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Original Article

Plasma Angiopoietin-Like Protein 2 Levels and Mortality Risk Among Younger-Old Japanese People: A Population-Based Case–Cohort Study

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Abstract

Aging is an important medical and social problem. Excessive angiopoietin-like protein (ANGPTL)-2 signaling causes chronic tissue inflammation, promoting development and progression of aging-related diseases. Moreover, circulating ANGPTL2 levels reportedly predict the risk of some aging-related diseases and subsequent death. However, there are, as yet, no reports of whether circulating ANGPTL2 levels predict vital prognosis in younger-old, community-dwelling populations. This study investigated associations between plasma ANGPTL2 levels and all-cause and specific-cause mortality in this population. The case–cohort study was abstracted from an ongoing, age-specific prospective cohort study: the New Integrated Suburban Seniority Investigation Project. This project enrolled 3 073 participants aged 64 years at the beginning of the investigation from 1996 through 2005. A subcohort of 714 randomly sampled participants plus 387 cases representing deceased participants followed through 2015 underwent survival analysis. Plasma ANGPTL2 concentrations were positively associated with >80% and 100% higher risk of all-cause mortality and cancer mortality, respectively, after adjustment for gender, smoking, alcohol consumption, walking time, sleep duration, caloric intake, medical status, disease history, BMI, and triglyceride, creatinine, uric acid, and high sensitivity C-reactive protein levels. A more robust association between ANGPTL2 levels and all-cause and cancer mortality was seen in participants with either frailties or with lifestyles of heavier drinking or current smoking. Elevated plasma ANGPTL2 levels are associated with high all-cause and cancer mortality in a community-dwelling sample of younger-old adults. These findings expand our knowledge of human aging and associated diseases.

Keywords: Biomarkers, Inflammation, Mortality

The number of older adults is increasing worldwide. Biological aging is associated with tissue and cellular dysfunction, decreased physiological reserves, and progressive loss of homeostasis due to combined lifestyle, environmental, and genetic factors. Chronic low-grade inflammation is a common characteristic of biological aging, further accelerating the aging process and contributing to age-related morbidity, mortality, and decreased longevity (1). Plasma levels of inflammatory biomarkers, such as interleukin (IL)-6, C-reactive protein (CRP), and tumor necrosis factor (TNF)-alpha, increase with age and are associated with increased risk for cancer, disability, or mortality (2).

Angiopoietin-like protein 2 (ANGPTL2) functions in tissue homeostasis by promoting adaptive inflammation and subsequent tissue remodeling (3). However, ANGPTL2 secretion increases in response to prolonged external and internal stress due to unhealthy lifestyle behaviors such as excessive caloric intake and lack of exercise. As a result, excessive ANGPTL2 signaling leads to maladaptive chronic inflammation and pathological irreversible tissue remodeling, speeding the development of diseases associated with aging such as cancer, metabolic syndrome, and atherosclerosis (4–7). Interestingly, levels of circulating ANGPTL2 increase with age in mice and humans (5,8). These observations suggest that risk of future development of chronic inflammation and/or aging-related disease could be predicted by monitoring circulating ANGPTL2 levels. Accordingly, recent epidemiologic studies confirm an association of circulating ANGPTL2 levels with aging- and lifestyle-related diseases, such as cancer (9,10) and noncancer-related diseases including cardiovascular diseases (CVDs) (5,11,12). Other studies indicate significant association of high-circulating ANGPTL2 levels with increasing risk for disease-related death in patients with diabetes (13) or on hemodialysis (14). Nonetheless, to date, there are no reports of correlation between circulating ANGPTL2 levels and vital prognosis, as estimated by all-cause death and/or specific-cause death in community-dwelling populations.

Here, we conducted such a study in community-dwelling Japanese populations. To do so, we employed samples of the New Integrated Suburban Seniority Investigation (NISSIN) age-specific cohort study, which enrolled only participants who were 64/65 years of age as a means to avoid the influence of conditions of extreme age that might significantly confound analysis. The targeted population of this study was the younger-old Japanese at the beginning of senescence in terms of physical and psychological status (15). We also conducted an exploratory evaluation of circulating ANGPTL2 levels on cause-specific mortality, such as cancer mortality and noncancer mortality, and performed a subgroup analysis of the presence of frailty, which is an aging-related syndrome of physiological decline characterized by marked vulnerability (16), as well as analysis of alcohol consumption and cigarette smoking, which enhance conditions of chronic inflammation and aging (17,18).

