Serum and Tissue Levels of Advanced Glycation End Products and Risk of Mortality in Patients on Maintenance Hemodialysis

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Keywords

Skin autofluorescence · N^ε-carboxymethyl-lysine · All-cause mortality · Cardiovascular diseases mortality · Hemodialysis patients

Abstract

Background: The relation of tissue and circulating advanced glycation end products (AGEs) with mortality in hemodialysis (HD) patients remains inconclusive. We aimed to investigate the association of serum AGEs (CML) and tissue AGEs estimated by skin autofluorescence (SAF) with all-cause and cardiovascular disease (CVD) mortality, and examine the possible modifiers for the association in HD patients with by far the largest sample size in any similar studies. **Methods:** A

total of 1,634 HD patients were included from the China Cooperative Study on Dialysis (CCSD), a multicenter prospective cohort study. The primary and secondary outcomes were all-cause mortality and CVD mortality, respectively. **Results:** The median follow-up duration was 5.2 years. Overall, there was a positive relation of baseline SAF levels with the risk of all-cause mortality (per 1 AU increment, adjusted hazard ratio (HR), 1.30; 95% confidence interval (CI): 1.12, 1.50) and CVD mortality (per 1 AU increment, adjusted HR, 1.36; 95% CI: 1.14, 1.62). Moreover, a stronger positive association between baseline SAF (per 1 AU increment) and all-cause mortality was found in participants with shorter dialysis vin-

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tage, or lower C-reactive protein levels (Both p interactions <0.05). Nevertheless, there was no significant association between serum CML and the risk of mortality. **Conclusions:** In patients undergoing long-term HD, baseline SAF, but not serum CML, was significantly associated with the risk of all-cause and CVD death.

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Introduction

Hemodialysis (HD) is a life-sustaining treatment for patients with ESRD. HD patients have a significantly increased risk of mortality than the general population [1, 2]. The leading cause of mortality in HD patients is cardiovascular disease (CVD), which is associated with not only the traditional risk factors but also a number of nontraditional risk factors, such as anemia, parathyroid hormone, mineral bone disorders, and uremic toxins [3–5].

Advanced glycation end products (AGEs) have been identified as uremic toxins, formed by the nonenzymatic reaction (the Maillard reaction) between sugars and the free amino groups of proteins, lipids, or nucleic acids [6]. HD patients show markedly increased AGE levels due to decreased renal clearance and increased endogenous formation [7]. Accumulation of AGEs has been indicated as a mechanism underlying inflammation, oxidative stress, and structural tissue damage leading to vascular diseases or mortality [8].

However, results from previous prospective studies of circulating AGE levels in HD patients have been conflicting, ranging from a protective factor [9], no clear association [10], or a risk factor of mortality [11]. Most of these studies did not measure the tissue level of AGEs, a better mirror of AGE-dependent tissue damage [12]. The development of skin autofluorescence (SAF) provided a noninvasive, easy-to-operate, and inexpensive method for measurement of tissue AGE levels [13]. However, few studies have observed the level of serum and tissue AGEs simultaneously and separately analyzed their impacts on the risk of mortality in patients undergoing long-term dialysis. The present study, using data from a multicenter cohort of 1,634 HD patients with a median follow-up of 5.2 years, investigates the association of serum and tissue AGEs with all-cause and CVD mortality, and examines the possible modifiers for the association.

Materials and Methods

Study Population and Design

The current cohort study consisted of patients enrolled in the China Cooperative Study on Dialysis (CCSD). The baseline data of the CCSD had been reported previously [14–17]. In brief, the CCSD is performed in 9 of the largest dialysis centers (at least 200 HD patients in each center) in 6 cities of China (Beijing, Shanghai, Guangzhou, Hangzhou, Wuhan, and Xi'an). Eligible participants were men and women aged ≥ 18 years with ESRD undergoing dialysis between January 1, 2005 and December 1, 2010. A total of 2,183 HD patients were screened in the CCSD [16]. The current cohort study enrolled 1,634 eligible HD participants from the CCSD, followed up from July 2010 to February 2016.

