CLINICAL RESEARCH STUDY

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Inflammation and Thrombosis Biomarkers and Incident Frailty in Postmenopausal Women

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ABSTRACT

BACKGROUND: The immune and blood coagulation systems have been implicated in the pathophysiology of the geriatric syndrome of frailty, but limited prospective data examining the relationship of clotting/inflammation biomarkers to risk of incident frailty exist.

METHODS: This prospective analysis was derived from a nested case-control study within the Women's Health Initiative. Among women 65 to 79 years free of frailty at enrollment, we randomly selected 900 incident cases from those developing frailty within 3 years; 900 non-frail controls were individually matched on age, ethnicity, and blood collection date. Biomarkers assessed for risk of incident frailty included fibrinogen, factor VIII, D-dimer, C-reactive protein, interleukin-6, and tissue plasminogen activator (t-PA).

RESULTS: When examined by quartiles in multivariable adjusted models, higher D-dimer and t-PA levels were each associated with increased risk of frailty (P trend = .04). Relative to the lowest quartile, the odds ratios for frailty compared with the upper quartile were 1.52 (95% confidence interval, 1.05-2.22) for t-PA and 1.57 (95% confidence interval, 1.11-2.22) for D-dimer. For women having high t-PA and high D-dimer compared with women having lower levels of both biomarkers, the odds of frailty was 2.20 (1.29-3.75). There was little evidence for association between coagulation factor VIII, fibrinogen, C-reactive protein, or interleukin-6 levels and incident frailty.

CONCLUSION: This prospective analysis supports the role of markers of fibrin turnover and fibrinolysis as independent predictors of incident frailty in postmenopausal women.

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KEYWORDS: D-dimer; Frailty; Tissue plasminogen activator; Women's Health Initiative

Frailty in older adults is defined as a syndrome consisting of involuntary weight loss, exhaustion, low physical activity, slowness, and weakness. The syndrome of frailty is associated with increased vulnerability to aging-related diseases and mortality.[1,2] The pathophysiology of frailty is not well understood, but multiple physiologic systems seem to be involved, including activation of immune/inflammation and blood coagulation systems.[3]

In cross-sectional analyses, frailty and functional decline have been associated with increased markers of coagulation, fibrinolysis, and inflammation, such as factor VIII, fibrinogen, and D-dimer, plasmin-antiplasmin complex, factor XI α1-antitrypsin, interleukin (IL)-6, C-reactive protein (CRP),

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total white blood count, and circulating T-lymphocytes expressing C-C chemokine receptor-5.[4-10] In an experimental mouse model of proinflammatory pathway activation due to deficiency of the anti-inflammatory cytokine IL-10, muscle weakness and higher IL-6 levels developed more rapidly with increasing age compared with control mice.[11] The connection among blood coagulation, fibrinolysis, and frailty is further supported by the recent report that community-dwelling older adults with frailty are at moderately increased risk of developing idiopathic venous thromboembolic disease.[12]

Although most existing reports support a cross-sectional relationship between hemostasis and inflammation markers and frailty, few prospective studies have examined the ability of biomarkers measured at baseline to predict incident frailty events during follow-up. In a recent prospective analysis of the Cardiovascular Health Study (CHS), baseline CRP level and, to a lesser extent, white blood count and factor VIII levels were associated with an increased risk of incident frailty during follow-up.[13] We examined the association of coagulation and fibrinolysis biomarkers measured at baseline enrollment and risk of incident frailty in postmenopausal women from Women's Health Initiative-Observational Study (WHI-OS). In addition to CRP, IL-6, and factor VIII, our analysis included fibrinogen, D-dimer, and tissue plasminogen activator (t-PA), which have not previously been assessed prospectively with respect to incident frailty.

CLINICAL SIGNIFICANCE

- In postmenopausal women, higher levels of the coagulation/fibrinolysis biomarkers D-dimer and t-PA were associated with increased risk of incident frailty.
- The combined effects of high D-dimer and high t-pa on risk of incident frailty were greater than the risk associated with either biomarker alone.
- Markers of fibrin turnover and fibrinolysis might serve as independent predictors of incident frailty in postmenopausal women.

