Table 3. Adjusted food-group scores for each cluster (JDOPPS data).

Cluster	*n*	Food-group score		
		Vegetables	Fish	Meat
Well-balanced	666 49.2%	0.297 (0.460)	0.216 (0.936)	0.319 (0.874)
Unbalanced	189 14.0%	1.522 (0.454)	0.528 (0.809)	0.315 (0.838)
Other	500 36.9%	−0.971 (0.643)	−0.488 (0.945)	−0.544 (0.980)

Each food-group score was adjusted for total daily energy intake by the residual method [20]. Values in parentheses are standard deviations.

doi:10.1371/journal.pone.0116677.t003

Dietary patterns in hemodialysis patients

Cluster analysis of the adjusted food-group scores revealed three clusters, which we call (1) "well-balanced diet", (2) "unbalanced diet," and (3) "other diet" (Table 3). Patients in the first of those three clusters, i.e. those whose diet was well-balanced, were those who ate approximately equal amounts of food from the meat, fish, and vegetable groups. Almost half of the JDOPPS patients had a well-balanced diet (49.2%). Patients in the second of the three clusters, i.e. those whose diet was unbalanced, were those who ate a much larger amount from the vegetable group than from the meat group and the fish group. They amounted to 14% of the JDOPPS patients.

Fig. 1 shows the amounts of micronutrients for each cluster of JDOPPS patients. According to clinical guidelines, protein intake was within the prescribed range among those who ate a well-balanced diet, too high among those who ate an unbalanced diet, and too low among the others. [1] The mean salt intake was more than 6 g/day in all groups, and was highest among those who ate an unbalanced diet. Potassium intake was within the prescribed range among those who ate a well-balanced diet, too high among those who ate an unbalanced diet, and too low among the others. Phosphorus intake was similar to potassium intake.

Patient characteristics by dietary pattern

Table 4 shows characteristics of the JDOPPS patients, stratified by the three dietary patterns. Patients who ate an unbalanced diet were older than those who ate a well-balanced diet, and fewer of them were male. Total daily energy intake, protein intake, salt intake, and potassium intake were highest among those whose diet was unbalanced.

Association between dietary pattern and clinical outcomes in hemodialysis patients

Table 5 shows associations between dietary patterns and the composite outcome. In Model 1, which included adjustments for age, gender, and dialysis duration, the unbalanced diet was associated with a higher event rate than the well-balanced diet (adjusted hazard ratio [HR] 1.81, 95% CI 1.15–2.85). A similar association was seen in Model 2 (adjusted HR 1.90, 95% CI 1.19–3.04), that is, after adjustment for serum albumin, BMI, and total daily energy intake, in addition to the covariates included in Model 1.

In the sensitivity analysis adjusted for the covariates included in Model 2 and also adjusted for hemoglobin level, ESA dose, and single-pool Kt/V, we also found a similar association between unbalanced diet and the composite outcome (adjusted HR 1.89, 95% CI 1.11–3.23). In the other sensitivity analysis, adjusted for the covariates included in Model 2 and also for smoking habit, we again found a similar association between unbalanced diet and adverse clinical events (adjusted HR 1.85, 95% CI 1.16–2.97).