Data Collection, Measurements, and Follow-Up

Baseline data used in the present study were derived from the database of the CCSD. All data were collected at enrollment on the bases of review of medical records by a group of experienced doctors and research nurses. The following data were collected: demographic data, underlying renal diseases, medication records, dialysis modality, dialysis program, and CVD, which was defined as the presence of clinically diagnosed ischemic heart disease, heart failure, and/or stroke after initiation of dialysis.

Blood pressure (BP) measurement was taken by using a sphygmomanometer before each of the 3 HD sessions, 3 times at 1-min intervals, all after 10 min of rest in a supine decubitus position. The mean of the 3 readings was calculated.

Participants were scheduled for a follow-up every 1–3 months in each center. At each follow-up visit, possible end point events were documented by trained research staff and physicians.

Hemodialysis Regimens

HD patients were dialyzed twice or thrice weekly with low-flux polysulphone or polyacrylamide dialyzer, either 1.5 or 1.7 m² (Fresenius, Germany; Gambro, Sweden; Nipro, Japan; B. Braun, Germany; Langsheng, China). All treatments were of 4- to 5-h duration with conventional glucose-free, bicarbonate-based dialysate containing 1.25–1.5 mM calcium, 2.0 mM potassium, and 138 mM sodium. Dialysate flow was 500 mL/min.

Laboratory Assays

Baseline fasting venous serum samples were collected prior to the HD sessions. Biochemical tests were performed using automatic clinical analyzers following the same standard protocol at each local dialysis center.

Determination of serum CML (a component of AGEs) was based on spectrofluorometric detection [18] at the core laboratory of Nanfang Hospital, Guangzhou, China. SAF was measured before the latest HD session follow-up by using a cutaneous autofluorescence device (AGE Reader; DiagnOptics Technologies, The Netherlands) [13]. The schematic diagram of SAF measurements is shown in online suppl. Fig. 1 (For all online suppl. material, see www.karger.com/doi/10.1159/000512385.) The values were compared with an age-matched non-CKD database contained within the device. The autofluorescence reader illuminates a skin surface of ~ 1 cm², guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation approximately 350 nm). Only light from the skin is measured with a spec-

trometer in the 300- to 600-nm range, using a 200-mm glass fiber (Farnell, Leeds, UK). All measurements were performed at room temperature in a semi-dark environment before dialysis. The non-dominant forearm rests on the device, and 3 readings, all taken within 1 cm of each other away from any areas of bruising or pigmentation, were averaged and recorded. A connected computer analyzed the level of autofluorescence and correlated that to know the normal range. Repeated autofluorescence measurements on 1 day and intraindividual seasonal variance showed an Altman error percentage of <6. The intra- and inter-day assay precision expressed as coefficients of variation for autofluorescence measurements were 2.5 and 4.6%, respectively.

Outcomes

All-cause mortality was the primary outcome, which included death due to any reason. The secondary outcome was CVD mortality, which included sudden cardiac death, stroke, myocardial infarction, heart failure, and death due to other known vascular causes. Evidence for death included death certificates from hospitals or reports from investigator visits.

Statistical Analysis

We assumed that the annual mortality rate of HD patients with low SAF levels was about 3%, with an alpha of 0.05, enrolled about 1,600 HD patients (high vs. low SAF: about 3:1) followed up for 5 years, would provide us >80% power to detect an effect of a hazard ratio (HR) of >1.5 between high and low SAF groups.

Baseline characteristics are presented as means $\pm$ standard deviations (SDs) or medians (interquartile range) and proportions for continuous and categorical variables, respectively. Statistical significance of differences in baseline characteristics was assessed in accordance with baseline SAF quartiles (<2.4, 2.4 to <2.9, 2.9 to <3.5, $\geq$ 3.5 AU) using ANOVA tests or χ^2 tests, accordingly.