Methods and Materials

Study Population

The NISSIN Project is an ongoing, age-specific prospective cohort study established between 1996 and 2005. The study protocol is described elsewhere in detail (15). Briefly, at the beginning of each survey year and based on the basic resident registry, all community-dwelling residents aged 64 years (classified as younger-old adults in Japan) in "N city" in central Japan were invited by letter to participate in a free comprehensive medical examination, complete a self-administered questionnaire, and provide a fasting blood sample each June from 1996 through 2005. A total of 3 073 participants (1 548 men and 1 525 women) agreed to register as cohort members (response rate: 43.9%) and 2 participants later moved away and were dropped from the analysis (Figure 1). Blood samples were obtained, laboratory blood testing was conducted at baseline, and plasma (1 mL, 1–2 replicates) was kept at −80°C at Nagoya University. The study proposal was approved by the Ethics Committee of Nagoya University Graduate School of Medicine, the National Center for Geriatrics and Gerontology of Japan, Aichi Medical University School of Medicine, Kumamoto University Graduate School of Medical Science, and Hokkaido University Graduate School of Medicine. Plasma analysis was conducted after approval from the Ethics Committee of Hokkaido University Faculty of Medicine (no. 14-037). Consent to respond to the questionnaire was obtained orally using an opt-out approach from 1996 through 2001 and thereafter in writing by an opt-in approach. Consent for blood sample donation was obtained by an opt-out approach up to 1999 and thereafter in writing by an opt-in approach.

Participants

A case–cohort design was employed to assess a potential correlation between ANGPTL2 levels and death in the NISSIN Project. In 2014, a subcohort (714 participants) stratified by survey year and gender with a sampling fraction of 25% (determined by the availability of research funds) was randomly abstracted from the NISSIN Project, regardless of whether participants had died. By the end of 2015, 426 of all participants had died and 98 of those were included in the subcohort. After excluding these participants with missing data relevant to ANGPTL2 (39 death cases, outside the subcohort), we analyzed the remaining 714 subcohort participants (including 98 death cases) and 387 death cases (Figure 1).

Laboratory Measurements

Baseline measurement

Serum levels of total cholesterol, hemoglobin A1c (HbA1c), fasting plasma glucose, creatinine, triglycerides, and uric acid were tested at baseline through routine blood examination in a single laboratory.

Inflammatory biomarker measurement

ANGPTL2 was blindly measured in 2014 for the subcohort members and death cases by the end of 2013 using the enzyme-linked immunosorbent assay method at the Department of Molecular

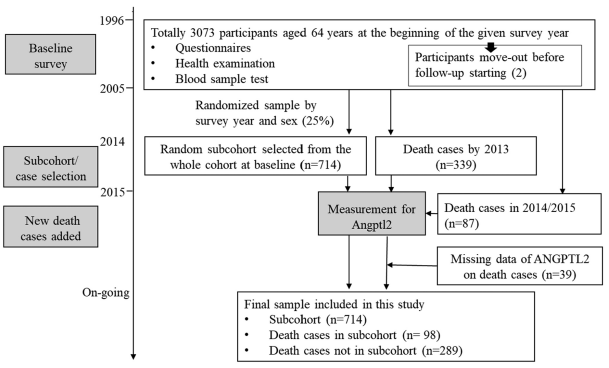

Figure 1. Flow chart showing study design and study population. There were 387 death cases and 714 subcohort (including 98 death cases). The subcohort was randomly abstracted from the baseline cohort, and the sampling fraction was 25%, which was determined based on the availability of research funding.

Genetics, Graduate School of Medical Science, Kumamoto University, Kumamoto, Japan. A Human ANGPTL2 assay kit was used (code no. 27745; IBL Co., Ltd, Fujioka, Japan) to measure plasma ANGPTL2 concentration following kit instructions (4). Additional ANGPTL2 analysis was conducted using the same method and at the same laboratory for new death cases between 2014 and 2015 (Figure 1). The lowest limit of detection was 0.05 ng/mL. Considering dilution of the sample by additives (such as sodium citrate), we adjusted ANGPTL2 concentration using total serum protein concentrations determined during the health examination, as follows: baseline ANGPTL2 = (baseline total protein/currently measured total protein) × currently measured ANGPTL2. Plasma hs-CRP levels were measured using a latex-enhanced immunoassay (Denka Seiken, Tokyo, Japan) with an automatic analyzer (Biolis 24i; Tokyo Boeki) at Fujita Health University; the detection limit was 0.01 mg/dL. Both intra- and interassay coefficients of variation were less than 10%. hs-CRP concentration was adjusted similarly to ANGPTL2. The adjustment method for ANGPTL2 and hs-CRP concentration was applied and published in the previous study (19).