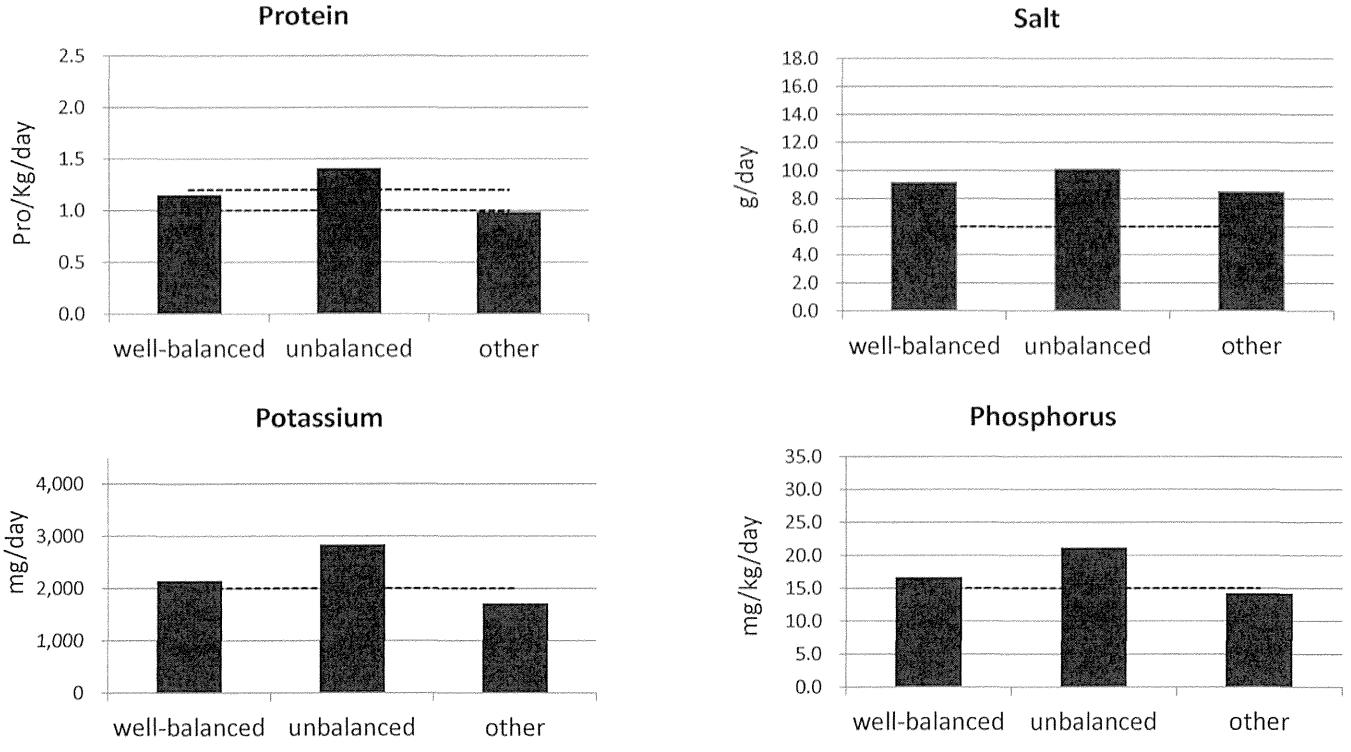

Figure 1. Micronutrient intake stratified by dietary pattern. Estimated micronutrient intake stratified by dietary pattern. Dotted lines show dietary standards according to Japan's clinical guidelines (Dietary recommendations for chronic kidney disease, 2007, Japanese Society of Nephrology).

doi:10.1371/journal.pone.0116677.g001

Discussion

Using PCA with data from a representative sample of the general population of Japan, we identified three food groups: meat, fish, and vegetables. We then found that hemodialysis patients could be said to have diets that were "well-balanced" or "unbalanced" with regard to those three food groups. (As noted previously, to identify dietary patterns based on foods or on food groups, as we did in this study, is common in nutritional epidemiology.[17–19]) The hemodialysis patients whose diet was unbalanced were more likely to have important clinical events. These findings suggest that limiting food portions, which is often recommended for hemodialysis patients to prevent severe adverse clinical outcome, is not enough. In addition to portion control, a diet that is balanced among meat, fish, and vegetables might help to prevent adverse outcomes.

Nutritional epidemiologic research in hemodialysis patients has largely focused on relationships between individual food items, micronutrients, and outcomes. For example, relationships between fish consumption, phosphate consumption, and outcomes in these patients have been reported.[7,28] However, hemodialysis patients do not eat only one specific food item, but rather various foods, and therefore dietary patterns should be determined on the basis of the combinations of foods that people actually eat. We began with PCA, from which we identified three groups of foods that are in fact eaten by people in Japan: meat, fish, and vegetables. We then used cluster analysis, from which we identified hemodialysis patients' actual patterns of food consumption with reference to those groups. Those patterns (well-balanced, unbalanced, and other) were associated with important clinical outcomes.

In hemodialysis patients, adequate protein intake (1.0 to 1.2 g/kg per day), such as can be obtained from the meat and fish groups we identified, is recommended to counteract loss of

Table 4. JDOPPS patient characteristics at baseline, by dietary pattern (n = 1,355).