MATERIALS AND METHODS

Study Sample

The WHI-OS is a prospective study of 93,676 women aged 50 to 79 years recruited from 1993 to 1998 from 40 clinical centers in the United States. Study details have been described.[14,15] Women were eligible for WHI-OS if they were postmenopausal, unlikely to relocate or die within 3 years, and not enrolled in any of the WHI clinical trials. The study was reviewed and approved by human subjects review committees at each participating institution. All women provided written informed consent.

The participants for the current analysis were derived from a case-control study of incident frailty nested within the WHI-OS. Women were considered eligible if they were at least 65 years old and not frail at study enrollment (see below for definition of frailty in WHI-OS), and did not report at baseline a diagnosis or disease that can manifest as frailty (Parkinson disease, severe autoimmune disease, multiple sclerosis, amyotrophic lateral sclerosis, congestive heart failure, coronary heart disease, stroke, cancer, or use of antidepressant medications). Cases and controls also were excluded if they developed cardiovascular disease or cancer events during follow-up within 4 years of enrollment. A total of 25,378 WHI-OS women were eligible. Cases of frailty (n = 900) were randomly selected from the 3453 WHI-OS participants who developed incident frailty by year 3 follow-up contact. Controls (n = 900) were selected from 15,198 participants with a WHI-OS frailty score of 0 (zero) at year 3 of follow-up and were matched to cases on age (±1 year), ethnicity, and date of blood collection, using a 1:1 matching ratio.

Definition of Frailty in Women's Health Initiative-Observational Study

The frailty phenotype developed in the WHI cohort was based on the criteria used by Fried et al[1] and has been found to be strongly associated with future mortality, disability, hospitalization, and hip fracture among older women in the WHI-OS.[2] Definition of the frailty phenotype in WHI was based on 5 component criteria (weakness, slowness, exhaustion, low physical activity, and unintended weight loss). Because physical performance measures were not available in WHI-OS, the CHS definition was modified to include the Rand-36 physical function scale,[16] which was used as a self-report indicator of muscle weakness and slow walking speed. The Rand-36 Vitality Scale (range 0-100) was used to measure exhaustion using 4 items pertaining to the past 4 weeks: "Did you feel . . . . worn out?; tired?; full of pep?; have a lot of energy?". Low physical activity was classified using a questionnaire that assessed weekly frequency and duration of 4 speeds of walking and activities. Kilocalories of energy expended in a week on leisure time activity was calculated (metabolic equivalent task score = kcal/wk * kg). A dichotomous variable was created indicating unintentional weight loss of more than 5% of body weight in the past 2 years, based on measured weight at the baseline and 3-year clinic visits in combination with a self-reported item on whether recent weight loss was intentional at the 3-year follow-up. For each measure described above, a frailty component was classified as present if the participant had a score in the lowest quartile of the distribution for that component or unintentional weight loss. To align the scoring with the frailty measure developed in CHS, poor physical function was scored as 2 points because both the muscle strength and walking ability components were measured by this scale. We then summed

the number of frailty components that were present, yielding a range of 0 to 5, and used a threshold score of ≥3 to create a dichotomous outcome of incident frailty.

Potential Confounders

Data on demographic (race/ethnicity, age, education), medical history, and health behavior characteristics were obtained by self-report in the WHI-OS at baseline. Smoking status was classified as current, past, or never. Medical conditions at baseline included self-reported physician diagnosis of arthritis, treated diabetes, hypertension (on medication and/or blood pressure >140/90 mm Hg), cancer, Parkinson disease, autoimmune disease, multiple sclerosis, and amyotrophic lateral sclerosis. Body mass index (BMI) was defined using measured height and weight at baseline as weight (kilograms) divided by height (meters squared). Depressive symptoms were assessed by an 8-item short form[17] of the Center for Epidemiologic Studies Depression Scale.[18] Postmenopausal hormone therapy use was ascertained by interview and categorized as current, past, or never use of any estrogen with or without progestin. Other medication use was assessed by having participants bring all current medications taken on a regular basis to their first screening interview. Clinic interviewers entered each medication name and strength directly from the containers into a database that assigned drug codes using Medi-Span software (First DataBank, Inc, San Bruno, Calif). During follow-up, incident cardiovascular events (myocardial infarction, definite or possible coronary death, angina, revascularization, carotid artery disease, congestive heart failure, and stroke) were ascertained, initially by annual self-report and confirmed through medical record review and adjudication by local clinic physicians and finally by a panel of central adjudicators.

