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Translational Article

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Translational

Plasma Protein Biomarkers of the Geriatric Syndrome of Frailty

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Frailty is a geriatric syndrome associated with physical decline with aging. Using a proteomics-based screening method to screen plasma for potential biomarkers, we previously found inflammatory glycoproteins to be increased with frailty. The purpose of this study was to confirm if plasma levels of these glycoproteins, as well as of interleukin-6, are increased with frailty in a larger sample (n = 65) of community-dwelling older adults. Plasma levels of transferrin, fibrinogen, haptoglobin, and interleukin-6 were determined with enzyme-linked immunosorbent assay. Differences in protein concentrations by frailty status were determined using analysis of variance. Higher levels of transferrin ($p < .001$), fibrinogen ($p < .0001$), and interleukin-6 ($p = .0035$) were associated with frailty status (nonfrail, prefrail, or frail) and frailty score (0–5) in this sample even after adjustment for age and sex. Haptoglobin did not differ by frailty status ($p = .05$). Our findings largely confirmed the findings of our nontargeted approach that inflammatory glycoproteins are increased with frailty. Future studies should include larger examinations of these associations and consider the potential usefulness of these glycoproteins as biomarkers for frailty.

Key Words: Frailty—Biomarkers.

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FRAILTY is a geriatric syndrome characterized by progressive physical decline and lack of resilience to stressors that eventually leads to disability and death. The syndrome is steadily gaining recognition as an important clinical and public health problem. Although the prevalence of frailty differs depending upon the definition used as well as the population being studied, it is estimated that the prevalence of frailty is between 4% and 59% in community-dwelling men and women aged 65 years or older (1). Again, although several definitions of frailty exist (2–4), perhaps the most widely used and applied criteria were developed by Dr. Fried and colleagues (4) using data from the Cardiovascular Health Study. Using the Cardiovascular Health Study criteria, frailty prevalence has been estimated at 10%–15% in the United States (4,5). It has been shown to be associated with inflammation, posing the threat of increased risk of age-related complications (6). Our prior work has used lectin affinity chromatography and two-dimensional polyacrylamide gel electrophoresis to screen plasma for glycoproteins that differed by frailty status (7). We found increased concentration of glycoproteins (transferrin, fibrinogen, and haptoglobin) in a small sample of older adults. The purpose of this study was to determine whether frailty is associated with increased inflammation generally, as measured by interleukin-6 (IL-6), and whether the concentrations of the specific glycoproteins found using our nontargeted proteomics approach (transferrin, fibrinogen, and haptoglobin) are increased by frailty status and frailty score in a larger sample of community-dwelling older adults.

Methods

Participants were 65 years older community-dwelling adults recruited from an independent living retirement community in San Antonio, TX. All participants were recruited with informed consent and approval by the Institutional Review Board at UT Health Science Center. Participants were recruited via the use of informational presentations and fliers posted at the retirement community. Only

individuals who were residents of the independent living facility were recruited; individuals residing in the assisted living or nursing facility of the retirement community were excluded. Frailty was defined according to validated criteria (4,5) as the presence of three or more of five criteria: weak grip strength, slow walking speed, unintentional weight loss, self-reported exhaustion, and low physical activity. Prefrailty was defined as the presence of one or two of these characteristics. Nonfrailty was defined as the absence of any of these criteria. Standardized criteria for these characteristics were derived from prior work in a population-based sample of community-dwelling older adults in San Antonio, TX and have been reported previously (5). Presence of common medical conditions was ascertained by self-report of physician-diagnosed disease using a standardized questionnaire format. For example, participants were asked the following question to assess for arthritis: "Have you ever been told by a doctor that you have arthritis?"

Whole blood was collected by venipuncture using antiseptic technique using citrate anticoagulant and centrifuged at 3,000 rpm for 10 minutes to extract plasma. Plasma levels of transferrin, fibrinogen, and haptoglobin were determined with enzyme-linked immunosorbent assay (Genway Biotech). High-sensitivity enzyme-linked immunosorbent assay for IL-6 was also performed (Abcam).

