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Original Article

Elevated Plasma Growth and Differentiation Factor 15 Predicts Incident Anemia in Older Adults Aged 60 Years and Older

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Abstract

Anemia is common in older adults and associated with greater morbidity and mortality. The causes of anemia in older adults have not been completely characterized. Although elevated circulating growth and differentiation factor 15 (GDF-15) has been associated with anemia in older adults, it is not known whether elevated GDF-15 predicts the development of anemia. We examined the relationship between plasma GDF-15 concentrations at baseline in 708 nonanemic adults, aged 60 years and older, with incident anemia during 15 years of follow-up among participants in the Invecchiare in Chianti (InCHIANTI) Study. During follow-up, 179 (25.3%) participants developed anemia. The proportion of participants who developed anemia from the lowest to highest quartile of plasma GDF-15 was 12.9%, 20.1%, 21.2%, and 45.8%, respectively. Adults in the highest quartile of plasma GDF-15 had an increased the risk of developing anemia (hazards ratio 1.15, 95% confidence interval 1.09, 1.21, $p < .0001$) compared to those in the lower 3 quartiles in a multivariable Cox proportional hazards model adjusting for age, sex, serum iron, soluble transferrin receptor, ferritin, vitamin B_{12}, congestive heart failure, diabetes mellitus, and cancer. Circulating GDF-15 is an independent predictor for the development of anemia in older adults.

Keywords: Anemia, Human aging, Proteomics, Senescence

Anemia is common in older adults and associated with cognitive decline (1), poor lower muscle strength (2), decline in physical performance (3), disability (2), increased risk of death (4), and independent of comorbidities (4). In adults ≥65 years, anemia affects an estimated 12% of those living in the community, 47% residing in nursing homes, and 40% who are admitted to the hospital (5). Anemia in older adults falls into 3 broad categories: nutritional deficiencies (iron, folate, and vitamin B_{12}), anemia of chronic disease (ACD), and unexplained anemia (UA) (6,7). Cancer, chronic kidney disease, myelodysplastic syndrome, and other blood disorders are more common with aging and can contribute to anemia in older adults (7). Age-related decline in kidney and bone marrow function may affect erythropoietin production or red cell production, respectively (7). An increased pro-inflammatory state, or "inflammaging" may contribute to the anemia of inflammation.

Growth and differentiation factor 15 (GDF-15), a divergent member of the transforming growth factor-β (TGF-β) superfamily, is involved in the regulation of energy homeostasis, suppression of the inflammatory response, and modulation of tumor progression (8,9). The *GDF15* gene is mapped to chromosome 19p13.11. GDF-15 is secreted as a 40-kDa propeptide that is cleaved in the endoplasmic reticulum to produce a mature active 25-kDa homodimer in the circulation (10). In health, GDF-15 is expressed in low amounts in liver, lung, and kidney, and in high amounts

by the placenta (8). GDF-15 expression increases in tissues in response to cellular stress such as inflammation, oxidative stress, hypoxia, acute tissue injury, telomere erosion, and oncogene activation (11,12). Plasma GDF-15 is elevated in cancer, cardiovascular disease, rheumatoid arthritis, liver injury, and inflammatory conditions (8).

Cross-sectional studies have shown that circulating GDF-15 concentrations are higher in adults with anemia compared with controls without anemia (13,14). Higher serum GDF-15 concentrations have been described among anemic adults compared with nonanemic controls in patients with type 2 diabetes (15), early chronic kidney disease (16), heart failure (17), kidney allograft (18), and heart allograft recipients (17). While cross-sectional studies have shown consistent associations between GDF-15 and anemia, it is not yet known whether circulating GDF-15 concentrations are an independent predictor of incident anemia.

We hypothesized that elevated plasma GDF-15 concentrations predict the development of anemia in older adults. To address this hypothesis, we examined the relationship between plasma GDF-15 concentrations in nonanemic older adults who were followed with longitudinal measurements of hemoglobin in a population-based study of aging for 15 years.

Materials and Methods

The study participants consisted of men and women, aged 60 years and older, who participated in the population-based Invecchiare in Chianti, "Aging in the Chianti Area" (InCHIANTI) study, conducted in 2 small towns in Tuscany, Italy. The rationale, design, and data collection have been described elsewhere (19). Participants were seen at baseline (1998–2000) and had 3, 6, 9, and 15-year follow-up visits. A total of 708 subjects aged 60 years and older with both plasma GDF-15 and hemoglobin measured at baseline with at least one follow-up visit were eligible in the present study. Participants received an extensive description of the study and participated after written, informed consent. The study protocol complied with the Declaration of Helsinki and was approved by the Italian National Institute of Research and Care on Aging Ethical Committee and by the Institutional Review Board of the Johns Hopkins University School of Medicine.