Laboratory Assays

Plasma biomarker assays were performed at the University of Washington Department of Laboratory Medicine on aliquots of citrated plasma (fibrinogen, factor VIII, D-dimer) or EDTA plasma (CRP, IL-6, t-PA) that had been collected at a single time point during the baseline WHI examination, processed under standard protocols, and stored at −80°C. High-sensitivity CRP was measured by immunonephelometry (BNII, Dade-Behring, Deerfield, IL). Plasma fibrinogen and factor VIII activity were measured on an automated hemostasis analyzer (STA Compact; Diagnostica Stago, Parsippany, NJ) using the modified thrombin time method (Clauss assay) and 1-stage clotting time assay, respectively. For the factor VIII assay, values are reported as a percentage of normal plasma pool. Quantitative D-dimer and t-PA levels were determined by enzyme-linked immunosorbent assays (Asserachrom D-Di, Diagnostica Stago). IL-6 was measured using a commercial BioSource high-sensitivity ELISA kit (Invitrogen, Carlsbad, Calif) according to manufacturer's instructions; the limit of detection for the IL-6 assay was 0.02 pg/mL.

Among the 1800 study participants, the number of participants with missing biomarkers values were 7 (0.4%) for fibrinogen, 7 (0.4%) for factor VIII, 7 (0.4%) for D-dimer, 8 (0.4%) for t-PA, 38 (2.1%) for IL-6, and 41 (2.3%) for CRP. A large number (847 or 47%) of participants had values below the detectable limit of the IL-6 immunoassay. One participant had a t-PA value of 153 mg/mL (>6 times higher than the next highest) and was excluded from the t-PA analyses. Eight participants (0.4%) had fibrinogen less than 100 mg/dL, and 42 participants (2.3%) had factor VIII less than 30%. To account for the possibility that some of these fibrinogen or factor VIII levels were spuriously low because of clot formation or sample degradation, we performed sensitivity analyses excluding these 50 subjects.

Statistical Analysis

Baseline demographic, medical, and health behavior characteristics were compared for women according to quartile of biomarker levels and by case-control status. Corresponding *P* values were based on chi-square tests of association. Mean biomarker levels were compared between incident frailty cases and controls using paired *t* tests. We also visually assessed the relationship between biomarkers and risk of incident frailty by fitting generalized additive models with biomarker levels as a continuous variable (log10 scale) using splines and after adjusting for age, ethnicity, hypertension, hormone use, BMI, education, alcohol consumption, arthritis, and smoking.

To formally test the relationship between biomarkers and risk of incident frailty, conditional logistic regression, matching on case-control pairs, was used to estimate odds ratios and 95% confidence intervals. This analytic approach, in the context of a matched case-control design, can be considered an adaptation of the Cox proportional hazards failure time model. Assessment of linear trend was approached by dividing the population into quartiles according to cut-points determined using the control distribution for each biomarker and calculating odds ratios for each quartile relative to the lowest quartile. Because of the large number of participants with undetectable IL-6 levels (<0.02 pg/mL), we performed a categoric analysis using tertiles, comparing individuals with values ≥ 1.0 pg/mL and those with values between 0.02 and 1.0 pg/mL with the baseline category of < 0.02 pg/mL. For each biomarker, we fit 2 covariate-adjusted regression models. Model 1 adjusted for hypertension, hormone use, BMI, and matched on case-control pair (minimally adjusted model). Model 2 adjusted for these covariates and education, alcohol consumption, arthritis, and smoking, and matched on case-control pair (fully adjusted model). For any statistically significant results, a sensitivity analysis was performed by stratifying the risk of frailty according to baseline use of medications with anti-inflammatory or anticoagulant properties (angiotensin-converting enzyme inhibitors, statins, non-statin anti-hyperlipidemics, nonsteroidal anti-inflammatory drugs, and warfarin). In additional sensitivity analyses, we adjusted our regression models for baseline use of any sedating medication (benzodiaz-

Table 1 Baseline Characteristic of Women's Health Initiative Observational Study Participants versus Incident Frailty