The relation of baseline SAF levels with all-cause and CVD mortality was explored using thin plate regression splines in generalized additive models implemented by the R package *mgcv*. To evaluate and compare the impact of baseline SAF and CML on study outcomes, SAF and CML were first individually and then simultaneously entered into the Cox proportional hazard regression models without and with adjustment for study centers, age, sex, smoking status, BMI, systolic BP, dialysis vintage, albumin, hemoglobin, intact parathyroid hormone, phosphate, calcium, sodium, potassium, fasting glucose, total cholesterol, CVD status, and the usage of renin-angiotensin-aldosterone system inhibitors at baseline. Our analysis showed that there was no obvious multicollinearity among the variables (online suppl. Table 1). The Kaplan-Meier curves were used to visualize and compare the cumulative hazards for all-cause and CVD mortality by baseline SAF categories. In addition, possible modifications to the association of baseline SAF levels with all-cause mortality were evaluated by stratified analyses and interaction testing. We also tested whether SAF improved 3- and 4-year risk prediction by comparing the performance of risk prediction models with versus without SAF using R package *survcomp*.

A 2-tailed $p < 0.05$ was considered statistically significant in all analyses. R software, version 3.6.3 (<http://www.R-project.org/>), was used to perform all statistical analyses.

Results

Characteristics of Study Participants

As illustrated in the flowchart of the study participants (online suppl. Fig. 2), of the 1,835 participants in the follow-up study, a total of 1,634 HD patients without a switch to PD, kidney transplantation, or lost to follow-up during the follow-up period were included in the final analysis.

The mean SAF and serum CML levels of the patients were 3.0 ± 0.8 AU and 154.3 ± 43.0 μ mol/L, respectively. The mean age was 57.4 ± 14.6 years, with a mean dialysis vintage of 50.0 ± 48.4 months. Baseline characteristics of the patients by SAF quartiles (<2.4, 2.4 to <2.9, 2.9 to <3.5, $\geq$ 3.5 AU) and CML quartiles (<123.7, 123.7 to <151.1, 151.1 to <181.9, $\geq$ 181.9 μ mol/L) are presented in Table 1 and online suppl. Table 2, respectively. The univariate analysis showed that there were significant differences in a lot of variables by SAF quartiles. The multivariable analysis further found that there was a positive relation of SAF with age, dialysis vintage, and CVD status; and a negative association of SAF with DBP and HDL cholesterol (online suppl. Table 3).

Association between Baseline SAF and Study Outcomes

During a median follow-up of 5.2 years (interquartile range, 3.2–5.3), all-cause or CVD death occurred in 553 (33.8%) and 375 (22.9%) patients, respectively. Overall, there was a positive relationship of baseline SAF levels with the risk of all-cause mortality (per 1 AU increment, adjusted oddsHR, 1.30; 95% confidence interval [CI]: 1.12, 1.50) (Fig. 1a). When baseline SAF levels were assessed as quartiles, the adjusted HRs and 95% CI in the second, third, and fourth quartiles were 2.06 (1.35, 3.14), 2.14 (1.41, 3.24), and 2.49 (1.64, 3.79), respectively, when compared with quartile 1 (p for trend <0.001). Kaplan-Meier curves of the cumulative event rate of all-cause mortality for the SAF categories (<2.4 vs. $\geq$ 2.4 AU) are shown in online suppl. Figure 3a. Consistently, a significantly higher risk of all-cause mortality (adjusted HR, 2.20; 95% CI: 1.51, 3.22) was found in patients in quartiles 2–4 than those in quartile 1 (Table 2).

Similarly, there was a positive association between baseline SAF levels and CVD mortality (per 1 AU increment, adjusted HR, 1.36; 95% CI: 1.14, 1.62) (Fig. 1b). Accordingly, participants in quartiles 2–4 had a significantly higher risk of CVD mortality (adjusted HR, 2.12; 95% CI: 1.35, 3.35) than with those in quartile 1 (Table 2; online suppl. Fig. 3b). In addition, further adjustment for

