Marked Elevation of Plasma Levels of Oxidative Stress-Responsive Apoptosis-Inducing Protein in Dialysis Patients

To the Editor: Cardiovascular injury is known to play a critical role in the morbidity and mortality associated with chronic kidney disease (CKD), especially in end-stage renal disease (ESRD), such as in dialysis patients. Although oxidative stress, rather than traditional cardiovascular risk factors such as diabetes, hypertension, hypercholesterolemia, and smoking, has been implicated in the mechanisms of cardiotoxicity in CKD,[1] the precise mechanism remains unclear. Recently, we identified an apoptosis-inducing humoral factor, in a conditioned medium from cardiac myocytes subjected to hypoxia/reoxygenation, to be a tyrosine-sulfated and more hypusinated secreted form of eukaryotic translation initiation factor 5A (eIF5A).[2] We found that eIF5A undergoes 69th tyrosine-sulfation in the *trans*-Golgi and is rapidly secreted from cardiac myocytes in response to hypoxia/reoxygenation, and then induces apoptosis by acting as a pro-apoptotic ligand. The apoptosis of cardiac myocytes induced by hypoxia/reoxygenation was suppressed by anti-eIF5A neutralizing monoclonal antibodies *in vitro*. Myocardial ischemia/reperfusion (but not ischemia only) rapidly and markedly increased plasma levels of eIF5A, which returned to the control levels within 60 minutes. Treatment with anti-eIF5A neutralizing monoclonal antibodies significantly reduced myocardial injury. These results demonstrated that a novel, posttranslationally modified, secreted form of eIF5A is a specific biomarker and a critical therapeutic target for oxidative stress—induced cell injury. We named this novel tyrosine-sulfated secreted form of eIF5A, oxidative stress-responsive apoptosis-inducing protein (ORAIP).[2] We confirmed that ORAIP (molecular weight 17 kD, isoelectric point 5.4) is specifically secreted in response to the oxidative stresses including ischemia/reperfusion, hypoxia/reoxygenation, ultraviolet-irradiation, ionizing radiation, cold/warm-stress (heat shock), and blood acidification,[2,3] then acts as a pro-apoptotic ligand to induce apoptosis of target cells such as cardiac myocytes. To investigate the roles of ORAIP in the oxidative stress—induced cytotoxicity in ESRD, we analyzed the plasma levels of ORAIP in ESRD patients just before and after dialysis. This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association, and was approved by the Institutional Ethical Committees. All patients and control subjects gave written informed consent after full explanation of the purpose, nature, and risks of all procedures used.

A total of 62 ESRD (dialysis) patients (37 male and 25 female [male/female = 1.48]), with a mean (± SE) age of 72.05 ± 1.37 years, and 40 age- and sex-matched control subjects without apparent CKD (24 male and 16 female [male/female = 1.50]), aged 70.55 ± 1.40 years, were studied. The characteristics of the ESRD (dialysis) and control groups are summarized in Table 1. The causative diseases of ESRD (dialysis) patients were (diabetic nephropathy, 24 cases; chronic glomerulo nephritis, 19; nephrosclerosis, 10; polycystic kidney diease, 2; IgA nephropathy, 2; focal glomerulosclerosis, 2; membranoproliferative glomerulonephritis, 1; chronic pyelonephritis, 1; and postoperative renal cell cancer, 1). Plasma ORAIP levels were analyzed by the sandwich enzyme-linked immunosorbent assay using blockingless type plates (Sumitomo Bakelite Co., Ltd, Tokyo, Japan) as described previously.[2]

In dialysis patients, plasma blood urea nitrogen, creatinine, and uric acid levels (mean ± SE) were markedly decreased by dialysis (from 62.91 ± 2.15 mg/dl to 19.39 ± 0.80 mg/dl; from 9.80 ± 0.37 mg/dl to 3.65 ± 0.16 mg/dl; and from 6.92 ± 0.13 to 2.02 ± 0.07 mg/dl, respectively. In contrast, plasma ORAIP levels before dialysis (mean ± SE 93.6 ± 5.1 ng/ml), which were markedly elevated as compared with those of control subjects (6.6 ± 1.5 ng/ml), significantly increased after dialysis (98.5 ± 5.7 ng/ml, $P = 0.0122$, paired t test) (Figure 1). This suggests that ORAIP may be somewhat concentrated but not eliminated by dialysis. To investigate the effects of marked elevation of plasma ORAIP levels on cardiovascular injury, we analyzed plasma levels of cardiac troponin T and brain natriuretic peptide (BNP). In all dialysis patients, plasma cardiac troponin T levels (mean ± SE, 67.9 ± 6.6 pg/ml) were elevated; however, there was no significant correlation ($r = 0.0945$, $P = 0.4651$) between plasma levels of ORAIP and cardiac troponin T (Figure 2A). In most dialysis patients, plasma BNP levels (mean ± SE, 164.7 ± 22.3 pg/ml) were markedly elevated; however, there was no significant correlation ($r = 0.1353$, $P = 0.2944$) between plasma levels of ORAIP and BNP (Figure 2B). No significant correlations