Demographic and health characteristics (sex, age, years of education, and smoking status [current smoker or not]) of the participants were assessed during a structured interview. Body mass index (BMI) was defined as kg/m^2. Anemia was defined as hemoglobin <12 g/dL for women and <13 g/dL for men (20). Serum C-reactive protein was measured using enzyme-linked immunosorbent assay (ELISA) (Roche Diagnostics, GmbH, Mannheim, Germany). Interleukin-6 (IL-6) was measured using ELISA (BioSource International, Camarillo, CA). Serum soluble transferrin receptor (sTfR) and ferritin was measured using a chemiluminescent immunoassay (Abbott Diagnostics, Abbott Park, IL). Serum iron was measured using a colorimetric assay (Roche Diagnostics, GmbH), and folate and vitamin B_{12} by radioimmunoassay (SimulTRAC-SNB Radioassay Kit, ICN Diagnostics Division, New York). Total cholesterol was measured with an enzymatic colorimetric assay using cholesterol esterase (Roche Diagnostics, GmbH). Estimated glomerular filtration rate (eGFR) was calculated based on serum creatinine. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medications (21). Chronic kidney disease was defined as an eGFR of <60 mL/min/1.73 m^2 or an eGFR of <30 mL/min/1.73 m^2 if aged 80 years and older (22,23). Depression was diagnosed with a Center for Epidemiologic Studies-Depression scale (CES-D) score ≥16 (24). History of chronic disease (angina, peripheral artery disease, heart failure, stroke, diabetes mellitus, and cancer) was ascertained using standard criteria combining information from self-reported medical history, medication use, and clinical examination by staff geriatrician.

Measurement of GDF-15

Venous blood was collected from participants in the early morning after an overnight fast. Blood samples were immediately stored at 4°C, centrifuged within 4 hours, then immediately aliquoted, and frozen at −80°C. Collection and processing of plasma in the InCHIANTI study was consistent with guidelines for analysis of protein biomarkers (25). Preanalytical studies of proteins measured using LC-MS/MS show that plasma proteins are stable for 14–17 years in storage at −80°C and for up to 25 freeze–thaw cycles (26,27).

Plasma GDF-15 concentrations were measured using the 1.3k HTS SOMAscan assay (SOMALogic, Boulder, CO) at the Trans-NIH Center for Human Immunology and Autoimmunity, and Inflammation (CHI), National Institute of Allergy and Infectious Disease, National Institutes of Health (Bethesda, MD). The abundance of GDF-15 was expressed in relative fluorescence units (RFU). Data were normalized using the following steps: (i) hybridization control normalization removed individual sample variance on the basis of signaling differences between microarray or Agilent scanner; (ii) median signal normalization removed inter-sample differences within a plate due to technical differences such as pipetting variation; (iii) calibration normalization removed variance across assay runs; and (iv) interplate normalization procedures using CHI site-specific calibrators from pooled healthy donors were performed to allow quality control of the normalization across all experiments conducted at the CHI (28). An interactive Shiny web tool was used during the CHI QC process (28). The overall technical variability of the assay was low, with a median intraplate coefficient of variation in the ~3%–4% range.

Statistical Analysis

Differences in baseline characteristics between individuals who did and did not develop anemia during follow-up were tested using the Kruskal–Wallis test for continuous variables and chi-square test for categorical variables. Plasma GDF-15 was log-transformed. Plasma GDF-15 was analyzed both as a continuous log-transformed variable and in quartiles, where the quartiles were defined as 2.91–3.18, 3.19–3.28, 3.29–3.39, and 3.40–4.20 RFU. Pearson's correlation coefficient was used to evaluate the association between BMI and plasma GDF-15. Multivariable Cox proportional hazards models were used to examine the relationship of plasma GDF-15 and other covariates at baseline with incident anemia. Covariates included age and sex in model 1; age, sex, log interleukin-6, serum iron, sTfR, ferritin, and vitamin B_{12} <200 pg/mL in model 2; congestive heart failure, diabetes mellitus, and cancer, in addition to the previous covariates in model 3. Hazards ratios (HR) and 95% confidential interval (95% CI) were expressed per 1 *SD* of plasma GDF-15 concentrations for the top quartile of plasma GDF-15 versus the lower 3 quartiles. Kaplan–Meier survival curves were compared using log-rank test. Data analyses were performed using SPSS version 25.0 for Windows (IBM Corp., Armonk, NY) with a statistical significance level of $p < .05$.