	Well-balanced (49.2%)	Unbalanced (14.0%)	Other (36.9%)
Mean (SD) age, years	62.3 (11.8)	64.2 (11.9)	59.2 (11.5)
Male (%)	57.2	40.7	74.8
Mean (SD) dialysis duration, years	7.7 (7.4)	7.2 (7.2)	7.6 (7.0)
Mean (SD) BMI	21.2 (3.3)	20.2 (3.0)	21.5 (3.1)
Comorbid conditions (%)			
Diabetes	31.7	32.8	32.2
Coronary Heart Disease	44.3	41.8	37.2
Cerebrovascular Disease	12.8	12.7	9.4
Other Cardiovascular Disease	28.5	36.0	31.2
Peripheral Vascular Disease	15.6	22.2	14.0
Cancer	8.7	9.6	10.3
Mean (SD) serum albumin, g/dL	3.8 (0.4)	3.8 (0.5)	3.9 (0.4)
Mean (SD) phosphorus, mg/dL	5.6 (1.3)	5.3 (1.4)	5.6 (1.4)
Mean (SD) serum potassium, mEq/L	5.1 (0.8)	5.1 (0.8)	5.0 (0.8)
Mean (SD) energy intake, cal/Kg/day	1592 (563)	1707 (538)	1640 (656)
Mean (SD) protein intake, g/Kg/day	1.16 (0.51)	1.41 (0.59)	0.99 (0.49)
Mean (SD) salt intake, g/day	9.16 (3.32)	10.10 (3.21)	8.48 (3.60)
Mean (SD) potassium intake, g/day	2.15 (0.86)	2.84 (1.01)	1.72 (0.84)
Mean (SD) phosphorus intake, mg/day	883 (370)	1018 (376)	793 (395)

The "well-balanced diet" was characterized by approximately equal intake of the three food groups (fish, meat, and vegetables). The "unbalanced diet" was characterized by relatively large vegetable intake compared with meat and fish intake, and the "other diet" refers to other intake patterns.

doi:10.1371/journal.pone.0116677.t004

protein via the dialysate.[29] Sufficient protein intake is critical to preventing malnutrition, but excessive protein intake may lead to hyperphosphatemia, which may in turn lead to cardiovascular events. Hemodialysis patients should also avoid excessive vegetable intake to prevent hyperkalemia, which, like hyperphosphatemia, is associated with cardiovascular events. It is therefore physiologically plausible that a diet well-balanced among food groups would be associated with good clinical outcomes, as was found in this study.

The present study had a number of strengths. First, the Hisayama study and the JDOPPS used representative samples of the general population of Japan and of hemodialysis patients in Japan, respectively. Therefore the findings should be generalizable to all hemodialysis patients in Japan. To the extent that differences in dietary patterns between hemodialysis patients in Japan and those in other countries can result in differences in clinical outcomes, the present findings might be used for nutritional research and possibly also for dietary recommendations to improve the prognosis of patients in, for example, the US and Europe. Second, the use of the

Table 5. Dietary patterns and the composite outcome (JDOPPS data).

Dietary patterns	Composite outcome rate (/100 person-years)	Model 1 Hazard ratio (95% CI)	Model 2 Hazard ratio (95% CI)
Well-balanced	7.4	Reference	
Unbalanced	10.3	1.81 (1.15–2.85)	1.90 (1.19–3.04)
Other	6.1	1.23 (0.82–1.83)	1.21 (0.81–1.82)

The composite outcome included hospitalization due to cardiovascular disease, and death due to any cause. Model 1: Adjusted for age, gender, and dialysis duration. Model 2: Adjusted for age, gender, dialysis duration, serum albumin, BMI, total daily energy intake, and comorbid conditions (diabetes, coronary heart disease, cerebrovascular disease, other cardiovascular disease, and peripheral vascular disease).

doi:10.1371/journal.pone.0116677.t005

BDHQ enabled us to measure food intake in clinical settings.[21–24] Third, results of the sensitivity analyses indicated that the association of dietary pattern with the composite outcome was robust with respect to hemoglobin level, ESA dose, Kt/V, and smoking habit.