Table 1. Characteristics of the CKD patient group and control group

	ESRD patients	Controls
n	62	40
Sex (male/female)	37/25 (1.48[a])	24/16 (1.50[a])
Age (yr)	72.05 ± 1.37	70.55 ± 1.40
Smoking (n)	24 (38.7%)	12 (30.0%)
Hypertension (n)	54 (87.1%)	27 (67.5%)
Diabetes mellitus (n)	26 (41.9%)	27 (67.5%)
Dyslipidemia (n)	20 (32.3%)	20 (50.0%)
Dialysis vintage (yr)	7.40 ± 0.95	
Dialysis time (h)	4.03 ± 0.03	
Removal amount (L)	2.32 ± 0.09	
Ultrafiltration rates (L/h)	0.58 ± 0.02	
Kt/V	1.40 ± 0.03	

CKD, chronic kidney disease; ESRD, end-stage renal disease.
Values expressed as (mean ± SE) or as numbers (%) unless otherwise specified.
[a]Male-to-female ratio.

were found between plasma levels of ORAIP and those of blood urea nitrogen ($r = -0.190$, $P = 0.139$), creatinine ($r = -0.111$, $P = 0.390$), and uric acid ($r = -0.078$, $P = 0.548$).

In the first report investigating the plasma levels of ORAIP in human samples, we have demonstrated that plasma levels of ORAIP were markedly elevated in dialysis patients and that ORAIP could not be eliminated by dialysis. From our previous *in vitro* and *in vivo* data in an animal model,[2] it is strongly

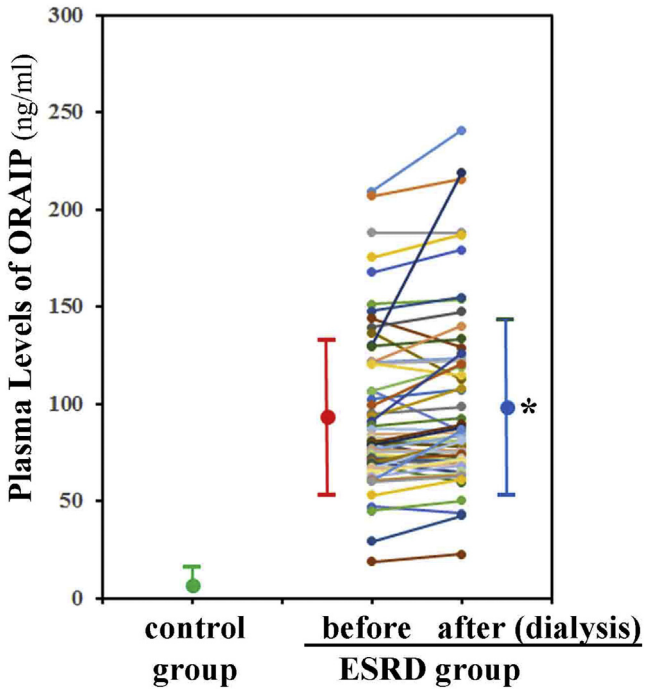

Figure 1. Plasma levels of oxidative stress-responsive apoptosis-inducing protein (ORAIP) in control subjects and chronic kidney disease (CKD) patients before and after dialysis. Plasma levels of ORAIP (mean ± SD) in control subjects, and individual values and (mean ± SD) of plasma levels of ORAIP in CKD patients before and after dialysis, are shown. *$P = 0.0122$ compared with before dialysis, as determined by a paired t test.