Assessment of Other Covariates

Questionnaire evaluation

Data relevant to lifestyle were obtained through questionnaires. Participants were asked about drinking status, with options of non- or current drinker. Current drinkers were asked about drinking frequency, type of alcohol consumed (beer, Japanese sake, Japanese spirits, whiskey, wine, and others), and average consumption volume per time using "gou" units (1 gou = 23 g). The average daily consumption volume was estimated using the formula: (average consumption volume per time × frequency of alcohol consumption × 23 g)/7 days. Alcohol consumers were categorized as occasional (non-drink or alcohol consumption <23 g/day) or heavier (alcohol consumption ≥23 g/day). Smoking status options were never, past, and current. The past smokers were further asked about their smoking cessation period. Daily walking time included walking for exercise or work or for household, social, or other activities each day. Participants were asked to state how many hours a day they walked. Answers included less than 30 minutes, 30 minutes to 1 hour, 1–2 hours, and ≥2 hours. Participants asked how long they slept each day over the last year provided answers in hours and/or minutes and were grouped based on a median number of <7, 7, or >7 hours/day. Caloric intake was estimated based on daily food intake and Japanese food composition tables according to a validated food frequency questionnaire (20). Missing daily calorie intake data were imputed based on gender-specific medians in the subcohort. Disease history included a self-reported history of clinically diagnosed heart disease, cerebrovascular disease, and cancer. Information provided on a self-report of hypertension, hyperlipidemia, diabetes mellitus, and medicine use based on given medical status was also collected. Exercise habit was also assessed by asking the participants to report their exercise frequency per week. Geriatric Depression Scale 15 (short version, GDS-15) was also conducted.

Health examination

Height, weight, and blood pressure were measured during the baseline health examination. Body mass index (BMI) was calculated by dividing weight (kg) by squared height (m). Usual walking speed was measured by asking the participants to walk 10 m at their usual walking speed. Grip strength was measured twice for left and right hands.

Follow-up and Outcome Confirmation

All participants were followed until death from any cause or until they moved out of the test city, or until December 31, 2015, whichever occurred first. Dates of death or moving were obtained from the basic resident registry. Information concerning the cause of death was accessed through the annual registry of the Ministry of Health, Labour and Welfare and coded by the 10th version of the International Classification of Diseases (ICD-10). The primary outcome was all-cause mortality, and mortality from cancer (ICD 00-97), or others.

Statistical Analysis

Plasma ANGPTL2 levels were expressed as quartiles with cutoffs based on gender-specific distribution in the subcohort. Participant characteristics were indicated by status in the subcohort or in death cases and by quartiles of ANGPTL2 concentration in the subcohort. Pearson's chi-square test (or Fisher's exact test) was used to test differences in category variables. Analysis of variance (or a *t* test) was used for continuous variables that were normally distributed, and the Wilcoxon test was used for continuous variables not normally distributed. Cox proportional hazard regression analyses modified for a stratified case–cohort study using Borgan's method were used to estimate multivariate-adjusted hazard ratios (HRs) of mortality and assess corresponding 95% confidence intervals (CIs) (21). Trend test was conducted by putting continuous plasma ANGPTL2 into the models.

Adjusted covariates included gender; survey year; cigarette smoking; alcohol consumption; daily walking time; sleep duration; daily caloric intake; medical status related to hypertension, hyperlipidemia, and diabetes mellitus; history of heart or cerebrovascular diseases, or cancer; BMI; and levels of creatinine, triglycerides, uric acid, and hs-CRP. Index of comorbidity was further defined into the sum of numbers of history diseases and medical status. Hypertension was further defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, self-reported hypertension, and/or use of antihypertensive medication. Hyperlipidemia was defined as a total cholesterol level ≥220 mg/dL and/or self-reported hyperlipidemia and/or medication use. Diabetes mellitus was defined as HbA1c ≥6.5%, fasting plasma glucose ≥126 mg/dL, and/or self-reported diabetes mellitus and/or medication use. In the NISSIN Project, HbA1c was measured according to the Japan Diabetes Society units. These results were transformed to National Glycohemoglobin Standardization Program (NGSP) values using the conversion formula: NGSP (%) = 1.02 × JDS (%) + 0.25% (22). All covariates were chosen based on previous studies.

Sensitivity analyses were conducted by excluding baseline participants with a history of cancer, heart disease, or cerebrovascular diseases (191 participants; 140 within the subcohort) or by excluding participants who died within 2 years of the start of follow-up, as these participants were likely to die at an early stage of the study because of health status rather than due to increased ANGPTL2 levels.

