Title page

Circulating Angiopoietin-like protein 2 levels and mortality risk in patients receiving maintenance hemodialysis: a prospective cohort study

Jun Morinaga, M.D., Ph.D.[1,2,3,4]*, Tatsuyuki Kakuma, Ph.D.[4], Hirotaka Fukami, M.D.[1,2], Manabu Hayata, M.D., Ph.D.[1], Kohei Uchimura, M.D., Ph.D.[5], Teruhiko Mizumoto, M.D., Ph.D.[1], Yutaka Kakizoe, M.D., Ph.D.[1], Taku Miyoshi, M.D., Ph.D.[1], Naoki Shiraishi, M.D., Ph.D.[1], Masataka Adachi, M.D., Ph.D.[1], Yuichiro Izumi, M.D., Ph.D.[1], Takashige Kuwabara, M.D., Ph.D.[1], Yusuke Okadome, MMSc.[2], Michio Sato, M.D., Ph.D. [2], Haruki Horiguchi, Ph.D. [2], Taichi Sugizaki, M.D., Ph.D. [2], Tsuyoshi Kadomatsu, Ph.D.[2], Keishi Miyata, M.D., Ph.D.[2], Saeko Tajiri, M.D.[6], Tetsuya Tajiri, M.D.[6], Kimio Tomita, M.D., Ph.D.[1], Kenichiro Kitamura, M.D., Ph.D.[5], Yuichi Oike, M.D., Ph.D.[2]*, Masashi Mukoyama, M.D., Ph.D.[1]*

[1]Department of Nephrology, [2]Department of Molecular Genetics, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto, Kumamoto, 860-8556, Japan; [3]Department of Clinical Investigation, Kumamoto University Hospital,

1-1-1 Honjo, Chuo-ku, Kumamoto, Kumamoto, 860-8556, Japan; [4]Biostatistics Center, Kurume University, 67 Asahimachi, Kurume, Fukuoka, 830-0011, Japan; [5]Third Department of Internal Medicine, Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan; [6]Medical Corporation, Jinseikai, 2-3-10 Toshima-nishi Higashi-ku, Kumamoto, Kumamoto, 861-8043, Japan;

*Address for correspondence:

Jun Morinaga, M.D., Ph.D., Department of Nephrology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan. TEL: 81-96-373-5164; FAX: 81-96-366-8458; E-mail: morijun@kumamoto-u.ac.jp

Yuichi Oike, M.D., Ph.D., Department of Molecular Genetics, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan. TEL: 81-96-373-5142; FAX: 81-96-373-5145; E-mail: oike@gpo.kumamoto-u.ac.jp

Masashi Mukoyama, M.D., Ph.D., Department of Nephrology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan. TEL: 81-96-373-5164; FAX: 81-96-366-8458; E-mail: mmuko@kumamoto-u.ac.jp

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Abstract

Background: Prognosis of patients undergoing hemodialysis treatment is poor, as many of them exhibit premature aging. Systemic inflammatory conditions often underlie premature aging phenotypes of the uremic population. Thus, we asked whether Angiopoietin-like protein (ANGPTL) 2, a factor that accelerates progression of aging-related and non-infectious inflammatory diseases, was associated with mortality of hemodialysis patients.

Methods: We conducted a multicenter prospective cohort study of 412 patients receiving maintenance hemodialysis treatment and evaluated relationships between circulating ANGPTL2 levels and risk for all-cause mortality. Circulating ANGPTL2 levels were log-transformed to correct for skewed distribution, and analyzed as continuous variable.

Results: Of 395 subjects analyzed statistically, time-to-event data analysis revealed high circulating ANGPTL2 levels associated with increasing risk for all-cause mortality after adjustment for age, sex, hemodialysis vintage, nutrition status, metabolic parameters, and circulating high sensitivity C-reactive protein values [HR: 2.04, 95%CI (1.10, 3.77)]. High circulating ANGPTL2 levels were also strongly associated with

increased mortality risk, particularly in patients with a relatively benign prognosis [HR: 3.06, 95%CI (1.86, 5.03)]. Furthermore, the relationship between circulating ANGPTL2 levels and mortality risk was especially strong in populations showing less senescent phenotypes, such as younger patients [HR: 7.99, 95%CI (3.55, 18.01)], short hemodialysis vintage [HR: 3.99, 95%CI (2.85, 5.58)], or non-diabetes [HR: 5.15, 95%CI (3.19, 8.32)].

Conclusion: We conclude that circulating ANGPTL2 levels are positively associated with mortality risk of patients receiving maintenance hemodialysis, and that ANGPTL2 may uniquely reflect progression of premature aging and subsequent mortality risk in that population in all but the most advanced senescent phenotypes.

Introduction

The number of chronic kidney disease (CKD) patients is increasing, with >30 million people in the US and 13 million in Japan estimated to be affected [1, 2]. In the end stage of CKD, a large proportion of patients has no choice other than to go life-saving renal replacement therapy, often, hemodialysis treatment. In 2016, >457,000 individuals underwent hemodialysis in the US and >329,000 in Japan [1, 3]. Clinically, prognosis of these patients is poor [4]. Therefore, evaluating mortality risk for these patients is extremely important.