	Frail Cases		Non-Frail Controls		
	N	%	N	%	*P* Value
Age group at screening, y					
60-69	485	53.9	485	53.9	1.00
70-79	415	46.1	415	46.1	.
Education					
0-8 y	12	1.3	6	0.7	<.001
Some high school	34	3.8	17	1.9	.
High school diploma/GED	192	21.5	135	15.1	.
School after high school	341	38.2	342	38.2	.
College degree or higher	314	35.2	395	44.1	.
Ethnicity					
White	865	96.1	865	96.1	1.00
Black	16	1.8	16	1.8	.
Hispanic	2	0.2	2	0.2	.
Asian/Pacific Islander	15	1.7	15	1.7	.
Unknown	2	0.2	2	0.2	.
BMI, kg/m^2 (full categories)					
Underweight (<18.5)	4	0.4	14	1.6	<.001
Normal (18.5-24.9)	271	30.2	461	51.5	.
Overweight (25.0-29.9)	352	39.3	320	35.8	.
Obesity I (30.0-34.9)	188	21.0	86	9.6	.
Obesity II (35.0-39.9)	55	6.1	10	1.1	.
Extreme obesity III (≥40)	26	2.9	4	0.4	.
Smoking					
Never	472	52.9	478	53.7	.005
Past	360	40.4	382	42.9	.
Current	60	6.7	30	3.4	.
Alcohol					
Non/past drinker	272	30.3	200	22.3	<.001
<1 drink/wk	314	35.0	251	28.0	.
1-14 drinks/wk	271	30.2	404	45.1	.
>14 drinks/wk	41	4.6	40	4.5	.
Hormone therapy use status					
Never used	378	42.0	400	44.5	.48
Past user	129	14.3	131	14.6	.
Current user	393	43.7	368	40.9	.
Depressive mood					
0	167	18.7	325	36.6	<.001
1-2	367	41.2	356	40.1	.
3-4	215	24.1	150	16.9	.
5+	142	15.9	57	6.4	.
Activity of daily living disability					
No	869	98.5	878	99.1	.27
Yes	13	1.5	8	0.9	.
Treated diabetes (pills or shots)					
No	862	95.8	888	98.8	<.001
Yes	38	4.2	11	1.2	.
Hypertensive					
No	448	49.9	564	62.7	<.001
Yes	450	50.1	336	37.3	.
History of arthritis					
No	330	36.7	529	58.8	<.001
Yes	570	63.3	371	41.2	.
History of hip fracture at age ≥55 y					
No	805	98.9	825	99.8	.03
Yes	9	1.1	2	0.2	.

Table 1 Continued

	Frail Cases		Non-Frail Controls		
	N	%	N	%	*P* Value
No. of falls in last 12 mo					
None	605	67.3	643	71.6	.02
1 time	170	18.9	171	19.0	.
2 times	81	9.0	61	6.8	.
≥3	43	4.8	23	2.6	.
Angiotensin-converting enzyme inhibitor use					
No	816	90.7	844	93.8	.01
Yes	84	9.3	56	6.2	.
Non-statin antihyperlipidemic medication use					
No	882	98.0	888	98.7	.27
Yes	18	2.0	12	1.3	.
Statin use					
No	798	88.7	835	92.8	.003
Yes	102	11.3	65	7.2	.
NSAID use					
No	521	57.9	588	65.3	.001
Yes	379	42.1	312	34.7	.
Coumarin anticoagulant use					
No	889	98.8	898	99.8	.01
Yes	11	1.2	2	0.2	.

GED = General Equivalency Diploma; BMI = body mass index; NSAID = nonsteroidal anti-inflammatory drug.

epines, hypnotics, opioids, antipsychotics, or neuroleptics), and all of our results were essentially unchanged.

In post hoc analyses, for each biomarker found to be individually associated with frailty (t-PA and D-dimer), we assessed whether the risk of incident frailty associated with the *combination* of the 2 biomarkers was greater than the risk associated with each biomarker individually. We divided participants into 4 categories: those being in the upper quartile distribution for both biomarkers, those being in the upper quartile for D-dimer, but not t-PA, those being in the upper quartile for t-PA but not D-dimer and the baseline category of being in the upper quartile for neither biomarker. A 3 degrees of freedom, chi-square test was performed to test risk differences across the 4 categories.