Subgroup analyses of the presence of frailty, alcohol consumption, or cigarette smoking were conducted. Frailty was assessed according to the modified Fried criteria (23), including shrinking, exhaustion, slowness, weakness, and low physical activity. Shrinking was defined as measured BMI <18.5 kg/m^2. Exhaustion was assessed by a question in GDS-15 by asking "Do you feel full of energy?"; the participants who answered no was considered as with exhaustion criteria. Slowness was defined as slow usual walking speed. Slow usual walking speed was defined as the lowest 20% of usual walking speed adjusted by sex and measured height. Weakness was the

lowest 20% of maximum grip strength of 2 hands adjusted by sex and BMI. Low physical activity was considered as exercise frequency per week less than once. All the cutpoints for the above-mentioned variables were based on their distribution in the subcohort. Frailty was further defined as follows: (a) participants whose total scores of shrinking, exhaustion, slowness, weakness, and low physical activity were ≥3 were defined as frail regardless of missing data; (b) participants whose total scores were 2 without missing data were defined as nonfrail; (c) participants whose total scores were 1 with ≤1 missing data point on any of the items were defined as nonfrail; (d) participants whose total scores were 0 with ≤2 missing data points on any of the items were defined as nonfrail; and (e) participants who did not meet any of the previously described criteria were deemed "missing" and excluded from the analysis (see Supplementary Table 2 for the detailed cutpoint of frailty criteria).

Statistical analyses were performed using R software (version 3.6.1) and the SAS statistical software package version 9.4 for Microsoft Windows (SAS Institute Inc., Cary, NC). *P* values were 2-tailed, and $p < .05$ was considered significant.

Results

Most participants who died during the survey were men who had participated in the first 5 years of the study. Most were heavy drinkers, current smokers, or long sleepers and showed a higher prevalence of diabetes mellitus, a lower prevalence of hyperlipidemia, and higher levels of creatinine, uric acid, and hs-CRP than participants in the subcohort (Table 1). Plasma ANGPTL2 concentrations in death cases were significantly higher than those seen in

Table 1. Participants' Baseline Characteristics for the Subcohort and Death Cases

	Subcohort (*n* = 714)		Death Cases (*n* = 387)		*p*
Sex, *n* (%)					<.001
Male	360	50.4	265	68.5	
Female	354	49.6	122	31.5	
Survey year, *n* (%)					<.001
1996–2000	348	48.7	248	64.1	
2000–2005	366	51.3	139	35.9	
Alcohol consumption, *n* (%)					.024
Occasional drinkers	572	80.1	287	74.2	
Heavier drinkers	142	19.9	100	25.8	
Cigarette smoking, *n* (%)					<.001
Never or ex-smoker (≥1 year)	582	81.5	247	63.8	
Current smokers	132	18.5	140	36.2	
Daily walking time, *n* (%)					.243
<1 h/day	319	44.7	193	49.9	
≥1 h/day	392	54.9	193	49.9	
Sleeping duration, *n* (%)					.030
< 7 h/day	259	36.3	115	29.7	
7 h/day	221	31.0	117	30.2	
> 7 h/day	234	32.8	155	40.1	
DCI (kcal), mean (*SD*)*	1982.3	765.4	1910.2	645.6	.098
Medical status, *n* (%)					
Hypertension	330	46.2	196	50.6	.160
Hyperlipidemia	355	49.7	140	36.2	<.001
Diabetes mellitus	83	11.6	77	19.9	<.001
Disease history, *n* (%)					
Heart disease	82	11.5	35	9.0	.210
Cerebrovascular disease	35	4.9	22	5.7	.576
Cancer	32	4.5	19	4.9	.747
Frailty status					
Nonfrailty	322	88.0	178	83.6	.136
Frailty	44	12.0	35	16.4	
BMI, kg/m^2, mean (*SD*)*	23.0	2.7	22.7	3.0	.159
Creatinine (mg/dL), median† (IQR)	0.8	0.7–0.9	0.8	0.7–0.9	<.001
Triglycerides (mg/dL), median† (IQR) †	104	77.0–145.0	116	83.0–149.0	.059
Uric acid (mg/dL), median† (IQR)	5.2	4.3–6.2	5.5	4.6-6.5	.003
Hs-CRP (mg/dL), median (IQR)*	0.05	0.02–0.1	0.1	0.02–0.1	.021
ANGPTL2 (ng/mL), mean (*SD*)*	4.3	1.3	4.7	2	<.001
ANGPTL2 (ng/mL), median (IQR)*	4.0	3.3–5.9	4.3	3.4–5.4	.004
Quartile 1, *N* (%)	179	25.1	82	21.2	.022
Quartile 2	178	24.9	82	21.2	
Quartile 3	178	24.9	93	24	
Quartile 4	179	25.1	130	33.6	

Notes: SD = standard deviation; IQR = interquartile range; ANGPTL2 = angiopoietin-like protein 2; DCI = daily calories intake; BMI = body mass index; Hs-CRP = high sensitivity C-reactive protein. The summed percentage was not equal to 1 because of the missing data.

*t test.

†Wilcoxon test, otherwise chi square test.

subcohort members (Table 1 and Figure 2). In the subcohort, participants in the highest quartile of circulating ANGPTL2 levels were more likely to exhibit a higher prevalence of diabetes, high BMI, and high levels of triglycerides, uric acid, and hs-CRP compared with those in the lowest quartile (Supplementary Table 1).