Patients receiving hemodialysis exhibit significant premature aging phenotypes relative to healthy individuals or patients undergoing renal transplant, a phenotype that increases mortality risk in these individuals [5]. Pathological mechanisms underlying premature aging in these patients are complex, as multiple considerations such as uremia, fluid overload, oxidative stress, comorbidities including heart failure, or exogenous factors including dialysis treatment itself may play a role in the phenotype [6, 7]. However, basically, each of these pathological mechanisms is associated with chronic inflammation [6, 7]. In advanced CKD, inflammatory triggers include activation of innate immune system, defective regulation of inflammatory processes, and

increased cytokine secretion from uremic senescent cells, a collection of events known as the senescence-associated secretory phenotype [8]. Subsequently, chronic systemic inflammation in uremia significantly promotes modification of metabolism in favor of increased catabolic pathways and pro-aging activity and away from anabolic pathways and anti-aging mechanisms [6, 7]. These changes accelerate phenotypes of premature aging such as muscle wasting, osteoporosis, vascular calcification, and cardiovascular hypertrophy, and contribute to patient mortality [5, 7]. Accordingly, we hypothesized that circulating levels of inflammation-related factor(s) may be correlated with premature aging phenotypes and associated mortality in uremic patients.

Angiopoietin-like protein (ANGPTL) 2, which possesses an N-terminal coiled-coil domain used for oligomerization and C-terminal fibrinogen-like domain, is a secreted protein structurally similar to angiopoietin but that does not bind to the angiopoietin receptor [9]. Previously, we demonstrated that ANGPTL2 functions in physiological tissue remodeling and plays crucial roles in pathological conditions associated with chronic noninfectious inflammation or aging [10-12]. ANGPTL2 plays pivotal roles in progression of multiple age-related diseases such as atherosclerosis, carcinogenesis, sarcopenia, frailty, and CKD, and is a significant inducer of chronic inflammation [12-16]. Interestingly, because circulating ANGPTL2 levels parallel local secretion of the protein

in tissues, analysis of circulating ANGPTL2 reportedly represents a useful biomarker of inflammation and aging-related outcomes, including *de novo* incidence of diabetes and cardiovascular disease in non-uremic subjects [17, 18]. However, an association of ANGPTL2 levels with clinical outcomes has not been made in the uremic population.

To address this need, we conducted a multicenter prospective cohort study of patients receiving maintenance hemodialysis in Kumamoto, Japan, to determine whether circulating ANGPTL2 levels predict mortality risk of patients receiving maintenance hemodialysis, after adjustment for confounding factors.

Materials and Methods

Study design

This study was conducted with an observational, multicenter prospective cohort design targeting a population receiving maintenance hemodialysis in five clinics in Japan. From March 2011 to March 2012, 412 subjects out of 515 patients who received hemodialysis treatment in those clinics were enrolled after submitting written informed consent to participate in the study (figure S1). Then, their clinical charts were followed for 6 years. This study was conducted in keeping with Helsinki Declaration and with approval of ethics committees for clinical research at Kumamoto University.

Measurement of circulating ANGPTL2 levels

Serum specimens were stored at -80°C, and then, in 2012, ANGPTL2 protein levels were measured at the Department of Nephrology, Kumamoto University, using a human ANGPTL2 enzyme-linked immune-sorbent assay (ELISA) kit designed to detect full length ANGPTL2 with antibodies targeting respective N- and C-termini of the protein (Immuno-Biological Laboratories., Gunma, Japan)[11, 17, 18]. Antibody specificity was confirmed, and antibodies did not cross-reacted with other ANGPTLs.

Statistical analysis

To prepare for statistical analysis, list-wise case deletion was applied to the dataset, and the number of subjects (412 subjects) was decreased to 395 (figure S1). All variables were visually examined, and ANGPTL2, hemodialysis vintage, high sensitive C-reactive protein (hs-CRP), whole parathyroid hormone (w-PTH), ferritin, iron (Fe), transferrin saturation (TSAT), triglycerides (TG), creatine kinase (CK), platelets (Plt), amylase, γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), total bilirubin (T-Bil), and frequency of percutaneous transluminal angioplasty (PTA) treatment were log-transformed to correct for skewed distribution.

Prognostic stage was determined in two steps. First, the random forest method was employed to rank strength of association with the outcome using all 52 variables collected from routine clinical data. After clinical consideration, the 10 highest risk factors were selected to establish prognostic stage. Then, the survival tree method was applied to create 9 patient groups with similar prognostic profiles[19]. Then, to generate the final prognostic stage, hazard ratios among 9 groups of subjects were compared and combined to obtain parsimonious model with 4 prognostic strata.