Results

The demographic and health characteristics of the 708 participants are given in Table 1. Of the 708 participants, 179 (25.3%) developed anemia during follow-up. Compared to participants who did not develop anemia, those who developed anemia were older ($p < .0001$), male ($p = .02$), had higher plasma GDF-15 ($p < .0001$), higher IL-6 ($p = .009$), lower serum iron ($p = .02$), higher sTfR ($p = .001$), lower ferritin ($p = .002$), and were more likely to have vitamin B_{12} <200 pg/mL ($p = .003$), congestive heart failure ($p < .0001$), and diabetes mellitus ($p = .048$). There were no significant differences in education, smoking, BMI, C-reactive protein, folate, eGFR, or prevalence of hypertension, angina, peripheral artery disease, stroke, depression, cancer, or chronic kidney disease between those who did or did not develop incident anemia. There was no correlation between BMI and GDF-15 ($p = .07$).

Multivariable Cox proportional hazards models were used to examine the association between plasma GFD-15 concentrations at baseline and incident anemia. Mean and median duration of follow-up were 8.9 and 9 years, respectively. The relationship between plasma GDF-15 concentrations as a continuous variable and incident anemia are given in Table 2. Plasma GDF-15 was significantly associated with increased risk of developing anemia after adjusting for age and sex (HR 1.51, 95% CI 1.32–1.73), additionally for IL-6, serum iron, sTfR, ferritin, and vitamin B_{12} (HR 1.51, 95% CI 1.32–1.73), and in a final multivariable model adjusting for the previous covariates and for congestive heart failure, diabetes mellitus, and cancer (HR 1.41, 95% CI 1.21–1.64). In an additional multivariable Cox proportional hazards model which included C-reactive protein and chronic kidney disease in addition to the previous covariates, plasma GDF-15 was associated with increased risk of developing anemia (HR 1.41, 95% CI 1.21–1.64).

The proportion of participants who developed anemia in the lowest to highest quartile of plasma GDF-15 was 12.9%, 20.1%, 21.2%, and 45.8%, respectively ($p < .0001$). The Kaplan–Meier survival curves for incident anemia by quartiles of plasma GDF15 concentration are shown in Figure 1. Incident anemia was greatest in the highest quartile of plasma GDF15 compared with the lower 3 quartiles ($p < .0001$).

The relationship between quartiles of plasma GDF-15 and incident anemia is given in Table 3. In multivariable Cox proportional hazards models, participants in the highest quartile of plasma GDF-15 had significantly higher incident anemia compared to those in the lowest quartiles after adjusting for age and sex, additionally plasma IL-6, serum iron, sTfR, ferritin, and vitamin B_{12}, and in a final multivariable model adjusting for the previous covariates and for congestive heart failure, diabetes mellitus, and cancer.

Table 2. Relationship Between GDF-15 and Incident Anemia in Multivariable Cox Proportional Hazards Models

Covariates in Models	HR (95% CI)[a]	*p* Value
Age, sex	1.51 (1.32, 1.73)	<.0001
Age, sex, log IL-6, serum iron, sTfR, ferritin, vitamin B_{12} <200 pg/mL	1.51 (1.31, 1.73)	<.0001
Age, sex, log IL-6, serum iron, sTfR, ferritin, vitamin B_{12} <200 pg/mL, congestive heart failure, diabetes mellitus, cancer	1.41 (1.21, 1.64)	<.0001

Note: [a]HR = Hazard ratios expressed per 1 *SD* of GDF-15; CI = Confidence interval.