We then performed survival analyses to evaluate the association between circulating ANGPTL2 levels and risk for all-cause mortality after adjustment for confounding factors. The gender-adjusted HR for all-cause mortality in the highest quartile of plasma ANGPTL2 (>4.98 ng/mL) was significantly higher (HR, 1.76; 95% CI, 1.20–2.56) in younger-old, community-dwelling participants (Model 1 in Table 2). After adjustments for potential confounding factors of gender, survey year, cigarette smoking, alcohol consumption, daily walking time, sleep duration, daily caloric intake, index of morbidity, BMI, and levels of triglycerides creatinine, and uric acid, the highest quartiles of circulating ANGPTL2 levels were also significantly associated with all-cause mortality (HR, 2.01; 95% CI, 1.33–3.03; Model 2 in Table 2). Furthermore, after adjustment for levels of hs-CRP, a biomarker of aging and inflammation, these associations remained significant, and we observed a more than 80% increase in HR for all-cause mortality (HR, 1.86; 95% CI 1.22–2.83; Model 3 in Table 2). Trend tests revealed that increased ANGPTL2 levels were significantly associated with increased risk for all-cause mortality (all p values for trend were <.001, Table 2). Furthermore, analyses using ANGPTL2 as a continuous variable revealed a significant association of increasing ANGPTL2 levels with increased risk for all-cause mortality in younger-old, community-dwelling participants following adjustment for the corresponding confounding factors indicated above (HR, 1.32; 95% CI 1.17–1.48; Table 2).

In an exploratory analysis for cancer mortality, HR in the highest quartile of plasma ANGPTL2 was increased up to 100% or more after adjustment for potential confounding factors, as in Model 3 above (HR, 2.08; 95% CI, 1.24–3.50; Table 2). Furthermore, an elevated risk for cancer mortality was significantly associated with increasing ANGPTL2 levels (all p values for trend were <.001, Table 2). Moreover, survival analyses using ANGPTL2 as a continuous variable revealed that increasing ANGPTL2 levels were significantly associated with increased risk for cancer mortality in participants following adjustment for corresponding confounding factors in Model 3 (HR, 1.35; 95% CI 1.16–1.57, Table 2).

For noncancer mortality, trend tests revealed that increased circulating levels of ANGPTL2 correlated with high risk (all p < .001, Table 2). However, the highest quartile of ANGPTL2 levels was not significantly associated with elevated risk for noncancer mortality after adjusting more potential confounders (Model 3, Table 2). In sensitivity analysis, the association between circulating ANGPTL2 levels and mortality risk did not change significantly after excluding participants with a history of cancer, heart disease, or cerebrovascular disease at baseline, or after exclusion of cases of individuals who died in the first 2 years from the start of follow-up (Supplementary Table 3).

To evaluate whether frailty, a condition resulting from chronic low-grade inflammation and aging (16), affects associations between circulating ANGPTL2 levels and outcomes, we performed an exploratory subgroup analysis of the presence of frailty at baseline. The HR of circulating ANGPTL2 levels for all-cause mortality was higher in both frailty and nonfrailty groups. The HR in the frailty group tended to be higher than that in the nonfrailty group, but the statistically significant difference in the HR between the 2 groups was not observed (subgroup analysis based on the presence of frailty, Table 3). Also, exploratory subgroup analysis based on unhealthy lifestyle showed that estimated HRs of circulating ANGPTL2 levels for both all-cause mortality and cancer mortality were relatively higher in current smokers and heavier drinkers than in participants lacking those behaviors, although there were no statistically significant interactions (subgroup analysis by smoking or drinking habits, Table 3). Focusing on noncancer mortality, HRs of circulating ANGPTL2 levels in current smokers or heavier drinkers showed incongruent trends (Table 3).

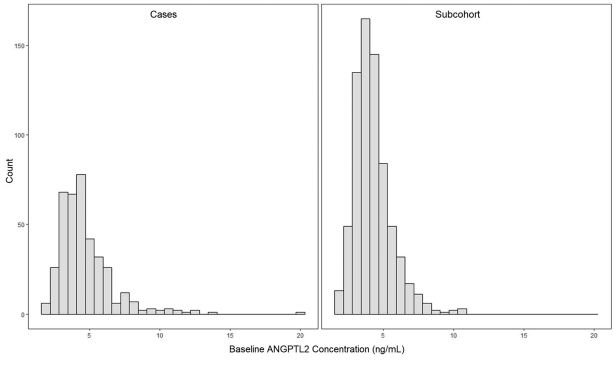

Figure 2. Distribution of baseline ANGPTL2 levels by death cases and subcohort.The median (IQR) for death cases was 4.32 ng/mL (3.41–5.37 ng/mL) and 4.03 ng/mL (3.32–4.86 ng/mL) for the subcohort.