To perform time-to-event data analysis, the Cox proportional hazard (PH) model was applied. In particular, in evaluating ANGPTL2 effects and adjusting for prognostic strata, strata-specific baseline hazard with strata-specific hazard parameter (1) and common hazard parameter (2) models shown below were fitted[20].

$$\lambda(t \mid x) = \lambda_{0S}(t)\exp(x'\beta_S)\ldots(1)$$
$$\lambda(t \mid x) = \lambda_{0S}(t)\exp(x'\beta)\ldots(2)$$
$$\lambda_{0S} : \textit{strata specific baseline hazard}$$
$$\beta_S : \textit{strata specific hazard parameter}$$
$$\beta : \textit{strata common hazard paraeter}$$

To ensure robustness of hazard estimates from the Cox PH model, bootstrap analysis with 10,000 replications were performed, and the average hazard ratio with a 95 percent confidence interval was evaluated. To evaluate relationships between circulating ANGPTL2 levels and aging-related parameters, we applied a mixed model adjusting for medical facility location to account for random effects. All statistical analysis was performed using STATA 15.0 (Stata corp. LLC., Lakeway Drive, College Station, TX), except for JMP Pro 13.2.1 (SAS Institute, Cary, NC) for random forest analysis. All P values were two-tailed, and $P<0.05$ was taken as statistically significant.

Results

Characteristics of study participants at baseline are shown in Table 1, and Figure 1 shows a histogram of circulating ANGPTL2 levels and the corresponding proportion of patients (%) receiving hemodialysis [median, 3.3 ng/ml, interquartile range: (2.5, 4.0)]. In the 395 subjects analyzed statistically, the median follow-up time for survival analysis was 2,213 days, and 94 of those 395 patients died. To evaluate a potential association between circulating ANGPTL2 levels and patient mortality risk using survival data, we applied the Cox proportional hazard (PH) model. That analysis revealed a significant association of high circulating ANGPTL2 levels with high mortality risk in patients undergoing hemodialysis after adjustment for age, sex, smoking, diabetes, hypertension, dyslipidemia, body mass index, cancer, hemodialysis vintage, albumin, cardio-thoracic ratio (CTR), and the product of adjusted Ca and inorganic phosphorus levels (Table 2, model 2). As shown in model 3 of the table, that association remained significant following adjustment for hs-CRP values (Table 2). These data suggest that circulating ANGPTL2 levels could serve as a useful biomarker to predict mortality risk in patients receiving maintenance hemodialysis following adjustment for confounding factors.

Establishment of prognostic profiles of hemodialysis patients

Next, to define clinical characteristics of patients in which circulating ANGPTL2 levels were strongly associated with mortality risk, we performed stratified analysis using a variable that indicated a patient's prognostic profile. To do so, we generated a synthetic stratum variable indicative of patients' mortality risk by employing 52 variables collected from routine medical assessment (table 1). In generating the stratum, we first performed random forest analysis to rank variables according to strength of association with patient mortality risk. After clinical consideration, we then selected 10 high-ranking risk factors as prognostic indicators (table S1). We then used the survival tree method to place patients into 9 groups, compared hazard ratios among those 9 groups, and then combined them to obtain a parsimonious model termed “Prognostic stage” consisting of 4 prognostic strata (figure S2, and table S2). In that analysis, patients were divided into 3 groups, a younger group (age≤69), a middle age-elderly group (age 70-80), and the most elderly group (age≥81) (figure S2). In the youngest group, patients with the most benign prognosis were categorized as stage 1, and decreasing levels of circulating creatinine (Cr) (<9.7mg/dl) or uric acid (UA) level (≤7.5mg/dl) were selected as indicators of worsening prognosis (figure S2). Next, patients of the middle-elderly group

(age 70 to 80) were categorized as prognostic stage 2 or more, and effectors indicative of worsening conditions for these patients included cardiomegaly (CTR>52.4%) or decreased circulating levels of UA (UA ≤6.3 mg/dl) (figure S2). Finally, in the highest-aged group (age≥81), prognostic stages, namely stages 2 and 4. In this group, higher hs-CRP (>992.3 ng/ml) or higher ferritin (>40 ng/ml) levels were significant effectors indicative of worsening mortality (figure S2). Overall, prognostic stages were defined as: Stage 1, patients of age≤69 with Cr≥9.7 and UA>7.5; Stage 2, patients of age≤69 with Cr≥9.7 and UA≤7.5, or patients of age≤69 with Cr<9.7 or patients aged 70-80 with CTR<52.4 and UA>6.3, or patients of age≥81 with hs-CRP≤992.3 and ferritin≤40; Stage 3, patients aged 70-80 with either CTR<52.4 and UA≤6.3 or with CTR>52.4; Stage 4, patients of age≥81 with either hs-CRP≤992.3 and ferritin>40 or with hs-CRP>992.3 (figure S2).