RESULTS

Of the baseline characteristics examined (Table 1), lower education, obesity, current smoking, infrequent alcohol consumption, worse health status, more depressive symptoms, diabetes, hypertension, arthritis, and nonsteroidal anti-inflammatory drug and statin use were more common among frailty cases than controls ($P \leq .01$). Cross-sectional univariate analyses of baseline characteristics in the 900 non-frail control women according to quartile of plasma biomarker levels showed significant associations ($P \leq .01$) of age with higher D-dimer, factor VIII, and t-PA; BMI with higher CRP and t-PA; hormone therapy use with higher CRP, fibrinogen, and t-PA; hypertension with higher CRP and t-PA; and alcohol consumption with higher fibrinogen.

In both minimally adjusted and fully adjusted regression models, women with t-PA or D-dimer levels in the upper quartile were at increased risk of incident frailty relative to women in the lowest respective quartiles; women in the second and third quartiles were at intermediate risk (Table 2). The trend *P* values associated with D-dimer and t-PA were each $P = .04$ for the fully adjusted models. Women with IL-6 levels ≥1 pg/mL and those with detectable IL-6 levels less than 1 pg/mL had a higher risk of frailty than women with undetectable IL-6 levels in the minimally adjusted model ($P = .03$), but the frailty-IL-6 association became nonsignificant on further covariate adjustment ($P = .27$). Baseline levels of CRP, fibrinogen, and factor VIII were not significantly associated with incident frailty in either minimally or fully adjusted models. Restricting the analysis to subjects with factor VIII levels > 30% and fibrinogen levels >100 did not appreciably alter these results.

Additional sensitivity analyses were performed by stratifying the fully adjusted frailty-biomarker models for D-dimer and t-PA according to baseline use of any anti-inflammatory and anticoagulant medication, including angiotensin-converting enzyme inhibitors, statins, non-statin anti-hyperlipidemics, nonsteroidal anti-inflammatory drugs, and warfarin. The frailty odds ratios by quartile of biomarker level were similar to those described above. For example, among the 936 participants (52%) not using anti-inflammatory or anticoagulant medications, the frailty odds ratio for the highest quartile of D-dimer was 1.81 (95% confidence interval, 1.12-2.91) compared with women in the lowest quartile of

Table 2 Risk of Frailty by Quartile of Inflammation and Coagulation Biomarkers

Biomarker Quartile	Model 1 Odds Ratio (95% CI)	Model 2 Odds Ratio (95% CI)
C-reactive protein Q1 (<1.1 mg/L)	1	1
C-reactive protein Q2 (1.1-2.3 mg/L)	1.12 (0.80-1.56)	1.24 (0.84-1.81)
C-reactive protein Q3 (2.3-4.8 mg/L)	1.13 (0.81-1.58)	1.14 (0.78-1.65)
C-reactive protein Q4 (≥4.8 mg/L)	1.15 (0.82-1.62)	1.05 (0.72-1.54)
P for trend	0.43	0.95
D-dimer Q1 (<0.24 ug/mL)	1	1
D-dimer Q2 (0.24-0.36 ug/mL)	1.14 (0.84-1.56)	1.27 (0.90-1.79)
D-dimer Q3 (0.36-0.61 ug/mL)	1.10 (0.81-1.49)	1.05 (0.75-1.48)
D-dimer Q4 (≥0.61 ug/mL)	1.37 (1.00-1.88)	1.57 (1.11-2.22)
P for trend	0.07	0.04
Factor VIII Q1 (<64%)	1	1
Factor VIII Q2 (64%-90%)	1.22 (0.90-1.64)	1.17 (0.84-1.64)
Factor VIII Q3 (90%-116%)	1.05 (0.76-1.44)	1.05 (0.74-1.48)
Factor VIII Q4 (≥116%)	1.02 (0.73-1.42)	1.10 (0.76-1.59)
P for trend	0.72	0.84
Fibrinogen Q1 (<261 mg/dL)	1	1
Fibrinogen Q2 (261-305 mg/dL)	0.71 (0.52-0.97)	0.70 (0.49-1.00)
Fibrinogen Q3 (305-352 mg/dL)	0.91 (0.66-1.25)	0.96 (0.67-1.36)
Fibrinogen Q4 (≥352 mg/dL)	0.95 (0.69-1.31)	0.93 (0.64-1.34)
P for trend	0.79	0.80
Tissue plasminogen activator Q1 (<6 mg/mL)	1	1
Tissue plasminogen activator Q2 (6-8 mg/mL)	1.14 (0.83-1.57)	1.23 (0.87-1.75)
Tissue plasminogen activator Q3 (8-10.3 mg/mL)	1.10 (0.80-1.51)	1.20 (0.84-1.72)
Tissue plasminogen activator Q4 (≥10.3 mg/mL)	1.48 (1.05-2.07)	1.52 (1.05-2.22)
P for trend	0.03	0.04
Interleukin-6 (0 pg/mL)	1	1
Interleukin-6 (0 < 1 pg/mL)	1.07 (0.74-1.54)	0.97 (0.64-1.45)
Interleukin-6 (≥1 pg/mL)	1.41 (1.00-1.98)	1.20 (0.82-1.76)
P for trend	0.03	0.27