Discussion

To the best of our knowledge, this is the first study to investigate an association between plasma ANGPTL2 levels and mortality risk in a community-dwelling, younger-old population. This age-specific case–cohort study reveals that plasma ANGPTL2 concentrations are positively associated with increased risk of all-cause and cancer-associated mortality. Notably, this association was stronger in participants with frailty characteristics and was also observed in participants with unhealthy lifestyle behaviors such as heavier drinking or current smoking.

Circulating ANGPTL2 Levels and All-Cause Mortality

It is well-known that low-grade chronic inflammation underlies various aging- and lifestyle-related diseases, such as cancer or other diseases associated with disability and mortality (16). As a result, various pro-inflammatory factors secreted from senescent cells, such as IL-6, have been characterized as senescence-associated secretory phenotypes factors (24). Because ANGPTL2 secretion increases in senescent and/or pathologically damaged cells and ANGPTL2 is a senescence-associated secretory phenotypes factor (8,25), high circulating ANGPTL2 levels likely indicate accelerated aging and associated disease plus the risk of death in younger-old adults.

Among inflammatory markers, elevated levels of circulating hs-CRP levels occur not only in acute inflammation caused by a bacterial or viral infection but also in chronic inflammation associated with aging and aging-related diseases (26,27). Interestingly, we observed a significant association between high circulating ANGPTL2 levels and increased all-cause mortality risk in community-dwelling participants after adjustment for hs-CRP levels, in addition to other candidate confounding factors.

To date, previous investigations from our group and other group targeting the general population of young, middle-aged, and younger-old people independently revealed that circulating

Table 2. Estimated Hazard Ratios of Circulating ANGPTL2 Levels for All-Cause Mortality, Cancer Mortality, and Noncancer Mortality in Younger-Old Adults (HR/95% CI)

	Subcohort/Cases	Model 1	Model 2	Model 3
All-cause mortality				
Q1 (≤3.28 ng/mL)	179/82	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Q2 (3.29 to 4.10 ng/mL)	178/82	0.94 (0.63, 1.40)	0.99 (0.65, 1.49)	0.972 (0.64, 1.476)
Q3 (4.11 to 4.98 ng/mL)	178/93	1.23 (0.83, 1.82)	1.33(0.88, 2.02)	1.29 (0.85, 1.96)
Q4 (>4.98 ng/mL)	179/130	1.76 (1.20, 2.56)	2.01 (1.33, 3.03)	1.86 (1.22, 2.83)
p for trend		<.0001		<.0001
1-unit increment		1.28 (1.16, 1.43)		1.32 (1.17, 1.48)
Cancer mortality				
Q1 (≤3.28 ng/mL)	179/41	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Q2 (3.29 to 4.10 ng/mL)	178/44	1.01 (0.62, 1.65)	1.10 (0.66, 1.83)	1.09 (0.65, 1.82)
Q3 (4.11 to 4.98 ng/mL)	178/49	1.27 (0.79, 2.06)	1.32 (0.80, 2.20)	1.31 (0.79, 2.18)
Q4 (>4.98 ng/mL)	179/71	1.85 (1.17, 2.92)	2.14 (1.29, 3.53)	2.08 (1.24, 3.50)
p for trend		.0001		.0001
1-unit increment		1.30 (1.14, 1.48)	1.35 (1.17, 1.56)	1.35 (1.16, 1.57)
Noncancer mortality				
Q1 (≤3.28 ng/mL)	179/41	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
Q2 (3.29–4.10 ng/mL)	178/38	0.86 (0.52, 1.44)	0.86 (0.50, 1.48)	0.84 (0.49, 1.45)
Q3 (4.11–4.98 ng/mL)	178/44	1.19 (0.72, 1.97)	1.36 (0.81, 2.30)	1.28 (0.75, 2.18)
Q4 (>4.98 ng/mL)	179/59	1.66 (1.03, 2.69)	1.84 (1.08, 3.12)	1.62 (0.95, 2.76)
p for trend		<.0001		<.0001
1-unit increment		1.27 (1.12, 1.45)	1.33 (1.16, 1.52)	1.27 (1.10, 1.47)

Notes: HR = hazard ratio; 95% CI = 95% confidence interval; ANGPTL2 = angiopoietin-like protein 2; *p* = probability. Model 1 was adjusted by gender and survey year; Model 2 was adjusted by gender, survey year, alcohol consumption, cigarette smoking, daily walking time, sleep duration, daily caloric intake, medical status, disease history, body mass index, and triglyceride, creatinine, and uric acid levels; Model 3 was adjusted by covariates in Model 2 and high sensitivity C-reactive protein. Levels of triglycerides, creatinine, uric acid, and high sensitivity C-reactive protein were log-transformed for analysis in Models.