suggested that chronically elevated plasma levels of ORAIP contribute, at least in part, to myocardial injury in these patients. Other cardiotoxic factors such as oxidized low-density lipoprotein, reactive oxygen species, parathyroid hormone, anemia, and others are known to be involved in the myocardial injury in CKD and ESRD, and we found that there were no significant correlations between the plasma levels of ORAIP and serum levels of parathyroid hormone as well as anemia, suggesting that ORAIP contributes to myocardial injury independently of other factors. Because numerous factors may contribute to myocardial injury, the absence of a significant positive correlation between plasma levels of ORAIP and cardiac troponin T does not exclude the possibility that ORAIP may contribute to the myocardial injury involved in dialysis patients, which may in turn, at least in part, exacerbate heart failure associated with these patients, resulting in the elevation of plasma BNP levels. Because we also found that ORAIP can induce apoptotic signaling in skeletal muscle cells,[2] it is suggested that elevated plasma levels of ORAIP affect skeletal muscles as well as cardiac muscle, and may contribute, at least in part, to sarcopenia in ESRD patients.[4] Oxidative stress has been implicated in the pathogenesis in dialysis patients,[5–7] and it was reported that inflammatory status and duration of dialysis treatment are the most important factors relating to the oxidative stress involved.[8] Dialysis therapies are known to enhance serum levels of cytokines as well as other uremic toxins, although the mechanisms have been controversial.[9] Nguyen *et al.*[10] reported that hemodialysis membrane induced activation of phagocytes that produce reactive oxygen species. Thus, oxidative stress is known to be induced by the dialysis procedure itself, and this may contribute in part to the increase in plasma ORAIP levels after dialysis. Xanthine oxidase is an enzyme involved in purine metabolism and also produces reactive oxygen species. Recently, it was reported that xanthine oxidase activity, but not uric acid levels, was an independent predictor of cardiovascular events in CKD and hemodialysis patients.[11] This suggests that oxidative stress induced by xanthine oxidase causes cardiovascular injury and supports that elevated levels of plasma ORAIP induced by oxidative stress mediate cardiovascular injury in ESRD patients. Because plasma ORAIP levels did not correlate with blood urea nitrogen, creatinine, and uric acid levels, ORAIP may be an independent biomarker of cardiovascular injury but not renal injury in ESRD patients. Although the primary mechanism of oxidative stress generation in ESRD is uncertain, the elevated levels of ORAIP,

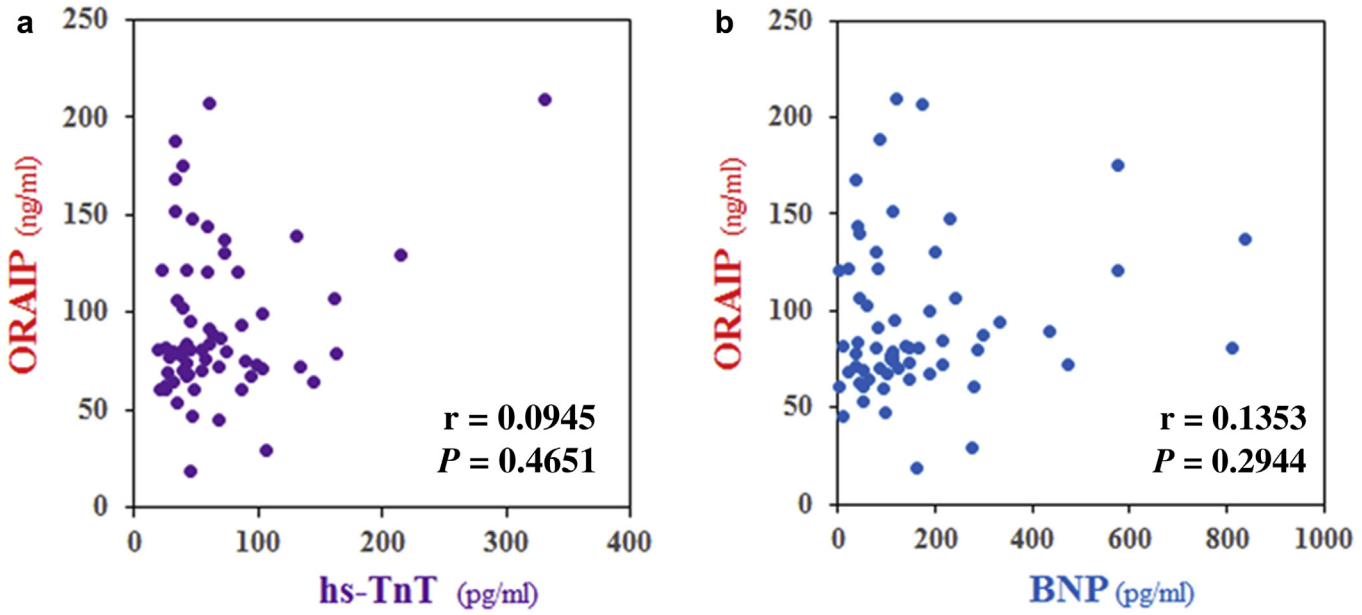

Figure 2. Correlation between plasma levels of oxidative stress-responsive apoptosis inducing protein (ORAIP) and biomarkers for cardiac injury. (a) Correlation between plasma levels of ORAIP and those of cardiac troponin T (hs-TnT). There was no significant correlation ($r = 0.0945$, $P = 0.4651$). (b) Correlation between plasma levels of ORAIP and those of brain natriuretic peptide (BNP). There was no significant correlation ($r = 0.1353$, $P = 0.2944$).

induced by oxidative stress, may cause renal microvascular injury, resulting in the progression of ESRD. Our findings warrant elimination of plasma ORAIP with a neutralizing antibody against ORAIP[2] to protect against cardiovascular injury and sarcopenia in dialysis patients.