One possible limitation of this study is that food intake was self-reported. Actual food intake might have differed from that estimated from the food-frequency questionnaire.[30] In particular, social-desirability bias might have caused hemodialysis patients, who were aware of their dietary proscriptions, to report inaccurately-low levels of food intake, and the estimated intake of micronutrients might therefore have been incorrect.

In summary, eating a diet that was not balanced among meat, fish, and vegetables was associated with important adverse clinical events, which suggests that hemodialysis patients should not only limit their food intake but should also strive for a proper balance among those three food groups.

Acknowledgments

We thank the residents of Hisayama for participating in the survey and the staff of the Division of Health and Welfare of Hisayama for cooperating in this study. We also thank the patients in the JDOPPS for participating in the survey, and the staff of the dialysis facilities participating in the JDOPPS. We thank Joseph Green for advice and suggestions.

Author Contributions

Conceived and designed the experiments: KT S. Fukuma TN S. Fukuhara. Performed the experiments: MN HY S. Fujimi YK TK KU TS T. Akizawa T. Akiba AS. Analyzed the data: S. Fukuma TW. Wrote the paper: KT S. Fukuma TW TN S. Fukuhara.

References

1. Kopple JD (2001) National kidney foundation K/DOQI clinical practice guidelines for nutrition in chronic renal failure. Am J Kidney Dis 37: S66–70. PMID: 11158865
2. Sanghavi S, Whiting S, Uribarri J (2013) Potassium balance in dialysis patients. Semin Dial 26: 597–603. doi: 10.1111/sdi.12123 PMID: 24073843
3. Sherman RA, Mehta O (2009) Dietary phosphorus restriction in dialysis patients: potential impact of processed meat, poultry, and fish products as protein sources. Am J Kidney Dis 54: 18–23. doi: 10.1053/j.ajkd.2009.01.269 PMID: 19376617
4. Mailloux LU (2000) The overlooked role of salt restriction in dialysis patients. Semin Dial 13: 150–151. PMID: 10833773
5. Heerspink HJ, Ninomiya T, Zoungas S, de Zeeuw D, Grobbee DE, et al. (2009) Effect of lowering blood pressure on cardiovascular events and mortality in patients on dialysis: a systematic review and meta-analysis of randomised controlled trials. Lancet 373: 1009–1015. doi: 10.1016/S0140-6736(09)60212-9 PMID: 19249092
6. Palmer SC, Hayen A, Macaskill P, Pellegrini F, Craig JC, et al. (2011) Serum levels of phosphorus, parathyroid hormone, and calcium and risks of death and cardiovascular disease in individuals with chronic kidney disease: a systematic review and meta-analysis. JAMA 305: 1119–1127. doi: 10.1001/jama.2011.308 PMID: 21406649
7. Noori N, Kalantar-Zadeh K, Kovesdy CP, Bross R, Benner D, et al. (2010) Association of dietary phosphorus intake and phosphorus to protein ratio with mortality in hemodialysis patients. Clin J Am Soc Nephrol 5: 683–692. doi: 10.2215/CJN.08601209 PMID: 20185606
8. Kalantar-Zadeh K, Regidor DL, Kovesdy CP, Van Wyck D, Bunnapradist S, et al. (2009) Fluid retention is associated with cardiovascular mortality in patients undergoing long-term hemodialysis. Circulation 119: 671–679. doi: 10.1161/CIRCULATIONAHA.108.807362 PMID: 19171851
9. Krishnamurthy VM, Wei G, Baird BC, Murtaugh M, Chonchol MB, et al. (2012) High dietary fiber intake is associated with decreased inflammation and all-cause mortality in patients with chronic kidney disease. Kidney Int 81: 300–306. doi: 10.1038/ki.2011.355 PMID: 22012132

10. Port FK, Eknoyan G (2004) The Dialysis Outcomes and Practice Patterns Study (DOPPS) and the Kidney Disease Outcomes Quality Initiative (K/DOQI): a cooperative initiative to improve outcomes for hemodialysis patients worldwide. Am J Kidney Dis 44: 1–6. PMID: 15486867