Kaplan-Meier survival curves for patients at each prognostic stage are shown in figure 2. The Cox PH model and corresponding bootstrap replication analysis also revealed that the hazard ratio for patient mortality increased with as prognostic stage advanced (table S3). In addition, circulating ANGPTL2 levels also increased with advanced prognostic stage (figure S3). Taken together, these data suggest that prognostic stages established here are useful as a statistical adjustment to suppress confounding factors

when evaluating the relationship between circulating ANGPTL2 levels and mortality risk. Thus, we conducted multivariate survival analysis to evaluate that relationship, following adjustment by our prognostic stage.

Clinical characteristics of patients whose circulating ANGPTL2 levels are strongly associated with mortality risk

We next analyzed a potential association between circulating ANGPTL2 levels and mortality at each prognostic stage using the Cox PH model and bootstrap analysis. This analysis revealed that high circulating ANGPTL2 levels are significantly associated with increased risk of mortality for hemodialysis patients in prognostic stages 1, 2, and 3, which are relatively benign prognostic groups, but that association was not significant at stage 4, the highest risk group (table 3). To simplify clinical interpretation of this analysis, we next evaluated the combined hazard ratio of circulating ANGPTL2 levels for mortality of prognostic stages 1, 2, and 3, but not stage 4. The combined hazard ratio for mortality was also significant (table 3). In addition, residual analysis of this model revealed that ANGPTL2 levels and the hazard ratio for mortality were almost linearly associated (figure S4). By contrast association between circulating ANGPTL2 levels and mortality risk was weaker in stage 4 patients, who had poor prognosis and exhibited advanced

senescence phenotypes such as older age and increases in circulating hs-CRP or ferritin levels (table 3, figure S2). Next, to determine whether relationships between circulating ANGPTL2 level and mortality risk were affected by senescence-associated clinical factors, we conducted stratification analyses using populations from prognostic stages 1, 2, and 3. These analyses revealed that the association between high circulating ANGPTL2 levels and increasing of mortality risk was strong, especially in subjects with less-senescent phenotypes such as younger than the median age (<63 years old), in the group without diabetes, and in shorter dialysis vintage than the median (<6.2 years) (table 4). In addition, our analysis revealed that these senescence-related parameters (age, complication of diabetes, or longer hemodialysis vintages) are positively correlated with circulating ANGPTL2 levels (table S4).

Discussion

In the current study, we conducted a prospective cohort study to evaluate whether circulating ANGPTL2 levels are associated with mortality risk of patients receiving hemodialysis treatment. We first demonstrated that high circulating ANGPTL2 levels are a significant risk for mortality. Next, exploratory stratified analysis revealed that high circulating ANGPTL2 levels are associated with high mortality risk, in particular in patients with relative benign prognosis and fewer senescence phenotypes. Ours is the first report indicating that circulating ANGPTL2 levels can predict clinically important outcomes in the uremic population.

Circulating ANGPTL2 levels and mortality in patients undergoing hemodialysis

Multivariate survival analysis reported here revealed that the relationship between high circulating ANGPTL2 levels and increasing mortality risk was significant after adjustment for age, sex, hemodialysis vintage, nutrition status, or metabolic parameters, such as complications of diabetes, dyslipidemia or hypertension. Moreover, this relationship remained significant after further adjustment for hs-CRP-related inflammatory status. Previously, our group reported that excess ANGPTL2 secretion

promotes progression of senescence-related disease such as metabolic syndrome, sarcopenia, cancer, chronic kidney disease or atherosclerosis in animal models or in vitro [11-16, 21]. In the progression of aging-related disease, ANGPTL2 potently accelerates senescence phenotypes through activation of reactive oxygen species or signaling through transforming growth factor β or nuclear factor-kappa B [9, 12, 13, 16]. These reports are in agreement with our current data from patients in the uremic population who exhibit phenotypes of accelerated premature aging relative to healthy individuals. Our analysis suggests that circulating ANGPTL2 levels reflect progression of premature aging in uremic individuals.

Interestingly, our analysis revealed significant relationships between high circulating ANGPTL2 levels and increased mortality risk in patients with a relatively benign prognostic profile (stages 1, 2, and 3), unlike the case in the elderly or the highest mortality group (stage 4). Accordingly, our exploratory analysis revealed that this association remained in the population showing less senescent phenotypes among younger people or individuals without diabetes or of shorter hemodialysis vintage. Evaluating mortality risk is difficult in hemodialysis patients, particularly in individuals who do not exhibit poor prognostic/senescent indicators, such as aging, accelerated inflammation, excess intracorporeal iron, long hemodialysis vintage, or diabetes

complications [22, 23]. Although both ANGPTL2 and CRP are inflammatory markers, calculation of Spearman's correlation coefficient between circulating ANGPTL2 levels and those of hs-CRP at baseline indicated a significant but not strong positive correlation (correlation coefficient: 0.27, $P<0.001$). Furthermore, our finding that a significant association of high circulating ANGPTL2 levels with high mortality risk in patients undergoing hemodialysis after adjustment for confounding factors including hs-CRP values supports the idea that circulating ANGPTL2 levels are unique and could serve as a useful biomarker of mortality risk not detected by CRP analysis. However, we note that in the oldest patients with advanced aging phenotypes related to diabetes or prolonged hemodialysis, circulating ANGPTL2 levels do not predict mortality risk for reasons currently unknown and which require further investigation.