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DISCLOSURE

All the authors declared no competing interests.

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Needle Size and the Risk of Kidney Biopsy Bleeding Complications

To the Editor: The percutaneous renal biopsy is an essential tool in the diagnosis and evaluation of kidney disease. Most renal biopsies are performed under direct ultrasound guidance with automated biopsy needles, techniques that have substantially reduced the risks of the procedure.[1–3] However, the kidney biopsy still carries a considerable risk of bleeding complications. Although certain risk factors for bleeding, including hypertension, acute kidney injury, female sex, and older age, have been well documented,[4] the association between the biopsy needle size and the rate of bleeding complications is unclear. Major complications, including hemorrhage requiring transfusion, angiographic intervention, nephrectomy, or death, have been reported in only around 2%–8% of renal biopsies, whereas minor complications, such as hematoma, are seen in anywhere from 17% to 33%.[4,5] Hematoma formation found on renal ultrasound is a direct assessment of post-biopsy bleeding and may be a more sensitive measure of bleeding than overt anemia or hemodynamic instability. At our institution, post-biopsy ultrasounds are routinely obtained as standard of care after kidney biopsy. We therefore conducted a retrospective cohort study of patients who underwent kidney biopsy, using post-biopsy hematoma as the endpoint of interest when comparing 14G versus 16G needles.

The study was conducted at Brigham and Women's Hospital in Boston, MA, between August 2014 and January 2016. We captured all biopsies that were performed under the supervision of a single nephrology attending, allowing for minimal variation in how procedures in our cohort were supervised and performed. The biopsies captured comprise the large majority of the ultrasound-guided renal biopsies conducted during the period of the study.

During the period of the study, the standard of care switched from the use of 14G to 16G needles, based on anecdotal observations of excessive numbers of hematomas noted in the 14G group. Spring-loaded, automated needles were used for all biopsies. All biopsies were conducted by or under the supervision of the same nephrology attending, and all patients were monitored for at least 6 hours after biopsy. A renal pathologist confirmed the adequacy of the sample at the time of the biopsy. The presence of a hematoma was ascertained by immediate post-biopsy ultrasound. We used electronic health record databases to capture demographic and clinical data. The outcome of interest was the occurrence of a post-biopsy hematoma. The secondary outcome was the change in hemoglobin concentration before and 6 hours after biopsy.

We used a χ^2 or *t*-test to compare baseline characteristics between patients with 16G and 14G needles. We used multivariate logistic regression to test the association between the needle size and the occurrence of a post-biopsy hematoma adjusted for clinical and demographic characteristics. We selected covariates that were previously shown to be associated with an increased risk of bleeding after biopsy including age, sex, needle size, platelet count, systolic blood pressure, and estimated glomerular filtration rate. We additionally adjusted for fellow performance of biopsies and the number of passes. Finally, we adjusted for the presence of >40% fibrosis on the biopsy as this was associated with a risk of hematoma in the univariate analysis. We used R, version 3.2.2, for all statistical analyses and considered $P < 0.05$ to be statistically significant.

Our cohort consisted of 86 patients with a mean age of 56.5 ± 16.9 years. Baseline characteristics, stratified by needle size and by the occurrence of a hematoma, are shown in Table 1. A 14G biopsy needle was used in 44 (51%) of patients and a 16G biopsy needle was used in the remaining patients. There were a similar proportion of patients who had inpatient biopsies in the 14G group compared with the 16G group (34% vs. 43%, respectively, $P = 0.54$). More passes were performed to obtain adequate samples in the 14G group compared with the 16G group (2.7 vs. 2.2, respectively, $P = 0.003$). There was no effect of the number of passes on the risk of hematoma ($P = 0.49$).

Hematoma formation was more frequent among individuals who underwent kidney biopsy using 14G needles compared with 16G needles (41% vs. 17%, respectively, $P = 0.03$). There was no difference in the diagnostic yield of glomeruli between the 2 groups (43 ± 21.6 vs. 37 ± 12.3 glomeruli, respectively, $P = 0.13$). There were no patients in whom the tissue obtained was inadequate to make a diagnosis. In a logistic regression analysis, the use of a 14G needle was associated with a significantly higher risk of hematoma (odds ratio 5.72, 95% confidence interval 1.54–25.7, $P = 0.01$) after multivariable adjustment for