CI = confidence interval.
Model 1 adjusted for hypertension, hormone use, and BMI.
Model 2 adjusted for model 1 covariates + education, alcohol consumption, hypertension, arthritis, and smoking.

D-dimer. Similarly, among non-medication users, the frailty odds ratio associated with the highest quartile of tPA was 1.52 (95% confidence interval, 0.93-2.50) compared with women in the lowest quartile of t-PA. There was no significant interaction between either D-dimer or t-PA and anti-inflammatory/anticoagulant medication use on risk of frailty (*P* values for interaction = .47 and .42, respectively).

Because D-dimer and t-PA measure different aspects of the blood coagulation and fibrinolysis pathways, and circulating levels were uncorrelated with one another among non-frail WHI-OS controls (Spearman's $r = -0.04$), we also explored whether the combination of high D-dimer and high t-PA levels was associated with a greater risk of frailty compared with each biomarker individually (Table 3). In the fully adjusted regression model, women in the upper quartile for t-PA had only a 1.3-fold increased risk of frailty and women in the upper quartile for D-dimer had only a 1.4-fold increased risk of frailty, compared with women with low levels of both markers. For women in the upper quartile of both t-PA and D-dimer, the risk of incident frailty was even larger (odds ratio, 2.2). Although there was some overlap in the confidence intervals for these risk estimates, the combined effect of t-PA and D-dimer levels on risk frailty was significantly greater than the effect of each biomarker individually ($P = .02$).

DISCUSSION

In a nested case-control study of incident frailty in postmenopausal women, higher levels of the coagulation/fibrinolysis biomarkers D-dimer and t-PA were each associated with increased risk of frailty at 3-year follow-up. The associations between frailty and D-dimer or t-PA were independent of the potential confounding effects of comorbid conditions and lifestyle factors that also are associated with increased coagulation and inflammation biomarkers levels and frailty. To our knowledge, a prospective association between D-dimer and t-PA and incident frailty have not been reported. Moreover, the combined effects of high D-dimer and high t-PA on risk of incident frailty were greater

Table 3 Risk of Frailty Comparing Levels of D-dimer and Tissue Plasminogen Activator in the Upper Range of the Distribution

Biomarker Quartile	Model 1 Odds Ratio (95% CI)	Model 2 Odds Ratio (95% CI)
Neither D-dimer nor t-PA in the upper quartile	1	1
Only t-PA in the upper quartile	1.38 (1.05-1.82)	1.29 (0.94-1.76)
Only D-dimer in the upper quartile	1.30 (0.97-1.74)	1.38 (0.99-1.91)
Both D-dimer and t-PA in the upper quartile	1.90 (1.17-3.08)	2.20 (1.29-3.75)
Overall *P* value (3 *df* test)	.02	.02

CI = confidence interval; t-PA = tissue plasminogen activator.
Model 1 adjusted for hypertension, hormone use, and BMI.
Model 2 adjusted for model 1 covariates + education, alcohol consumption, hypertension, arthritis, and smoking.

than the risk associated with either biomarker alone. The risks of frailty associated with D-dimer and t-PA were not modified by baseline use of medications with anticoagulant or anti-inflammatory properties. Finally, we observed little evidence for association between coagulation factor VIII, fibrinogen, CRP, or IL-6 levels and development of frailty.