Table 1. Baseline characteristics of study participants by SAF quartiles^a

Variable	SAF, AU				p value
	Q1 (<2.4)	Q2 (2.4 to <2.9)	Q3 (2.9 to <3.5)	Q4 (≥3.5)	
N	379	370	442	443	
Male, n (%)	214 (56.5)	221 (59.7)	240 (54.3)	234 (52.8)	0.225
Age, years	50.4±14.8	55.4±14.5	59.2±13.2	63.4±13.0	<0.001
Dialysis vintage, months	38.8±40.0	48.7±47.6	47.6±42.7	62.9±57.3	<0.001
Dialysis adequacy, kt/v ^b	1.6±0.4	1.5±0.4	1.5±0.4	1.5±0.4	0.199
BMI, kg/m ²	21.2±3.5	21.6±3.5	21.8±3.5	21.7±3.6	0.113
SBP, mm Hg	143.9±21.8	143.2±22.5	142.5±22.6	139.9±22.6	0.054
Diastolic BP, mm Hg	85.3±13.7	82.9±13.2	80.9±11.5	77.4±11.8	<0.001
Smoking, n (%)	29 (7.7)	49 (13.2)	58 (13.1)	61 (13.8)	0.028
CVD, n (%)	146 (38.5)	149 (40.3)	178 (40.3)	226 (51.0)	<0.001
Diabetes, n (%)	114 (30.1)	139 (37.6)	189 (42.8)	210 (47.4)	<0.001
Hypertension, n (%)	337 (88.9)	344 (93.0)	404 (91.4)	400 (90.3)	0.257
<i>Laboratory results</i>					
TC, mmol/L	4.1±1.0	4.1±1.1	4.2±1.0	4.1±1.1	0.592
Triglyceride, mmol/L	1.4±1.0	1.6±1.2	1.6±1.1	1.7±1.2	0.011
HDL cholesterol, mmol/L	1.1±0.4	1.1±0.4	1.1±0.4	1.1±0.4	0.082
FG, mmol/L	5.0±1.8	5.6±2.4	5.8±2.4	5.8±2.2	<0.001
Cr, μmol/L	938.5±376.6	870.5±348.4	793.3±346.5	781.5±333.6	<0.001
Uric acid, μmol/L	422.0±151.6	403.8±152.1	380.3±153.7	377.0±153.5	<0.001
Calcium, mmol/L	2.2±0.3	2.2±0.3	2.3±0.3	2.3±0.3	<0.001
Phosphate, mmol/L	2.0±0.7	2.0±0.7	1.9±0.6	1.8±0.7	<0.001
Sodium, mmol/L	140.8±3.9	139.8±3.4	139.5±3.9	139.2±4.1	<0.001
Potassium, mmol/L	5.0±0.8	4.9±0.8	5.0±0.9	4.9±0.8	0.230
Albumin, g/L	39.9±6.0	39.7±6.6	39.1±6.7	38.8±6.4	0.055
Hemoglobin, g/L	99.6 (21.3)	104.4 (20.9)	105.2 (19.1)	104.1 (19.2)	<0.001
CRP, mg/L	3.5 (2.0–9.7)	3.9 (2.1–12.3)	3.7 (2.2–9.7)	5.3 (2.9–12.2)	0.009
iPTH, pg/mL	284.0 (136.4–562.7)	255.5 (128.2–496.2)	238.6 (117.4–491.1)	252.1 (127.9–586.4)	0.378
SAF, AU	1.9±0.3	2.6±0.1	3.1±0.2	4.0±0.5	<0.001
<i>Medication use, n (%)</i>					
RAAS inhibitors	173 (45.6)	170 (45.9)	234 (52.9)	218 (49.2)	0.124
Glucose-lowering drugs	28 (7.4)	47 (12.7)	66 (14.9)	95 (21.4)	<0.001
Lipid-lowering drugs	24 (6.3)	30 (8.1)	49 (11.1)	60 (13.5)	0.003
Phosphorus binder	124 (32.7)	157 (42.4)	194 (43.9)	181 (40.9)	0.007
Erythropoietin	302 (79.7)	312 (84.3)	372 (84.2)	363 (81.9)	0.274

BP, blood pressure; SBP, systolic blood pressure; iPTH, intact parathyroid hormone; HDL, high-density lipoprotein; RAAS, renin-angiotensin-aldosterone system; SAF, skin autofluorescence; CVD, cardiovascular disease; FG, fasting glucose; TC, total cholesterol; CRP, C-reactive protein. ^a Continuous variables are presented as mean±SD, or median (25th–75th percentile). ^b kt/v was per session in HD.

baseline serum CML (online suppl. Table 4), or baseline Cr and the use of glucose-lowering drugs, lipid-lowering drugs, and phosphorus binder (online suppl. Table 5), or uric acid (online suppl. Table 6), or dialysis adequacy (online suppl. Table 7) did not substantially change the results.