Descriptive statistics were used to summarize the sample baseline characteristics, using *t* tests for continuous variables and chi-squared test statistic for categorical variables. Differences in protein concentration by frailty status (nonfrail vs prefrail vs frail) were determined using analysis of variance. For proteins that were found to differ by frailty category using analysis of variance, multiple linear regression was used to determine the association between protein concentration and frailty category (nonfrail, prefrail, or frail) and frailty score (0, 1, 2, 3, 4, 5). Unadjusted regression analyses were first conducted to determine the association between frailty category and frailty score without the addition of covariates. Subsequently, analyses were adjusted for age and sex.

Results

The participant characteristics are shown in Table 1. Twenty-two (33.9%) of the participants were nonfrail, 31 (47.7%) were prefrail, and 12 (18.5%) were frail. Mean age for the total sample was 80.6 ± 6.4 years and was higher across frailty category. Few common medical conditions, only hypercholesterolemia, differed in proportion across frailty category. None of the individuals in the study were current smokers. Greater levels of transferrin (ng/mL) was associated with frailty status ($p < .001$), as was fibrinogen (g/L; $p < .0001$), and IL-6 ($p = .0035$), as shown in Table 2. Haptoglobin concentration (mg/mL) did not differ by frailty status (nonfrail: 1.1 ± 0.6, prefrail: 1.3 ± 0.6, frail: 1.3 ± 0.6, $p = 0.5$). Table 3 shows the unadjusted and adjusted linear regression analyses for the association between IL-6, transferrin, and fibrinogen concentration and frailty category and frailty score. Frailty category and frailty score remained significantly associated with increased concentration of IL-6, transferrin, and fibrinogen, after adjustment for age and sex. IL-6 levels increased by 0.29 for each increase in frailty status, from nonfrail to frail ($p = .006$), and increased by 0.15 for each increase in frailty score, from 0 to 5 ($p = .017$). Similar findings were seen with transferrin and fibrinogen.

Discussion

Frailty, a clinical geriatric syndrome that has been shown to be predictive of disability and mortality in older adults, has been associated with subclinical inflammation. As the etiology is not well understood, it has been suggested that the identification of blood markers may help to understand the pathophysiology underlying the syndrome and possibly assist in the identification of at-risk older adults (8). In this study, we found that frailty is associated with higher plasma concentrations of the inflammatory glycoproteins (transferrin, fibrinogen, and IL-6). This confirmed our prior work in which we found these glycoproteins to be increased in frail compared with nonfrail using a nontargeted glycoproteomics approach that combined lectin affinity chromatography and two-dimensional polyacrylamide gel electrophoresis (7). One of the first studies to document that frailty is associated with inflammation was by Walston and colleagues (2002), who found increased C-reactive protein in frail individuals (6). Later studies have also shown that frailty is associated with increased inflammation with increased levels of IL-6 (9) and CXC chemokine ligand-10 (10). Further, increased inflammation (C-reactive protein and IL-6) as well as the metabolic syndrome has been found to be predictive of incident frailty (11). In addition to inflammation, frailty has also been associated with alterations in other physiologic systems, such as the endocrine (12) and hematologic systems (6). Notably, frailty has frequently been associated with anemia (13,14), and there appears to be an interplay between the increased inflammation and anemia observed with aging and frailty. Leng and colleagues (15) found an inverse relationship between IL-6 and hemoglobin, such that increased IL-6 was associated with anemia in frail older adults. Elevated levels of proinflammatory cytokines, such as IL-6, lead to the development of anemia by directly inhibiting erythropoietin or by interfering with normal iron metabolism (16).

To our knowledge, this, combined with our prior study (7), is the first to use a high-throughput proteomics-based approach to screen for possible frailty biomarkers, and later confirm the findings with the present study. Our study also showed a step-wise higher concentration of inflammatory proteins with worsening frailty status and frailty score, corroborating other studies that have shown that prefrailty (presence of one or two frailty characteristics) is an increased risk state that is associated with physiologic derangements (6) as well as increased risk for transitioning to overt frailty (three or more frailty characteristics) and mortality (17).