11. Kuriyama S (2008) Characteristics of the clinical practice patterns of hemodialysis in Japan in consideration of DOPPS and the NKF/DOQI guidelines. Clin Exp Nephrol 12: 165–170. doi: 10.1007/s10157-007-0020-7 PMID: 18185907

12. Kubo M, Kiyohara Y, Kato I, Tanizaki Y, Arima H, et al. (2003) Trends in the incidence, mortality, and survival rate of cardiovascular disease in a Japanese community: the Hisayama study. Stroke 34: 2349–2354. PMID: 12958323

13. Fukuhara M, Arima H, Ninomiya T, Hata J, Yonemoto K, et al. (2012) Impact of lower range of prehypertension on cardiovascular events in a general population: the Hisayama Study. J Hypertens 30: 893–900. doi: 10.1097/HJH.0b013e328351d380 PMID: 22388232

14. Katsuki S (1966) Epidemiological and clinicopathological study on cerebrovascular disease in Japan. Prog Brain Res 21: 64–89. PMID: 5917289

15. Hata J, Ninomiya T, Hirakawa Y, Nagata M, Mukai N, et al. (2013) Secular trends in cardiovascular disease and its risk factors in Japanese: half-century data from the Hisayama Study (1961–2009). Circulation 128: 1198–1205. doi: 10.1161/CIRCULATIONAHA.113.002424 PMID: 23902756

16. Pisoni RL, Gillespie BW, Dickinson DM, Chen K, Kutner MH, et al. (2004) The Dialysis Outcomes and Practice Patterns Study (DOPPS): design, data elements, and methodology. Am J Kidney Dis 44: 7–15. PMID: 15486868

17. Reedy J, Wirfalt E, Flood A, Mitrou PN, Krebs-Smith SM, et al. (2010) Comparing 3 dietary pattern methods—cluster analysis, factor analysis, and index analysis—With colorectal cancer risk: The NIH-AARP Diet and Health Study. Am J Epidemiol 171: 479–487. doi: 10.1093/aje/kwp393 PMID: 20026579

18. Okubo H, Sasaki S, Horiguchi H, Oguma E, Miyamoto K, et al. (2006) Dietary patterns associated with bone mineral density in premenopausal Japanese farmwomen. Am J Clin Nutr 83: 1185–1192. PMID: 16685064

19. Wirfalt E, Midthune D, Reedy J, Mitrou P, Flood A, et al. (2009) Associations between food patterns defined by cluster analysis and colorectal cancer incidence in the NIH-AARP diet and health study. Eur J Clin Nutr 63: 707–717. doi: 10.1038/ejcn.2008.40 PMID: 18685556

20. Flood A, Rastogi T, Wirfalt E, Mitrou PN, Reedy J, et al. (2008) Dietary patterns as identified by factor analysis and colorectal cancer among middle-aged Americans. Am J Clin Nutr 88: 176–184. PMID: 18614739

21. Kobayashi S, Honda S, Murakami K, Sasaki S, Okubo H, et al. (2012) Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. J Epidemiol 22: 151–159. doi: 10.2188/jea.JE20110075 PMID: 22343326

22. Sasaki S, Yanagibori R, Amano K (1998) Self-administered diet history questionnaire developed for health education: a relative validation of the test-version by comparison with 3-day diet record in women. J Epidemiol 8: 203–215. PMID: 9816812

23. Sasaki S, Ushio F, Amano K, Morihara M, Todoriki O, et al. (2000) Serum biomarker-based validation of a self-administered diet history questionnaire for Japanese subjects. J Nutr Sci Vitaminol (Tokyo) 46: 285–296. PMID: 11227800

24. Kobayashi S, Murakami K, Sasaki S, Okubo H, Hirota N, et al. (2011) Comparison of relative validity of food group intakes estimated by comprehensive and brief-type self-administered diet history questionnaires against 16 d dietary records in Japanese adults. Public Health Nutr 14: 1200–1211. doi: 10.1017/S1368980011000504 PMID: 21477414

25. Cattell RB (1965) Factor Analyis: An Introduction to Essentials. Ii. The Role of Factor Analysis in Research. Biometrics 21: 405–435. PMID: 14338675