Association between Baseline Serum CML and Study Outcomes

Overall, there was no significant relation of baseline serum CML with all-cause mortality (per 1 SD increment, adjusted HR, 1.03; 95% CI: 0.88, 1.20) and CVD mortality (per 1 SD increment, adjusted HR, 0.91; 95% CI: 0.74, 1.10) (online suppl. Table 8).

Fig. 1. Relation of baseline SAF with the risk of all-cause mortality (**a**) and CVD mortality (**b**) in HD patients. Adjusted for study center, age, sex, smoking status, BMI, SBP, dialysis vintage, albumin, hemoglobin, iPTH, phosphate, calcium, sodium, potassium, FG, TC, CVD status, and the usage of RAAS inhibitors. HR, hazard

ratio; HD, hemodialysis; SAF, skin autofluorescence; CVD, cardiovascular disease; SBP, systolic blood pressure; iPTH, intact parathyroid hormone; FG, fasting glucose; TC, total cholesterol; RAAS, renin-angiotensin-aldosterone system.

Stratified Analyses for the Association of Baseline SAF with All-Cause Mortality

We further performed stratified analyses to assess the relation of baseline SAF (per 1 AU increment) with all-cause mortality in various subgroups. A stronger positive association between baseline SAF and all-cause mortality was found in participants with dialysis vintage <34.0 months (median) (adjusted HR, 1.51; 95% CI: 1.25, 1.82 vs. ≥ 34.0 months: adjusted HR, 1.13; 95% CI: 0.92, 1.38; p interaction = 0.028), or with a C-reactive protein (CRP) level <11.1 mg/L (quartile 3) (adjusted HR, 1.44; 95% CI: 1.17, 1.76 vs. ≥ 11.1 mg/L: adjusted HR, 1.04; 95% CI: 0.80, 1.35; p interaction = 0.042) (Fig. 2). However, other variables at baseline, including sex, age, dialysis adequacy, systolic BP, diabetes, total cholesterol, albumin, and concomitant CVD, did not significantly modify the association between SAF and the risk of all-cause mortality (all p interactions >0.05) (Fig. 2).

Assessment of Discrimination and Model Accuracy

We next compared whether the addition of SAF improved model prediction for the all-cause and CVD mortality. The addition of SAF to the fully adjusted clinical model significantly improved the C-statistic for the 4-year all-cause mortality from 0.72 (95% CI: 0.69, 0.74) to 0.73

(95% CI: 0.70, 0.76, $p = 0.009$) and improved the prediction of CVD mortality with C-statistics from 0.73 (95% CI: 0.70, 0.76) to 0.74 (95% CI: 0.70, 0.77, $p = 0.044$). Similar results were found for the 3-year all-cause and CVD mortality (online suppl. Table 9). However, the addition of SAF to the clinical model only resulted in a slightly greater improvement in risk reclassification (online suppl. Table 10).

Discussion

Our study demonstrated that there was a positive relation of baseline tissue AGE levels with all-cause and CVD mortality, independent of circulating AGEs and other important confounders, among patients undergoing long-term HD. Moreover, our study expanded the results of previous studies by demonstrating that the positive relation between tissue AGEs and all-cause mortality was more pronounced in patients with shorter dialysis vintage or lower CRP levels, suggesting that the accumulative level of tissue AGEs might be an early predictor for the risk of all-cause or CV death.