Next, based on our preliminary analysis, circulating ANGPTL2 levels in uremic patients analyzed here were higher than those in the general population of the Hisayama study based on multivariate analysis and adjusted by age and gender [17, 18]. Further investigations using the same assay conditions in one cohort are needed to determine whether ANGPTL2 levels in uremic populations are increased relative to healthy subjects

Prognostic staging of patients receiving hemodialysis

Survival tree analysis (figure S2) revealed that decreased levels of Cr (<9.7mg/dl at ages ≤69) or UA (≤7.5mg/dl at ages ≤69, and ≤6.3mg/dl at ages 70-80) were indicators of poor prognosis. Others have reported that high circulating Cr levels are associated with lowered risk of mortality in hemodialysis patients [22, 24, 25]. Circulating Cr levels reportedly reflect changes in muscle mass altered by imbalanced anabolic/catabolic metabolism in uremic individuals[25]. In addition, others report that high circulating UA levels are associated with lowered risk for mortality in hemodialysis patients and that those levels may reflect patient nutritional status [26-28]. Moreover, UA reportedly antagonizes adverse effects of uremic toxins such as indoxyl sulfate through antioxidant activity or by enhancing nitric oxide bioavailability in vascular endothelial cells [26, 29]. These reports are in agreement with our data, which indicates that decreased circulating Cr or UA levels are associated with higher all-cause mortality in hemodialysis patients. Next, survival tree analysis (figure S2), also revealed that increased CTR (>52.4% at ages 70-80) as an indicator of poor prognosis. Our current findings are supported by a previous report indicating that cardiomegaly accelerates patient mortality risk in uremic population [30, 31]. Finally, higher hs-CRP levels (>992.3 ng/ml at ages ≥81) or higher ferritin levels (>40 ng/ml at ages ≥81) were significant effectors

indicative of increased mortality risk, findings that coincided with previous reports showing that circulating CRP or ferritin levels were significantly associated with increased risk of mortality in uremic patients [23, 32, 33]. In the highest aged group (≥81), patients may be less tolerant of inflammatory stress due to either uremia or infection or to the presence of excess intracorporeal iron due to decreased iron bioavailability or excess iron loading.

Taken together, our prognostic staging of the uremic population indicates that prognostic risk factors differ across age groups, an outcome important to recognize when assessing mortality risk.

One limitation of our study is that we did not evaluate whether circulating ANGPTL2 levels predict cause-specific death, such as cardiovascular or cancer death, or death from infection. Moreover, we collected clinical data and assayed ANGPTL2 levels at baseline and did not evaluate an association between time-dependent changes in ANGPTL2 level and clinical outcomes. Further clinical cohort studies using larger sample sizes may be needed to assess uremic hemodialysis patients.

In summary, we demonstrate that circulating levels of the senescence-associated factor ANGPTL2 are significantly associated with mortality risk in patients receiving

hemodialysis who have a relatively benign prognostic profile. We conclude that ANGPTL2 could serve as a biomarker for progression of premature aging in the uremic population in all but the most advanced senescent phenotypes.

Disclosure

None.

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Supplementary information

Table S1. Ten of the highest-ranking valuables selected by the random forest methods for all-cause mortality in patients receiving maintenance hemodialysis.

Table S2. Hazard ratios (HR) for mortality of 9 groups of subjects based on survival tree analysis.

Table S3. Hazard ratio for mortality in each prognostic stage.

Figure S1. Recruitment and follow-up flow diagram.

Figure S2. Determination of prognostic stage of patients undergoing hemodialysis. Numbers in squares indicate groups derived from survival tree analysis (1 to 9), and square colors indicate prognostic stage grades.

Figure S3. Association between prognostic stages and circulating ANGPTL2 levels (n=395).

Figure S4. Martingale residual of combined survival analysis in the population including prognostic stages 1, 2, and 3 (n=354). Dark blue dots, martingale residuals. Red line, lowess smoothing of martingale residuals.

Supplementary methods

Clinical evaluation and laboratory testing

Supplementary information is available at Nephrology Dialysis Transplantation's website.