Table 1. Characteristics at Baseline Visit, Aged ≥60 Years, From the InCHIANTI Study

Characteristics	Overall (*n* = 708)	Censored (*n* = 529)	Developed Anemia (*n* = 179)	*p* Value
Age, years	72.7 ± 6.5	71.8 ± 6.2	75.8 ± 6.6	<.0001
Sex, female, %	55.5	58.2	47.5	.02
Education, years	5.7 ± 3.4	5.6 ± 3.2	6.0 ± 3.9	.19
Current smoking, %	15.2	15.2	15.0	.19
Body mass index, kg/m^2	27.7 ± 4.1	27.8 ± 4.1	27.4 ± 4.1	.35
Log plasma GDF-15, RFU	3.30 ± 0.16	3.27 ± 0.15	3.36 ± 0.17	<.0001
Log C-reactive protein, mg/L	0.40 ± 0.44	0.39 ± 0.43	0.43 ± 0.44	.29
Log interleukin-6, pg/mL	0.12 ± 0.34	0.10 ± 0.35	0.18 ± 0.33	.009
Serum iron, µg/dL	86.1 ± 24.0	87.3 ± 23.4	82.6 ± 25.5	.02
Soluble transferrin receptor, nmol/L	16.2 ± 4.7	15.8 ± 4.2	17.2 ± 5.8	.001
Ferritin, mg/L	150.2 ± 123.0	158.6 ± 127.6	125.6 ± 104.9	.002
Vitamin B_{12}, pg/mL	456.1 ± 334.1	461.5 ± 332.4	439.7 ± 339.5	.46
Vitamin B_{12} <200 pg/mL, %	8.9	7.1	14.9	.003
Folate, nmol/L	7.5 ± 4.5	7.4 ± 4.4	7.9 ± 4.6	.21
Folate <5.89 nmol/L, %	40.5	42.4	38.5	.38
eGFR, mL/min/1.73 m^2	77.3 ± 20.8	77.6 ± 20.8	76.4 ± 20.8	.49
Hypertension, %	47.5	46.9	49.2	.11
Angina, %	4.0	3.6	5.0	.45
Peripheral artery disease, %	7.9	6.8	11.2	.17
Congestive heart failure, %	3.0	1.9	6.1	<.0001
Stroke, %	3.2	2.8	4.5	.46
Diabetes mellitus, %	7.6	6.2	11.7	.048
Depression, %	4.4	4.0	5.7	.40
Cancer, %	5.6	4.7	8.4	.09
Chronic kidney disease, %	14.5	15.7	11.2	.18

Note: eGFR = Estimated glomerular filtration rate; GDF-15. = Growth and differentiation factor 15; RFU = Relative fluorescence unit.

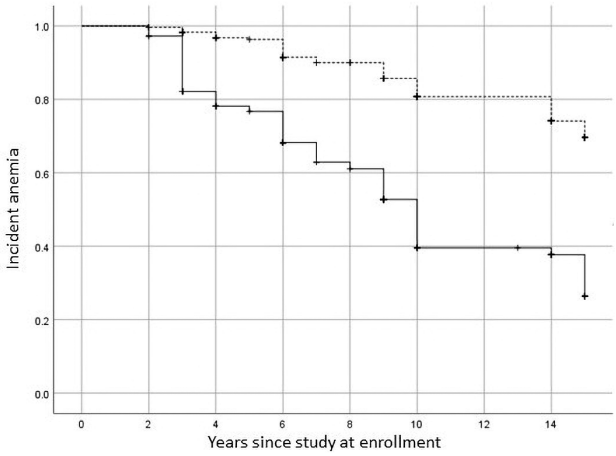

Figure 1. Kaplan–Meier survival curve of incident anemia among participants in the top quartile of plasma GDF-15 (solid line) compared with the lower 3 quartiles (dotted line). GDF-15 = Growth and differentiation factor 15.

Table 3. Relationship Between Quartiles of GDF-15 and Anemia in Multivariable Cox Proportional Hazards Models

Covariates in Models[a]	HR (95% CI)	*p* Value
Age, sex	1.17 (1.11, 1.23)	<.0001
Age, sex, log interleukin-6, serum iron, soluble transferrin receptor, ferritin, vitamin B_{12} <200 pg/mL	1.17 (1.11, 1.24)	<.0001
Age, sex, log interleukin-6, serum iron, soluble transferrin receptor, ferritin, vitamin B_{12} <200 pg/mL, congestive heart failure, diabetes mellitus, cancer	1.15 (1.09, 1.21)	<.0001

Note: [a]These models compare the top quartile with the lower 3 quartiles.

Discussion

This study shows that elevated circulating GDF-15 is an independent predictor of anemia in older adults. The relationship between elevated plasma GDF-15 and the development of anemia was significant after adjusting for demographic factors and other causes of anemia such as inflammation, iron status, vitamin B_{12}, and chronic diseases. There was also a graded relationship between quartiles of plasma GDF-15 and the proportion of participants who developed anemia. The present longitudinal study extends findings from cross-sectional studies of circulating GDF-15 and anemia in older community-dwelling adults (13,14) and shows that elevated plasma GDF-15 predicts incident anemia.