Studies of older adults consistently support associations between markers of thrombosis and inflammation and measures of disability, physical performance, or frailty.[4-12] However, most previous studies assessing the outcome of frailty were cross-sectional analyses, in which the temporal relationships underlying the observed associations are more difficult to tease apart. To our knowledge, there has been one other prospective analysis examining the relationship of biomarkers measured at baseline to development of incident frailty during follow-up in CHS.[13] In the report by Barzilay et al,[13] CRP and insulin resistance were associated with increased risk of incident frailty in CHS independently of potential confounders, whereas coagulation factor VIII levels had a borderline significant association. The lack of association between CRP and incident frailty in the WHI-OS might reflect differences in gender or other study population characteristics between women from WHI compared with men and women from CHS. D-dimer and t-PA were not evaluated in the analysis of incident frailty by Barzilay et al,[13] although higher D-dimer levels were previously reported to be increased with baseline frailty in a cross-sectional analysis from CHS.[5] Therefore, additional prospective studies of older adults are required to confirm the role of D-dimer and t-PA as independent risk factors for incident frailty.

D-dimer results from fibrin formation and degradation, and is a marker of activation of the coagulation and fibrinolytic systems. Fibrinolytic activity is largely regulated by tissue-type plasminogen activator (t-PA) released from endothelial cells and by a circulating inhibitor, plasminogen activator inhibitor type 1. t-PA antigen measures free, active t-PA, and t-PA complexed to plasminogen activator inhibitor type 1. Therefore, t-PA and D-dimer each summarize different components of the coagulation/fibrinolytic systems. Both t-PA antigen and D-dimer levels increase with age[19,20] and have been reported to predict future venous thromboembolic[21] and arterial thrombotic events[22,23] independently of traditional cardiovascular risk factors. Together with a recent report that frailty predicts increased risk of idiopathic venous thrombotic events,[12] the current WHI findings support a possible pathophysiologic connection among aging, activation of the blood coagulation and fibrinolytic systems, and occurrence of frailty. The association between D-dimer and t-PA and development of frailty in postmenopausal women from WHI was present in women without clinical cardiovascular disease, because women with overt cardiovascular disease were excluded from our analyses. Because frailty has been correlated with the extent of underlying atherosclerotic disease,[24] studies that include subclinical noninvasive measures of vascular disease are needed to more accurately assess the interrelationships among coagulation/fibrinolysis biomarkers, vascular disease, and frailty.

Frailty has been conceptualized as a pathophysiologic state of global functional decline characterized by increased vulnerability to acute and chronic physical and psychosocial stressors due to impaired homeostatic mechanisms. Acute or chronic stress contributes to activation of the coagulation system and impaired fibrinolysis, including increases in circulating levels of D-dimer and t-PA.[25-28] Therefore, D-dimer and t-PA might hasten the clinical transition to frailty or other aging-related disorders in susceptible older individuals who experience sustained or repeated physical or psychosocial trauma, stress, injury, or "allostatic load."[29] Strengths of the current study include the use of a prospective study design in which we assessed incident cases of frailty that occurred subsequent to measurement of the biomarkers. Although we excluded women with chronic diseases that manifest as frailty and also performed sensitivity analyses according to use of medications with anti-inflammatory or anticoagulant properties, we cannot fully exclude the possibility that residual confounding due to unmeasured factors, such as subclinical atherosclerosis,[24] might account for the observed frailty-biomarker associations. Because our study population comprised postmenopausal women of predominantly white race, whether the associations with t-PA and D-dimer with incident frailty are generalizable to men and other ethnic groups also requires additional study.

Other study limitations include the relatively short follow-up duration and lack of objective physical performance measurements of muscle weakness and walking speed, which may be prone to error. Nonetheless, our "modified definition" of frailty has been validated previously through strong association with future mortality, disability, hospitalization, and hip fracture events among older women in the WHI-OS.[2] Because of assay and plasma specimen issues that we were not able to fully resolve, a sizeable proportion

of our study participants had undetectable IL-6 levels; thus, our ability to assess the risk of frailty with IL-6 was limited, and longitudinal assessment of IL-6 in other frail populations is warranted.

CONCLUSIONS

Plasma levels of D-dimer and t-PA, 2 markers of fibrin turnover and fibrinolysis, were associated prospectively with risk of incident frailty in postmenopausal women. These findings support the role of activation of coagulation and fibrinolytic systems in the pathophysiology and occurrence of frailty in older adults. If these results are confirmed in other study populations, screening of D-dimer and t-PA may be useful for identifying otherwise healthy older individuals at risk for developing frailty or other functional consequences of aging.

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