Table 1. Participant Characteristics (n = 65)

Characteristics	Nonfrail (n = 22), Mean ± SD or n (%)	Prefrail (n = 31), Mean ± SD or n (%)	Frail (n = 12), Mean ± SD or n (%)	p Value for Frailty Difference or n (%)
Age, y	76.5 ± 4.7	81.8 ± 6.7	85.2 ± 3.8	.0001
Female, n (%)	16 (72.7)	19 (61.3)	4 (33.3)	.08
Education, y	16.0 (2.4)	14.9 (2.1)	16.1 (2.1)	.84
BMI, kg/m^2	26.2 ± 4.0	26.6 ± 4.0	29.3 ± 7.4	.16
Hypertension	16 (72.7)	23 (76.7)	5 (41.7)	.077
Hypercholesterolemia	16 (72.7)	23 (76.7)	4 (33.3)	.021
Arthritis	13 (59.1)	14 (45.2)	7 (58.3)	.725
Diabetes	1 (4.6)	4 (13.3)	0 (0.0)	.271
Myocardial infarction	2 (9.5)	8 (28.6)	4 (33.3)	.185
Congestive heart failure	1 (4.6)	1 (3.2)	2 (16.7)	.434
History of malignancy, skin or nonskin	12 (54.6)	13 (41.9)	9 (75.0)	.330
Fall within the last year	7 (31.8)	8 (25.8)	5 (41.7)	.732
Depression	4 (18.2)	7 (22.6)	2 (16.7)	.848
Memory loss	1 (5.6)	1 (3.2)	1 (8.3)	.809
Grip strength, kg	28.2 ± 7.2	22.9 ± 7.7	25.5 ± 6.0	.04
Walking speed, s	2.8 ± 0.5	2.8 ± 0.7	3.9 ± 1.4	.0003
Physical activity, kcal/wk	3,432.5 ± 3,559.6	1,676.8 ± 1,516.9	954.5 ± 1,705.6	.009
Frailty score	0	1.3 ± 0.4	3.3 ± 0.5	<.0001

Note: BMI = body mass index.

Table 2. Plasma Protein Concentrations by Frailty Category

	Nonfrail (n = 22), Mean ± SD or n (%)	Prefrail (n = 31), Mean ± SD or n (%)	Frail (n = 12), Mean ± SD or n (%)	p Value for Frailty Difference or n (%)
Transferrin, ng/mL	43.4 ± 11.4	54.3 ± 11.9	58.3 ± 10.2	<.001
Fibrinogen, g/L	40.6 ± 9.3	51.2 ± 19.5	70.4 ± 17.5	<.0001
Haptoglobin, mg/mL	1.1 ± 0.6	1.3 ± 0.6	1.3 ± 0.6	.51
Interleukin-6, pg/mL	0*	0.13 ± 0.33	0.60 ± 0.98	.0035

Note: *All samples in the nonfrail group were found to be below the detectable level for interleukin-6.

Table 3. Multiple Linear Regression Analyses (unadjusted and adjusted) of Plasma Protein Concentration by Frailty Category and Frailty Score

	Interleukin-6		Transferrin		Fibrinogen	
	Coefficient (95% CI)	p Value	Coefficient (95% CI)	p Value	Coefficient (95% CI)	p Value
Unadjusted models						
Frailty category (nonfrail, prefrail, frail)	0.27 (0.11–0.45)	.002	7.97 (3.94–11.99)	<.001	14.4 (8.4–20.3)	<.001
Frailty score (0, 1, 2, 3, 4, 5)	0.14 (0.04–0.24)	.006	4.63 (2.27–6.98)	<.001	10.2 (7.1–13.3)	<.001
Models adjusted for age and sex						
Frailty category (nonfrail, prefrail, frail)	0.29 (0.08–0.5)	.006	9.33 (4.60–14.06)	<.001	13.8 (6.6–20.9)	<.001
Frailty score (0, 1, 2, 3, 4, 5)	0.15 (0.03–0.27)	.017	5.26 (2.48–8.04)	<.001	10.5 (6.8–14.3)	<.001

Note: CI = confidence interval.