ANGPTL2 levels increase with aging (5) and that high levels are a candidate biomarker predictive of future development and progression of diabetes and CVD (13,28,29). Interestingly, we recently reported that circulating ANGPTL2 levels were not associated with advanced aging in the oldest-old population (aged 85–110 years or older), however, circulating levels of IL-6 or TNF-α were associated with the oldest-old population (30). The oldest-old, including centenarians, have a very low prevalence of diabetes and CVD (31,32), suggesting that they are exceptional survivors of common aging-related diseases, including cardiometabolic disease and cancer. Accordingly, we hypothesized there would be no association between circulating ANGPTL2 and advancing aging and increased mortality in these individuals, who likely evade inflammatory conditions induced by excessive ANGPTL2 secretion. By contrast, the current study indicates that high circulating ANGPTL2 levels predict all-cause death in younger-old adults. Overall, we conclude that high circulating ANGPTL2 levels may represent an in vivo specific inflammatory pathological condition different from inflammatory conditions estimated by hs-CRP, IL-6, or TNF-α levels. Thus, high circulating ANGPTL2 levels are more than an inflammatory marker but rather serve as a unique biomarker to predict future all-cause mortality in younger-old adults.

Circulating ANGPTL2 Levels and Cancer Mortality

This study indicates that high plasma ANGPTL2 levels are associated with the risk of cancer mortality, a finding consistent with previous reports linking ANGPTL2 levels with cancer pathologies. In brief, patients with gastric (33) and esophageal (9) cancer show higher circulating ANGPTL2 levels than do noncancer healthy controls, and such levels are associated with metastasis in patients with lung (34), breast (35), and liver (36) cancer and with recurrence of colorectal cancer (37). Moreover, using a tumor cell-implanted mouse model, we previously reported that tumor cell-derived ANGPTL2 increased cancer cell invasivity and metastasis and decreased animal survival (34,38). We have also found that ANGPTL2-induced chronic tissue inflammation contributes to increased carcinogenesis in a chemically induced carcinoma mouse model (6). Overall, it is suggested that high plasma ANGPTL2 concentrations may associate with cancer progression that is more likely to result in mortality.

Circulating ANGPTL2 Levels and Noncancer Mortality

Previously, we also showed that ANGPTL2-induced chronic tissue inflammation accelerates the development of noncancer diseases, such as CVDs, diabetes, and sarcopenia, in mouse models (4,5,7,39). Moreover, we reported that high circulating ANGPTL2 concentrations are associated with severity of CVD and renal disease (11,40). Notably, high ANGPTL2 concentrations may also predict a higher risk for future development and poor prognosis of CVD and diabetes (12,29). As expected, here we found that increased ANGPTL2 levels were significant predictors of increased mortality risk from noncancer disease; however, interestingly, the highest quartiles of circulating ANGPTL2 levels were not significantly associated with noncancer mortality. This discrepancy could be due either to the wide variety of diseases represented by these cases (eg, 43.4% died of CVD, 21.4% of respiratory disease, 7.1% from injury or intoxication, and 6.0% from gastroenterological disease) or to the smaller number of participants who died in this group. Further studies of large numbers of noncancer-associated events conducted over a longer period are needed to evaluate associations between ANGPTL2 levels and mortality risk in these cases.

Table 3. Estimated Hazard Ratios of Circulating ANGPTL2 Levels for All-Cause Mortality, Cancer Mortality, and Noncancer Mortality, as Estimated by Subgroup Analyses in Younger-Old Adults

Outcomes	Subcohort/Cases	HR (95% I)	*P* for interaction
Frailty			
All-cause mortality			.436
Non frailty	322/178	1.35 (1.16, 1.56)	
Frailty	44/35	1.94 (1.01, 3.71)	
Cancer mortality			.680
Non frailty	322/94	1.43(1.17, 1.75)	
Frailty	44/16	5.57 (0.64, 48.36)	
Noncancer mortality			.385
Non frailty	322/84	1.25 (1.04, 1.50)	
Frailty	44/19	1.66 (0.68, 4.07)	
Drinking status			
All-cause mortality			.201
Occasional drinker	572/287	1.30 (1.13, 1.49)	
Heavier drinker	142/100	1.49 (1.12, 1.96)	
Cancer mortality			.097
Occasional drinker	572/156	1.30 (1.10, 1.55)	
Heavier drinker	142/49	1.77 (1.22, 2.56)	
Noncancer mortality			.794
Occasional drinker	572/131	1.27 (1.08, 1.50)	
Heavier drinker	142/51	1.29 (0.87, 1.91)	
Smoking status			
All-cause mortality			.345
Never or ex-smoker (≥1year)	582/247	1.26 (1.10, 1.44)	
Current smokers	132/140	1.65 (1.26, 2.16)	
Cancer mortality			.163
Never or ex-smoker (≥1year)	582/127	1.22 (1.02, 1.45)	
Current smokers	132/78	1.69 (1.16, 2.46)	
Noncancer mortality			.919
Never or ex-smoker (≥1year)	582/120	1.29 (1.10, 1.51)	
Current smokers	132/62	1.55 (1.03, 2.32)	