Previous studies have linked the tissue AGEs with all-cause and/or CVD mortality in HD patients, but the re-

Table 2. Association between SAF and study outcomes

Skin AGE, AU	N	Events (%)	Crude model		Adjusted model ^a	
			HR (95% CI)	p value	HR (95% CI)	p value
<i>All-cause mortality</i>						
Continuous	1,634	553 (33.8)	1.51 (1.38, 1.67)	<0.001	1.30 (1.12, 1.50)	<0.001
Quartiles						
Q1 (<2.4)	379	67 (17.7)	1.00 (ref.)		1.00 (ref.)	
Q2 (2.4 to <2.9)	370	122 (33.0)	2.01 (1.50, 2.71)	<0.001	2.06 (1.35, 3.14)	<0.001
Q3 (2.9 to <3.5)	442	163 (36.9)	2.29 (1.72, 3.04)	<0.001	2.14 (1.41, 3.24)	<0.001
Q4 (≥3.5)	443	201 (45.4)	3.02 (2.29, 3.99)	<0.001	2.49 (1.64, 3.79)	<0.001
p for trend			<0.001		<0.001	
Categories						
Q1 (<2.4)	379	67 (17.7)	1.00 (ref.)		1.00 (ref.)	
Q2–4 (≥2.4)	1,255	486 (38.7)	2.45 (1.90, 3.16)	<0.001	2.20 (1.51, 3.22)	<0.001
<i>CVD mortality</i>						
Continuous	1,634	375 (22.9)	1.50 (1.34, 1.69)	<0.001	1.36 (1.14, 1.62)	<0.001
Quartiles						
Q1 (<2.4)	379	46 (12.1)	1.00 (ref.)		1.00 (ref.)	
Q2 (2.4 to <2.9)	370	84 (22.7)	2.02 (1.41, 2.90)	<0.001	1.88 (1.13, 3.14)	0.015
Q3 (2.9 to <3.5)	442	112 (25.3)	2.29 (1.62, 3.23)	<0.001	2.17 (1.32, 3.58)	0.002
Q4 (≥3.5)	443	133 (30.0)	2.92 (2.08, 4.08)	<0.001	2.43 (1.46, 4.04)	<0.001
p for trend			<0.001		0.001	
Categories						
Q1 (<2.4)	379	46 (12.1)	1.00 (ref.)		1.00 (ref.)	
Q2–4 (≥2.4)	1,255	329 (26.2)	2.42 (1.77, 3.29)	<0.001	2.12 (1.35, 3.35)	0.001

SAF, skin autofluorescence; iPTH, intact parathyroid hormone; SBP, systolic blood pressure; FG, fasting glucose; CVD, cardiovascular disease; TC, total cholesterol; RAAS, renin-angiotensin-aldosterone system; HR, hazard ratio; CI, confidence interval; AGE, advanced glycation end product. ^a Adjusted for study centers, age, sex, smoking status, SBP, dialysis vintage, albumin, hemoglobin, iPTH, phosphate, calcium, sodium, potassium, FG, TC, CVD status, and the usage of RAAS inhibitors.

ported results are inconsistent [19–24]. Some studies suggest a positive association between SAF levels and all-cause [19–23] or CVD mortality [19, 20, 24], while others find no significant impacts of tissue AGEs on CVD mortality [21, 22]. A recent study [25] in patients with CKD stage 5 indicates that the skin AGE level is not associated with all-cause mortality or CVD mortality in the fully adjusted models. Of note, all the previous studies had a smaller sample size ($n = 105$ – 332) [19–25] and could be defined as exploratory. To overcome the problem, a meta-analysis [26] was conducted and showed that higher SAF levels were associated with the risk of CVD (RR, 1.97; 95% CI: 1.11–3.49) and all-cause mortality (RR, 2.08; 95% CI: 1.41–3.06) in HD patients. However, the obvious heterogeneity across the studies (I -square = 62.4%; $p = 0.046$) in the meta-analysis suggests that the results may be dominated by the studies with relatively larger sample size and are unreliable. These results call for a large-scale cohort

study to further confirm the association between tissue AGEs and the risk of mortality in HD patients.

Our study provided a chance to evaluate the dose-response relation of SAF levels with mortality in HD patients with by far the largest sample size ($n = 1,634$) in many similar studies and included a comprehensive adjustment and stratified analysis for the important confounders. In the current study, the annual mortality rate in low-SAF patients was 3.4%, and a sample size of 1,634 (high vs. low SAF levels: 3:1), with a type I error rate of 5%, provide us >90% power to detect an effect of an HR of 2.2 (high vs. low SAF levels) during the 5.2-year follow-up.

