-1.49)	0.97 (0.57-1.66)
			$P = 0.22$	$P = 0.42$	$P = 0.48$

AMH, antimüllerian hormone; CRP, C-reactive protein; IL6, interleukin 6; MV, multivariable; sTNFR2, soluble fraction of tumor necrosis factor alpha receptor 2.
[a]OR from unconditional logistic regression adjusted for age at blood collection in months (continuous).
[b]MV model adjusted for age at blood collection (continuous, months); fasting status at blood collection (<8, ≥8 h); BMI (<18.5, 18.5 to <25, 25 to <30, ≥30 kg/m^2); ethnicity (Hispanic, non-Hispanic); years until cycle regularity (<1, 1-2, 3-4, ≥5, never); pack-years of smoking (continuous, years); duration of oral contraceptive use (0-23, 24-71, ≥72 mo); parity (0, 1-2,3-4, ≥5 term pregnancies); duration of exclusive breast feeding (continuous, months); alcohol intake (none, <1, 1, ≥1/d); autoimmune disorders (yes, no); and exogenous hormone use (yes, no).
[c]MV model additionally adjusted for antimüllerian hormone levels (modeled as continuous AMH levels and AMH^2).
[d]*P* values from likelihood ratio tests.

TABLE 4. *Geometric means of level of antimüllerian hormone and years until menopause by quartiles of inflammatory biomarker levels among early menopause cases and controls[a], Nurses' Health Study II, 1996-2011*

	AMH, ng/mL[b]	Years until menopause, y	
	MV[c] Geometric mean (95% CI)	MV model[c] Geometric mean (95% CI)	MV model + AMH[d] Geometric mean (95% CI)
sTNFR2			
Q1	0.23 (0.13-0.41)	5.2 (3.7-6.7)	5.2 (4.0-6.4)
Q2	0.28 (0.15-0.52)	6.0 (4.4-7.5)	5.9 (4.7-7.1)
Q3	0.27 (0.14-0.50)	5.3 (3.7-6.8)	5.3 (4.1-6.5)
Q4	0.20 (0.11-0.36)	4.7 (3.2-6.3)	4.9 (3.7-6.1)
	$P = 0.05$[e]	$P = 0.008$	$P = 0.007$
CRP			
Q1	0.23 (0.12-0.42)	4.9 (3.4-6.4)	4.8 (3.6-6.0)
Q2	0.24 (0.13-0.45)	5.1 (3.5-6.7)	5.1 (3.8-6.3)
Q3	0.24 (0.13-0.45)	5.4 (3.8-7.0)	5.5 (4.3-6.7)
Q4	0.21 (0.12-0.39)	5.2 (3.7-6.7)	5.4 (4.2-6.6)
	$P = 0.82$	$P = 0.60$	$P = 0.11$
IL6			
Q1	0.25 (0.14-0.47)	5.0 (3.5-6.6)	5.0 (3.8-6.2)
Q2	0.23 (0.12-0.41)	5.3 (3.7-6.8)	5.3 (4.0-6.5)
Q3	0.20 (0.11-0.37)	5.1 (3.5-6.6)	5.2 (4.0-6.4)
Q4	0.23 (0.13-0.43)	5.2 (3.6-6.7)	5.2 (4.0-6.4)
	$P = 0.44$	$P = 0.92$	$P = 0.84$

AMH, antimüllerian hormone; CRP, C-reactive protein; IL6, interleukin 6; MV, multivariable; sTNFR2, soluble fraction of tumor necrosis factor alpha receptor 2.
[a]$n = 328$ cases included in all analyses. Analyses of AMH included all controls ($n = 492$). Analyses of years until menopause was restricted to controls experiencing natural menopause ($n = 449$).
[b]AMH levels log-transformed for analysis, and then exponentiated.
[c]MV model adjusted for age at blood collection, fasting status, BMI, ethnicity, age at menarche, years until cycle regularity, pack-years of smoking, duration of oral contraceptive use, parity, duration of exclusive breast feeding, alcohol intake, autoimmune disorders, exogenous hormone use. Please see footnote to Table 3 for variable categorization.
[d]MV model additionally adjusted for AMH and AMH^2.
[e]*P* values are from *F* test.

low fertility in TNFR1 knockout mice, though not in TNFR2 knockouts.[16]

Correlations between ovarian dysfunction and lower TNFa levels have been observed in a small number of clinical studies. Vital Reyes et al compared inflammatory factor levels in women with POI ($n = 15$), low ovarian reserve ($n = 8$), chronic anovulation ($n = 7$), and normal controls ($n = 10$).[11] TNFa levels were significantly lower in women with POI (0.15 pg/mL) than controls (0.4 pg/mL; $P = 0.01$), whereas IL6 levels were similar. Comparable findings were reported by Naz et al,[12] who found TNFa levels significantly lower in 16 POI patients than 16 age-matched controls. The authors hypothesized that lower TNFa levels in women with POI could be attributed to diminished ovarian reserve and thus decreased synthesis of TNFa by granulosa cells. In their cross-sectional study, the authors concluded that abnormal ovarian function and premature ovarian failure may affect TNFa levels. The prospective design of our study provides information on the temporality of this relation, allowing us to hypothesize and evaluate the reverse—that alterations in TNFa levels may be present before detectable symptoms of oocyte depletion and menopause onset.