Notes: HR = hazard ratio; 95% CI = 95% confidence intervals; ANGPTL2 = angiopoietin-like protein 2; *p* = probability. The models were adjusted by gender, survey year, alcohol consumption/cigarette smoking/body mass index (corresponding with the subgroup analysis), daily walking time, sleep duration, daily caloric intake, medical status, disease history, and levels of triglycerides, creatinine, uric acid, and high sensitivity C-reactive protein. Levels of triglycerides, creatinine, uric acid, and high sensitivity C-reactive protein were log-transformed for analysis in models.

Circulating ANGPTL2 Levels and Mortality in Participants With Frailty

As noted above, increased ANGPTL2 secretion accelerates the development of aging-related conditions, including cancer and noncancer diseases (3–5,34,35). Given that aging is also a cause of frailty, we hypothesized that the HR of high circulating ANGPTL2 levels for all-cause mortality would be significantly greater in participants with frailty relative to the nonfrailty group. However, frailty does not exacerbate the effects of high ANGPTL2 levels. One explanation for our wrong hypothesis is the small number of cases evaluated in the frailty group. Therefore, more study is needed to determine whether circulating levels of A2 have differential effects on participants with or without frailty.

Circulating ANGPTL2 Levels and Mortality in Participants With Unhealthy Lifestyle Behaviors

In current smokers or heavier drinkers, the HR for all-cause mortality and cancer mortality of plasma ANGPTL2 levels was relatively higher than participants without this unhealthy lifestyle, although there was no interaction between plasma ANGPTL2 levels and the unhealthy lifestyle against mortality. Heavy smoking and drinking are known to accelerate induction of aging-related chronic inflammation (17,18), which in turn enhances development and progression of aging-related diseases (16). ANGPTL2 expression in human endothelial cells is reportedly increased by smoking (41). Moreover, excessive ANGPTL2 signaling in endothelial cells induces chronic vascular tissue inflammation, promoting development of aging-related atherosclerotic vascular disease (5). These findings support our observation that circulating ANGPTL2 levels were positively associated with mortality risk in participants who currently smoke. On the other hand, there are no reports of an association between ANGPTL2 expression and excessive drinking, although here, increased ANGPTL2 levels were associated with increased mortality risk in participants with heavier drinking habits. Because heavy drinking also accelerates induction of aging-related chronic inflammation, further investigation is needed to determine whether ANGPTL2 contributes to chronic inflammation induced by drinking.

Strengths and Limitations to the Current Study

In terms of strengths, our study used an age-specific design in a case–cohort study, which eliminated the effect of age on association between ANGPTL2 and subsequent mortality. The gender- and survey-year-stratified design of subcohort selection improved the efficiency and representativeness of this case–cohort study, and the design reduced costs of measuring plasma ANGPTL2. Moreover, adjustment for physical activity and daily caloric intake helped to identify a specific association between plasma ANGPTL2 levels and

mortality, as physical inactivity and being overweight may induce ANGPTL2 secretion (42). As for limitations, long-term storage of biological samples could have promoted protein degradation or altered assay accuracy. However, we normalized ANGPTL2 levels to total serum protein concentrations (for details, see *Methods*), such that even if degradation had occurred, associations observed here would have been underestimated. Our baseline study population originally included participants with cancer or CVD, and these patients are likely to express increased levels of ANGPTL2 (5,6,43). However, after excluding those participants, sensitivity analyses did not alter the predictive value of ANGPTL2 on mortality risk.

Conclusion

We revealed that elevated plasma ANGPTL2 is associated with high all-cause and specific-cause mortality in a community-dwelling sample of younger-old adults. These findings confirm that high plasma levels of ANGPTL2 reflect accelerated aging and associated diseases and death in younger-old adults. Further study is needed to determine whether circulating ANGPTL2 levels could serve as a good indicator for improving risk stratification against future development of aging/lifestyle-related diseases and their prognosis.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None declared.

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Author Contributions

A.T., T.K., and K.W. designed the study; A.T., T.K., K.W., K.T., and M.A. conducted the investigation; A.T., S.U., T.K., Y.O., and W.J.Z. obtained funding to support this study; W.J.Z., J.M., and Y.O. analyzed data and drafted the manuscript; J.M., M.E., and H.Y. measured biomarkers in this study; S.U., J.M., M.E., T.K., K.W., K.T., M.A., Y.O., K.S., and A.T. reviewed and edited the manuscript.

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