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Variable categories	Number	Percentage
Male gender	250	63.3
Current smoking	66	16.7
Obesity	57	14.4
Hypertension	325	82.3
Diabetes	162	41
Dyslipidemia	205	51.9
Cancer	32	8.1
Rheumatoid arthritis	5	1.3
PAD	85	21.5
Stroke	65	16.5
Statin	82	20.8
RAS inhibitor	211	53.4
Continuous variables	**Median**	**IQR**
Age (years)	65	(58, 74)
BMI ($kg \cdot m^{-2}$)	21.17	(19.2, 23.6)
systolic BP (mmHg)	148	(134, 161)
diastolic BP (mmHg)	77	(67, 87)
Total protein (g/dl)	6.6	(6.3, 7.0)
Albumin(g/dl)	3.8	(3.6, 4.0)
BUN (mg/dl)	60.7	(52.3, 71.3)
Creatinine (mg/dl)	11.09	(9.2, 12.5)
Uric acid (mg/dl)	7.8	(6.9, 8.7)
T-Cho (mg/dl)	151	(131, 172)
HDL-Cho (mg/dl)	45	(37, 57)
LDL-Cho (mg/dl)	78	(63, 94)
Triglyceride (mg/dl)	84	(59, 126)
aCa (mg/dl)	9.4	(8.9, 9.9)
IP (mg/dl)	5.3	(4.6, 6.2)
Ca x P $(mg \cdot dl^{-1})^2$	48.5	(42.2, 57.0)
Mg (mg/dl)	2.7	(2.5, 2.9)
AST (U/l)	13	(9, 17)
ALT (U/l)	10.0	(7, 13)
LDH (IU/l)	182	(163, 212)
GGT (IU/l)	19	(13, 28)
ALP (IU/l)	216	(171, 279)

T-Bil (mg/dl)	0.3	(0.3, 0.4)
Amylase (IU/l)	112	(87, 147)
CK (U/l)	86	(58, 132)
WBC (/μl)	5430	(4420, 6580)
Hb (g/dl)	10.7	(9.9, 11.4)
Plt (x10,000/μl)	15.9	(12.9, 19.5)
Fe (μg/dl)	60	(46, 79)
Ferritin (ng/ml)	42.6	(23.1, 92.9)
TSAT (%)	25	(18, 30)
whole PTH (pg/ml)	45	(24, 93)
hs-CRP (ng/ml)	706	(295, 1830)
CTR (%)	48.4	(45.0, 52.4)
Vintage (years)	5.8	(2.9, 12.2)
Dialysis time (hour)	4.5	(4.0, 5.0)
QB (ml/min)	200	(180, 200)
KT/V	1.44	(1.25, 1.62)
Increasing body weight (kg)	2.8	(2.1, 3.5)
PTA treatment frequency	0	(0, 0)

Table 1. Patient characteristics at baseline (n=395); PAD, peripheral artery disease; RAS inhibitor, renin angiotensin system inhibitor. IQR, inter quartile range; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; T-Cho, total cholesterol; HDL-Cho, high density lipoprotein cholesterol; LDL-Cho, low density lipoprotein cholesterol; aCa, adjusted calcium; IP, inorganic phosphorus; Mg, magnesium; AST, aspartate aminotransferase; ALT, alanine transaminase; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; T-Bil, total bilirubin; CK, creatine kinase; WBC, white blood cell; Hb, hemoglogin; Plt, platelets; Fe, iron; TSAT,

transferrin saturation; Whole-PTH, whole parathyroid hormone, Ca x P, the product of aCa and IP; Hs-CRP, high sensitivity C-reactive protein; CTR, cardio-thoracic ratio; QB, quantity of blood flow; KT/V, dialysis dose; PTA, percutaneous transluminal angioplasty.

Model	HR	95% CI	P
1	2.83	(1.54, 5.19)	0.001
2	2.32	(1.43, 3.75)	0.001
3	2.04	(1.10, 3.77)	0.023

Table 2. Circulating ANGPTL2 levels and mortality risk in hemodialysis patients (n=395). Hazard ratio of log(ANGPTLT2) for mortality, 95% CI, and P value are indicated. Model 1: Crude. Model 2: multivariate Cox proportional hazard model adjusted by age, sex, smoking habit, diabetes, hypertension, dyslipidemia, BMI, cancer, hemodialysis vintage, albumin, CTR, Ca x P and medical facility location. Model 3: model 2 plus hs-CRP. Hs-CRP and ANGPTL2 were transformed to natural-log values. HR, Hazard ratio; 95% CI, 95 percent confidence interval; P, probability; BMI, body mass index; CTR, cardio-thoracic ratio; Ca x P, a product of aCa and IP. hs-CRP, high sensitivity C-reactive protein; ANGPTL2, Angiopoietin-like protein 2.

Prognostic stage	Number at risk	Number of events	HR	95%CI	P	HR (BS)	95%CI (BS)
1	141	5	8.88	(5.35, 14.74)	<0.001	8.25	(1.61, 3.04)
2	168	31	1.72	(1.20, 2.49)	0.004	1.65	(1.21, 2.51)
3	45	25	6.56	(1.93, 22.26)	0.003	6.37	(2.07, 25.57)
(Combined stages 1, 2, and 3)	354	61	3.06	(1.86, 5.03)	<0.001	2.90	(1.72, 4.92)
4	41	33	1.47	(0.78, 2.79)	0.235	1.44	(0.78, 2.97)

Table 3. Relationship between circulating ANGPTL2 levels and hazard ratio for mortality at each prognostic stage of hemodialysis patients. The Cox proportional hazard model was used and adjusted by prognostic stage and medical facility location. (n=395). Combined analysis of stages 1, 2, and 3 were adjusted for prognostic stages and medical facility location (n=354). HR, hazard ratio; 95% CI, 95 percent confidence interval; P, probability; HR (BS), average HR estimated by bootstrap replication; 95%CI (BS), 95%CI estimated by bootstrap replication.