Other work suggests that high TNFa levels may be associated with poorer fertility. In rats, TNFa administration suppressed ovulation while promoting granulosa cell apoptosis and autophagy.[17] In humans, Wang et al[18] found that administration of TNFa at low and moderate doses significantly increased granulosa cell proliferation in IVF patients, but at higher doses, proliferation was not significantly increased compared with controls. Additional evidence supporting a role of TNFa in ovarian function and menopause timing comes from genetic studies. A meta-analysis of genome-wide association studies aimed at identifying genetic predictors of menopause timing identified several candidate genes in novel loci, including TNF, TNFRSF17, and CSNK2B genes implicated in immune function and NFKappaB signaling.[19] Collectively, these studies suggest that the role of TNF in ovarian function is complex, and that healthy ovarian aging may be the product of a delicate balance of TNFa's actions affecting follicle recruitment versus atresia.

In contrast to sTNFR2, CRP and IL6 levels were not associated with early menopause. This is consistent with cross-sectional results by Yildirim et al,[20] who found similar CRP levels in 43 POI patients and 41 normal controls. In this study, POI was, however, significantly associated with low neutrophil-to-lymphocyte ratio, a newly identified marker of inflammation for which higher levels have been associated with cancer and cardiovascular mortality.[21,22] The heterogeneity in associations between inflammatory factors and risk of early menopause and/or POI suggested that relations are complex, and that dysfunction in specific immune pathways may be related to menopause timing.

Our study has several limitations requiring attention. Although premenopausal, participants in our analyses were already aged 35 to 44 years at the time of blood collection. Thus, we had insufficient power to evaluate whether inflammatory factors may be clinically relevant as predictors of POI. Second, we assessed inflammatory factors levels at a single time before menopause onset. Although a single measure may not capture long-term levels, data suggest that inflammatory factors are stable over time; we previously observed high intraclass correlations for inflammatory factor levels measured 4 years apart (sTNFR2 = 0.78; CRP = 0.67; IL-6 = 0.47).[23] In addition, biomarkers were measured in plasma samples after long-term frozen storage, and some sample degradation may have occurred, though this would not be expected to be related to early menopause status. Samples were kept frozen at -130°C or colder and each aliquot was used only once to minimize freeze-thaw cycles. Various studies support long-term stability of the biomarkers we assessed under these conditions,[15,24,25] and prior studies using biospecimens from the same repository have observed relations between these inflammatory factors and risk of heart disease and ovarian cancer, among other outcomes.[26,27]

Self-report of menopause may contribute to misclassification, which we attempted to limit in several ways. First, at blood collection, participants reported menstrual cycle regularity and confirmed they were premenopausal. Second, menopause status was queried prospectively biennially between blood collection and the end of follow-up. This method for assessing menopause timing has been well validated in a similar prospective study of US nurses.[28] Finally, as our study participants are registered nurses, our population is quite homogenous with respect to race/ethnicity and socioeconomic status. Although this may limit the generalizability of our findings, we would not expect the physiologic relation of inflammatory factor and early menopause to vary by these factors. Future studies to confirm these findings in more diverse populations, however, will be essential.

CONCLUSIONS

In conclusion, our findings suggest that sTNFR2 is associated with early menopause and menopause timing, independent of AMH and established risk factors. Whether sTNFR2 is a novel risk factor for early menopause warrants replication in additional prospective studies.

REFERENCES

1. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. *Endocr Rev* 2009;30:465-493.
2. Muka T, Oliver-Williams C, Kunutsor S, et al. Association of age at onset of menopause and time since onset of menopause with cardiovascular outcomes, intermediate vascular traits, and all-cause mortality: a systemic review and meta-analysis. *JAMA Cardiol* 2016;1:767-776.
3. Shuster LT, Rhodes DJ, Gostout BS, Grossardt BR, Rocca WA. Premature menopause or early menopause: long-term health consequences. *Maturitas* 2010;65:161-166.
4. Nelson LM. Clinical practice. Primary ovarian insufficiency. *N Engl J Med* 2009;360:606-614.
5. Simpson JL. Trilogy 8: Premature ovarian failure and perimenopause. Genetics of premature ovarian failure. In: Tarlatzis, BC, Bulun, SE, editors. *Proceedings of the International Federation of Fertility Societies 21st World Congress on Fertility and Sterility and the 69th Annual Meeting of the American Society for Reproductive Medicine*, Boston, MA; 2013: 123-136.