This study is limited because it is a small sample of older community-dwelling adults at a large senior living facility; therefore, caution must be used with regard to the generalization of these findings. The individuals in this study are retired military officers and their spouses living in an independent living retirement community in San Antonio. The overall education level is high (average of 15.8 years) in this sample compared with the general population, and the prevalence of comorbid medical conditions is generally lower. For example, the prevalence of diabetes was 8% overall in this sample compared with the estimated 13% prevalence of diabetes in older adults in the United States (18). However, because this convenience sample is biased toward an overall healthier older adult population than would likely be observed in a population-based sample, the advantage for the purposes of this study is that there is less likelihood that chronic disease has confounded these results. In fact, the only comorbid condition that we found to differ significantly across frailty categories was hypercholesterolemia, which was actually found to be higher in the nonfrail group compared with the prefrail and nonfrail groups. Therefore, because we found no difference in comorbid medical conditions across frailty categories, we did not include these factors in the multiple linear regression analyses of plasma protein concentration by frailty category and score, and

we only include age and sex in these analyses. Because the presence of comorbid disease was measured by self-report of physician-diagnosed disease only, measurement error could have occurred. However, this highly educated patient population had a high level of health literacy and knowledge of their medical conditions and are therefore unlikely to underreport comorbid medical disease. In spite of these potential sources of bias, our results of greater levels of inflammatory markers are consistent with what has been found in prior studies (6).

Fried and colleagues (2001) were the first to operationalize frailty as a medical syndrome of increased vulnerability to stress using a definition of three of five frailty criteria (wasting, exhaustion, low energy expenditure, weakness, and slowness), as previously described (3). This landmark study found that this frailty phenotype predicts falls, worsening disability, hospitalization, and death. Several subsequent groups have applied these criteria and found similar results in other cohort studies including the Women's Health and Aging Study (19), the Women's Health Initiative (20), the Hispanic Established Populations for the Epidemiologic Study of the Elderly (21), and the San Antonio Longitudinal Study of Aging (5,12). Taking a more clinical approach, using information obtained from a comprehensive geriatric assessment, Rockwood and colleagues (2) have defined frailty as the cumulative effect of the accumulation of individual deficits in multiple systems. This model has also been shown to be predictive of death and institutionalization (22). Studies that have compared the two models have shown that they are comparable in predicting poor outcomes (23); however, it is suggested that a continuous frailty index, as opposed to categorical, may have optimal predictive ability (24). In the present study, we chose to use the Fried model to define frailty (nonfrail, prefrail, and frail) in our participants because we have prior experience operationalizing these criteria in participants of the San Antonio Longitudinal Study of Aging. However, in order to determine whether the inflammation markers differ across a continuum of frailty, we also examined the criteria by frailty score (0–5). Indeed, we found IL-6, transferrin, and fibrinogen to increase by frailty category as well as frailty score.

We focused on glycoproteins because they result from the most common type of protein posttranslational modification and comprise up to half of all circulating proteins; and, therefore are likely to be the most useful subproteome to study using clinical blood samples. Our findings are also supportive of the increasing body of literature that frailty is a geriatric syndrome associated with inflammation (25), as these glycoproteins are known to increase with inflammatory states. Although IL-6 is a known correlate of inflammation and predictor of disability and mortality with age (26,27), it is not readily available in clinical practice. Transferrin and fibrinogen are often ordered during routine patient care, primarily to screen for hematologic conditions. Our study suggests that these proteins may be helpful in the assessment of frailty and overall inflammation, in conjunction with a clinician's evaluation and assessment. We do acknowledge, however, that this study is limited by its small sample size compared with many cohort studies. These results support using an initial high-throughput approach to screen for novel protein biomarkers for frailty as well as for other conditions and disease states.

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References

1. Clegg A, Young J, Iliffe S, Rikkert MO, Rockwood K. Frailty in elderly people. *Lancet*. 2013;381:752–762.
2. Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty in elderly people. *CMAJ*. 2005;173:489–495.
3. Studenski S, Hayes RP, Leibowitz RQ, et al. Clinical global impression of change in physical frailty: development of a measure based on clinical judgment. *J Am Geriatr Soc*. 2004;52:1560–1566.
4. Fried LP, Tangen CM, Walston J, et al.; Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001;56:M146–M156.
5. Espinoza SE, Hazuda HP. Frailty in older Mexican-American and European-American adults: is there an ethnic disparity? *J Am Geriatr Soc*. 2008;56:1744–1749.
6. Walston J, McBurnie MA, Newman A, et al.; Cardiovascular Health Study. Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the Cardiovascular Health Study. *Arch Intern Med*. 2002;162:2333–2341.
7. Shamsi KS, Pierce A, Ashton AS, Halade DG, Richardson A, Espinoza SE. Proteomic screening of glycoproteins in human plasma for frailty biomarkers. *J Gerontol A Biol Sci Med Sci*. 2012;67:853–864.
8. Walston J, Hadley EC, Ferrucci L, et al. Research agenda for frailty in older adults: toward a better understanding of physiology and etiology: summary from the American Geriatrics Society/National Institute on Aging Research Conference on Frailty in Older Adults. *J Am Geriatr Soc*. 2006;54:991–1001.
9. Leng SX, Xue QL, Tian J, Walston JD, Fried LP. Inflammation and frailty in older women. *J Am Geriatr Soc*. 2007;55:864–871.
10. Qu T, Yang H, Walston JD, Fedarko NS, Leng SX. Upregulated monocytic expression of CXC chemokine ligand 10 (CXCL-10) and its relationship with serum interleukin-6 levels in the syndrome of frailty. *Cytokine*. 2009;46:319–324.
11. Barzilay JI, Blaum C, Moore T, et al. Insulin resistance and inflammation as precursors of frailty: the Cardiovascular Health Study. *Arch Intern Med*. 2007;167:635–641.