Stratum	Number at risk	Number of events	HR	95%CI	P
Age (years)					
<63	157	13	7.99	(3.55, 18.01)	<0.001
≥63	197	48	2.02	(0.76, 5.33)	0.157
Diabetes					
No	209	32	5.15	(3.19, 8.32)	<0.001
Yes	145	29	1.48	(0.74, 2.88)	0.278
Vintage (years)					
<6.2	177	30	3.99	(2.85, 5.58)	<0.001
≥6.2	177	31	2.17	(1.01, 4.68)	0.047

Table 4. Relationship between circulating ANGPTL2 levels and hazard ratio for mortality in each senescence-related category in patients receiving hemodialysis. Patients at stages 1, 2 and 3 were analyzed (n=354). The Cox proportional hazard model was used and adjusted by prognostic stage and medical facility location. ANGPTL2 levels were transformed to natural-log values. HR, Hazard ratio; 95% CI, 95 percent confidence interval; P, probability.

Figure Legends

Figure 1. Histogram showing circulating ANGPTL2 levels and the corresponding proportion of patients (%) receiving hemodialysis. Median, 3.3ng/ml; interquartile range, (2.5, 4.0).

Figure 2. Kaplan-Meier survival curve of patients receiving hemodialysis at each prognostic stage. Table below graph indicates the number of patients at risk in each prognostic stage. Blue line, stage 1; green line, stage 2; yellow line, stage 3; and red line, stage 4.