Our study provided some new insights. First, we demonstrated that higher SAF associated with increased all-cause and CVD mortality in HD patients, independent of serum CML and other traditional or suspected risk factors. Consistently, previous studies have reported that tis-

Fig. 2. Relation of baseline SAF (per 1 AU) with the risk of all-cause mortality in various groups. ^aAdjusted, if not stratified, for study center, age, sex, smoking status, BMI, SBP, dialysis vintage, albumin, hemoglobin, iPTH, phosphate, calcium, sodium, potassium, FG, TC, CVD status, and the usage of RAAS inhibitors. ^bDiabetes

was defined as an FG ≥ 7.0 mmol/L or using of glucose-lowering drugs or having history of diabetes. SAF, skin autofluorescence; CVD, cardiovascular disease; SBP, systolic blood pressure; iPTH, intact parathyroid hormone; FG, fasting glucose; TC, total cholesterol; RAAS, renin-angiotensin-aldosterone system.

sue, but not circulating, AGEs were related to cardiovascular surrogate, such as tissue velocity imaging on echocardiography [27] and low circulating endothelial progenitor cells [28]. Second, our results showed that dialysis vintage and CRP levels significantly modified the association between tissue AGEs and the risk of mortality among HD patients. A stronger association was found in those with shorter dialysis vintage or lower CRP levels. It has been recognized that the mortality-related confounders would increase as the increment of dialysis vintage and inflammatory condition. It is possible that the detrimental effect of tissue AGEs accumulation may be affected by some unidentified confounders. Our studies underscore the importance of measuring the SAF in the early stage of dialysis and suggest that the optimal control of

tissue AGE accumulation may be a strategy for improving the outcomes in HD patients.

The potential mechanisms by which tissue accumulation of AGEs increases mortality risk are unclear, but it is biologically plausible. Accumulation of AGEs may lead to cross-links between structural proteins including collagen and elastin in arterial walls, resulting in arterial stiffness [29]. AGEs bind to its receptor (RAGE), alter signaling cascades, and provoke endothelial dysfunction [30], inflammatory responses, and oxidative stress [31, 32]. Consistently, higher SAF has been reported to be independently associated with arterial stiffness [33] and endothelial dysfunction determined by flow-mediated dilation [34].

The current study has some limitations. First, although the regression models were adjusted for a broad array of covariates, residual confounding from unmeasured factors cannot be excluded. Second, our analyses were based on a single baseline measurement of SAF and CML and therefore may not reflect levels over a longer period. Third, since SAF measurements had only been validated in subjects with Fitzpatrick skin types 1–4, the results of the present study may not generalize to patients with other skin types. In addition, skin disorders, such as pallor, hyperpigmentation, and xerosis, are common and diverse in HD patients [35–37]. However, we did not collect the detailed information about those skin disorders in the current study. Therefore, although corrections using differences in skin reflectance may resolve confounding effects of skin pigmentation on SAF [38], we could not have a detailed analysis to evaluate the possible effect of the skin disorders on the SAF measurements. Fourth, we did not have available data on BUN, glycohemoglobin, and glycated albumin. Finally, due to the modestly improved C-statistics, NRI and IDI, the predictive performance and the clinical usefulness of measuring SAF should be further evaluated and confirmed in more studies. In summary, the tissue level of AGEs measured by SAF was associated with the risk of all-cause and CVD mortality among HD patients, especially in those with shorter dialysis vintage or lower CRP levels.

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Statement of Ethics

Our study was approved by the local Ethics Committee in each center, and all participants provided written informed consent.

Conflict of Interest Statement

The authors declare that they have no competing interests.

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Author Contributions

Study concept and design: F.F.H. and J.J. Conduct of study: F.F.H., J.J., J.C., X.Y., C.M., F.X., W.S., W.Z., X.L., S.S., P.Z., Y.Z., Y.Z., S.L., Z.Z., Q.L., Y.Y., J.T., and W.L. Data collection and analysis: J.J., Y.Z., and X.Q. Drafting of the manuscript: J.J., Y.Z., and X.Q. Critical review and revision of the manuscript: F.F.H.

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