12. Leng SX, Cappola AR, Andersen RE, et al. Serum levels of insulin-like growth factor-I (IGF-I) and dehydroepiandrosterone sulfate (DHEA-S), and their relationships with serum interleukin-6, in the geriatric syndrome of frailty. *Aging Clin Exp Res*. 2004;16:153–157.
13. Chaves PH, Semba RD, Leng SX, et al. Impact of anemia and cardiovascular disease on frailty status in community-dwelling older women: the Women's Health and Aging Studies I and II. *J Geron Med Sci*. 2005;60A(6):729–735.
14. Roy CN. Anemia in frailty. *Clin Geriatr Med*. 2011;27:67–78.
15. Leng S, Chaves P, Koenig K, Walston J. Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: a pilot study. *J Am Geriatr Soc*. 2002;50:1268–1271.
16. Ershler WB. Biological interactions of aging and anemia: a focus on cytokines. *J Am Geriatr Soc*. 2003;51(suppl 3):S18–S21.
17. Espinoza SE, Jung I, Hazuda H. Frailty transitions in the San Antonio Longitudinal Study of Aging. *J Am Geriatr Soc*. 2012;60:652–660.
18. California Healthcare Foundation/American Geriatrics Society Panel in Improving Care for Elders with Diabetes. Guidelines for improving the care of the older person with diabetes mellitus. *J Am Geriatr Soc*. 2003;51(5s):265–280.
19. Bandeen-Roche K, Xue QL, Ferrucci L, et al. Phenotype of frailty: characterization in the women's health and aging studies. *J Gerontol A Biol Sci Med Sci*. 2006;61:262–266.
20. Woods NF, LaCroix AZ, Gray SL, et al.; Women's Health Initiative. Frailty: emergence and consequences in women aged 65 and older in the Women's Health Initiative Observational Study. *J Am Geriatr Soc*. 2005;53:1321–1330.
21. Ottenbacher KJ, Graham JE, Al Snih S, et al. Mexican Americans and frailty: findings from the Hispanic established populations epidemiologic studies of the elderly. *Am J Public Health*. 2009;99:673–679.
22. Rockwood K, Mitnitski A, Song X, Steen B, Skoog I. Long-term risks of death and institutionalization of elderly people in relation to deficit accumulation at age 70. *J Am Geriatr Soc*. 2006;54:975–979.
23. Rockwood K, Andrew M, Mitnitski A. A comparison of two approaches to measuring frailty in elderly people. *J Gerontol A Biol Sci Med Sci*. 2007;62:738–743.
24. Kulminski AM, Ukraintseva SV, Kulminskaya IV, Arbeev KG, Land K, Yashin AI. Cumulative deficits better characterize susceptibility to death in elderly people than phenotypic frailty: lessons from the Cardiovascular Health Study. *J Am Geriatr Soc*. 2008;56:898–903.
25. Fedarko NS. The biology of aging and frailty. *Clin Geriatr Med*. 2011;27:27–37.
26. Ferrucci L, Penninx BW, Volpato S, et al. Change in muscle strength explains accelerated decline of physical function in older women with high interleukin-6 serum levels. *J Am Geriatr Soc*. 2002;50:1947–1954.
27. Harris TB, Ferrucci L, Tracy RP, et al. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med*. 1999;106:506–512.