GDF-15 is normally expressed in low amounts in liver, lung, and kidney, and higher expression occurs chronic diseases (8). GDF-15 is also expressed in response to an inflammatory and stress-induced cytokine (8). Circulating GDF-15 increases with age (29), independently of chronic diseases (30). GDF-15 has been identified as a biomarker of cellular senescence (31). Higher plasma GDF-15 concentrations in older adults may reflect a higher overall burden of senescent cells in tissues. Cellular senescence is considered a stress response mechanism that protects cells and organisms from cancer development and is generally considered one of the fundamental mechanisms of aging (32). Senescent cells, which accumulate with aging, are in a state of cell-cycle arrest but remain viable and metabolically active. Factors that increase cellular senescence include DNA damage, oncogene mutation, tumor suppressor loss, oxidative damage, telomere erosion, chromatin alterations, and epigenetic stress (32). Senescent cells have a bioactive secretome known as the senescence-associated secretory phenotype (SASP) (33). SASP is cell type-specific and depends on the tissue environment, the stimulus for senescence, time course, and other factors (34). SASP is characteristic of senescent cells such as satellite cells in skeletal muscle, fibroblasts, intraplaque foam cells, and astrocytes. GDF-15 is expressed by many types of senescent cells (31,35,36). Circulating SASP proteins can cause deleterious effects to other peripheral tissues and accelerate aging phenotypes (33). The selective elimination of senescent cells, also known as senolysis, has been shown to ameliorate aging-related diseases in mouse models (37–40).

The biological mechanisms by which elevated circulating GDF-15 could relate to anemia have not been established. The receptor for GDF-15, GDNF-family-α-like (GFRAL), is highly expressed in a discrete region of the brainstem, the area postrema, and nucleus tractus solitarius (8). GFRAL also has low levels of expression in liver, adipose, testis, and hematopoietic cells of bone marrow (41). Stimulation of GFRAL by GDF-15 leads to a reduction in appetite, food intake, and body weight in mouse models (8). GDF-15 may influence signaling pathways in other cell types outside of the brain without GFRAL, as GDF-15 has been shown to inhibit transcriptional regulation of the Smad pathway after translocation into the nucleus (42). Elevated GDF-15 could potentially stimulate GFRAL in the brainstem to reduce appetite, decrease food intake, and lower body weight in older adults, such a mechanism could contribute to the anemia associated with anorexia (43). Elevated GDF-15 could potentially modulate erythropoiesis through GFRAL in bone marrow or through signaling pathways that do not require GFRAL and depend upon translocation of GDF-15 into the nucleus (42). Circulating GDF-15 was identified as a suppressor of the iron regulatory protein hepcidin (44). However, erythropoietin administration to healthy young human volunteers has been shown to reduce circulating hepcidin without changes in circulating GDF-15, suggesting that GDF-15 does not play a direct role in modulating hepcidin expression (45,46).

In the InCHIANTI study, 25.3% of the participants developed anemia over 15 years. The reported incidence of anemia in older adults appears to vary widely. The annual incidence of anemia in older adults was estimated as 90.3 per 1000 among men and 69.1 per 1000 among women in Olmsted County, Minnesota (47). In the Cardiovascular Health Study, 9% of nonanemic adults, aged ≥65 years, developed anemia over 3 years of follow-up (48).

The strengths of this study include the population-based sample of participants, standardized data collection, and longitudinal follow-up of 15 years. Plasma GDF-15 was measured using a aptamer-based assay; our previous studies show that plasma GDF-15 measured using SOMAscan and plasma GDF-15 measured using ELISA have a correlation of 0.82 (30). The participants in the InCHIANTI study are all Italian, and the findings of the study cannot necessarily be generalized to other study populations and ethnic groups. A limitation of the study is that plasma GDF-15 was only measured once at baseline.

Conclusion

Older community-dwelling adults aged 60 years and older with elevated circulating GDF-15 are at an increased risk of subsequently developing

anemia. These findings need corroboration in other independent study populations. The biological mechanisms by which GDF-15 could contribute to the development of anemia require further investigation.

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Author Contributions

R.D.S. and L.F. designed the study; Y.Y. and T.T. created the dataset; Y.Y., M.Z., and R.D.S. analyzed the data; Y.Y. and R.D.S. drafted the manuscript; all authors revised the manuscript; and all authors approved the final version.

Conflict of Interest

None declared.

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