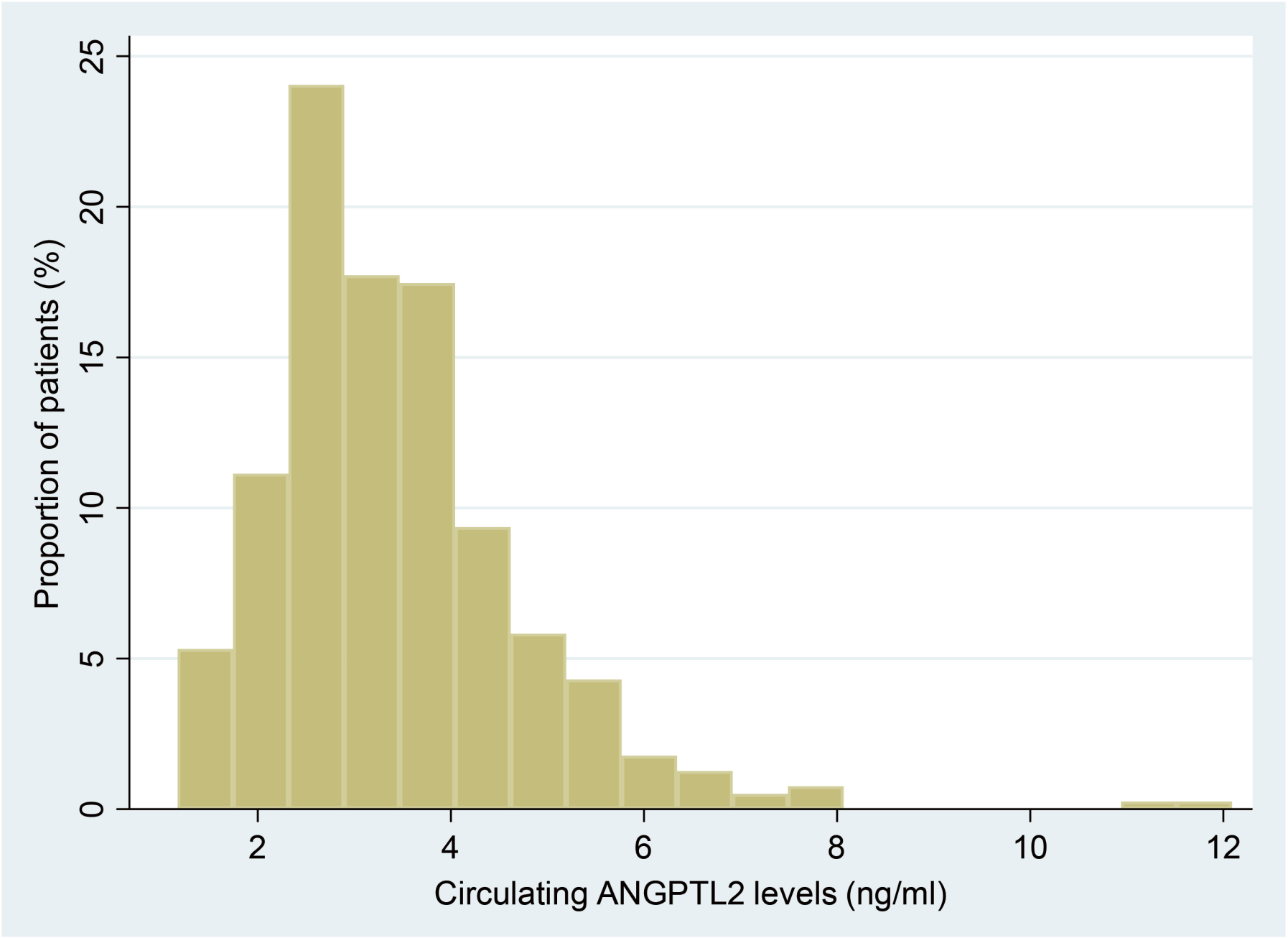

Figure 1. (Morinaga J *et al*.)

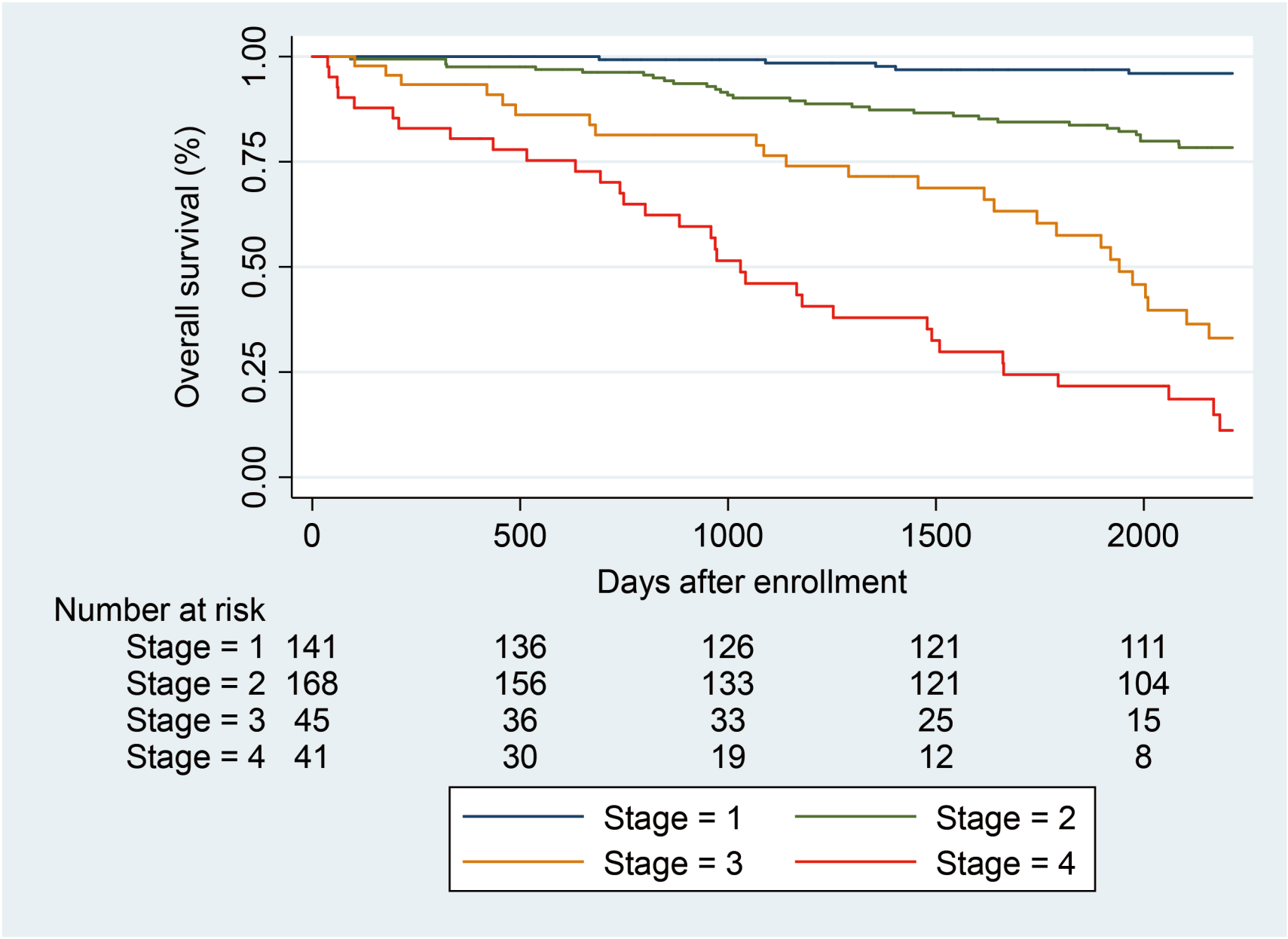

Figure 2. (Morinaga J *et al.*)