6. Whitcomb BW, Mumford SL, Perkins NJ, et al. Urinary cytokine and chemokine profiles across the menstrual cycle in healthy reproductive-aged women. *Fertil Steril* 2014;101:1383-1391.
7. Wander K, Brindle E, O'Connor KA. C-reactive protein across the menstrual cycle. *Am J Phys Anthropol* 2008;136:138-146.
8. von Wolff M, Thaler CJ, Strowitzki T, Broome J, Stolz W, Tabibzadeh S. Regulated expression of cytokines in human endometrium throughout the menstrual cycle: dysregulation in habitual abortion. *Mol Hum Reprod* 2000;6:627-634.
9. Cui Ll. Yang G, Pan J, Zhang C. Tumor necrosis factor alpha knockout increases fertility of mice. *Teriogenology* 2011;75:867-876.
10. Sakumoto R, Okuda K. Possible actions of tumor necrosis factor-alpha in ovarian function. *J Reprod Dev* 2004;50:39-46.
11. Vital Reyes VS, Téllez Velasco S, Hinojosa Cruz JC, Ortiz Romero Mde J, Chavarría Olarte ME, Reyes Fuentes A. Serum levels of IL-1beta, IL-6 and TNF-alpha in infertile patients with ovarian dysfunction [Spanish]. *Ginecol Obstet Mex* 2005;73:604-610.
12. Naz RK, Thurston D, Santoro N. Circulating tumor necrosis factor (TNF)-alpha in normally cycling women and patients with premature ovarian failure and polycystic ovaries. *Am J Reprod Immunol* 1995;34:170-175.
13. Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. *Cancer Res* 2006;66:2476-2482.
14. Aderka D. The potential biological and clinical significance of the soluble tumor necrosis factor receptors. *Cytokine Growth Factor Rev* 1996;7: 231-240.
15. Diez-Ruiz A, Tilz GP, Zangerle R, Baier-Bitterlich G, Wachter H, Fuchs D. Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. *Eur J Haematol* 1995;54:1-8.
16. Roby KF, Son DS, Terranova PF. Alterations of events related to ovarian function in tumor necrosis factor receptor type 1 knockout mice. *Biol Reprod* 1999;61:1616-1621.
17. Yamomoto Y, Kuwahara A, Taniguchi Y, et al. Tumor necrosis factor alpha inhibits ovulation and induces granulosa cell death in rat ovaries. *Reprod Med Biol* 2015;14:107-115.
18. Wang LJ, Brännström. Robertson SA, Norman RJ. Tumor necrosis factor alpha in the human ovary: presence in follicular fluid and effects on cell proliferation and prostaglandin production. *Fertil Steril* 1992;58:934-940.
19. Stolk L, Perry JR, Chasman DI, et al. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. *Nat Genet* 2012;44:260-268.
20. Yildirim G, Tokmak A, Kokanal MK, et al. Association between some inflammatory markers and primary ovarian insufficiency. *Menopause* 2015;22:1000-1005.
21. Wang X, Zhang G, Jiang X, Zhu H, Lu Z, Xu L. Neutrophil to lymphocyte ratio in relation to risk of all-cause mortality and cardiovascular events among patients undergoing angiography or cardiac revascularization: A meta-analysis of observational studies. *Atherosclerosis* 2014;234:206-213.
22. Faria SS, Fernandes PC Jr, Silva MJB, et al. The neutrophil-to-lymphocyte ratio: a narrative review. *Ecancermedicalscience* 2016;10:702.
23. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. *Circulation* 2003; 108:155-160.
24. Doumatey AP, Zhou J, Adeyemo A, Rotimi C. High sensitivity C-reactive protein (Hs-CRP) remains highly stable in long-term archived human serum. *Clin Biochem* 2014;47:315-318.
25. Panicker G, Meadows KS, Lee DR, Nisenbaum R, Unger ER. Effect of storage temperatures on the stability of cytokines in cervical mucous. *Cytokine* 2007;37:176-179.
26. Pai JK, Pischon T, Ma J, et al. Inflammatory markers and the risk of coronary heart disease in men and women. *NEJM* 2004;351:2599-2610.
27. Poole EM, Lee IM, Ridker PM, Buring JE, Hankinson SE, Tworoger SS. A prospective study of circulating C-reactive protein, interleukin-6, and tumor necrosis factor α receptor 2 levels and risk of ovarian cancer. *Am J Epidemiol* 2013;178:1256-1264.
28. Colditz GA, Stampfer MJ, Willett WC, et al. Reproducibility and validity of self-reported menopausal status in a prospective cohort study. *Am J Epidemiol* 1987;126:319-325.