26. Willett W, Stampfer MJ (1986) Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 124: 17–27. PMID: 3521261

27. Everitt BLS, Lesse M (2001) Cluster analysis. 4th ed. London: Arnold.

28. Kutner NG, Clow PW, Zhang R, Aviles X (2002) Association of fish intake and survival in a cohort of incident dialysis patients. Am J Kidney Dis 39: 1018–1024. PMID: 11979345

29. Combe C, McCullough KP, Asano Y, Ginsberg N, Maroni BJ, et al. (2004) Kidney Disease Outcomes Quality Initiative (K/DOQI) and the Dialysis Outcomes and Practice Patterns Study (DOPPS): nutrition guidelines, indicators, and practices. Am J Kidney Dis 44: 39–46. PMID: 15486873

30. Mahabir S, Baer DJ, Giffen C, Subar A, Campbell W, et al. (2006) Calorie intake misreporting by diet record and food frequency questionnaire compared to doubly labeled water among postmenopausal women. Eur J Clin Nutr 60: 561–565. PMID: 16391574

REVIEW

Cardiorenal syndrome in chronic kidney disease

Kazuhiko Tsuruya[a,b] *and Masahiro Eriguchi*[b]

Purpose of review
The purpose of this study is to review current perspectives regarding the pathogenesis of cardiorenal syndrome (CRS) in chronic kidney disease (CKD), and current treatment guidelines for this condition.

Recent findings
The pathophysiological mechanisms underlying the development of CRS in CKD include neurohumoral, haemodynamic and CKD-related mechanisms. Recent evidence suggests that sympathetic nerve activity plays a role in CRS, but the SYMPLICITY HTN-3 trial failed to show reduction of blood pressure after catheter-based renal denervation in patients with resistant hypertension. Kidney injury in patients with heart failure was previously considered to result from arterial underfilling due to low cardiac output, but the role of renal venous hypertension in this process has also recently been investigated. It would be useful to develop a reliable treatment option for CRS due to haemodynamic mechanism other than volume control using diuretics. Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone that has recently been identified as a CKD-related factor affecting CRS. FGF23 treatment has both advantages and disadvantages in terms of CRS progression.

Summary
Multiple disorders underlie the development of CRS. Current treatment options include renin-angiotensin system blockade and volume control, but remain limited. A multidisciplinary approach is required to prevent CRS, including renal sympathetic denervation, treatment of renal venous hypertension and FGF23 treatment.

Keywords
fibroblast growth factor 23, nitric oxide, renal venous hypertension, renin-angiotensin system, sympathetic nerve activity

INTRODUCTION

Chronic kidney disease (CKD) is an independent risk factor for cardiovascular disease (CVD), and there is a high prevalence of CVD among patients with CKD. Mortality due to CVD is 10–30 times higher in dialysis patients than in the general population [1], and patients with CVD often have CKD. This interaction between CKD and CVD is known as cardiorenal syndrome (CRS).

Ronco *et al.* [2,3▪▪] proposed division of CRS into five categories according to the associated etiologic and chronologic factors. Each category is characterized as follows: CRS type 1; acute worsening of cardiac function [e.g. acutely decompensated congestive heart failure (CHF)] leading to acute kidney injury and/or dysfunction, CRS type 2; chronic abnormalities in cardiac function (e.g. chronic CHF) causing progressive and permanent CKD, CRS type 3; acute worsening of kidney function leading to acute cardiac injury and/or dysfunction, such as acute myocardial infarction, CHF or arrhythmia, CRS type 4; primary CKD contributing to decreased cardiac function, cardiac hypertrophy, fibrosis and/or increased risk of adverse cardiovascular events, CRS type 5; acute cardiac and renal injury and dysfunction in the setting of an overwhelming systemic insult.

This classification describes the clinical setting associated with CRS, but is not based on pathophysiological mechanisms. CVD is common in patients with CKD and is associated with substantially increased risk of end-stage renal disease (ESRD) and all-cause mortality before the development of

[a]Department of Integrated Therapy for Chronic Kidney Disease and [b]Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Correspondence to Kazuhiko Tsuruya, MD, PhD, Department of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel: +81 92 642 5843; fax: +81 92 642 5846; e-mail: tsuruya@intmed2.med.kyushu-u.ac.jp

Curr Opin Nephrol Hypertens 2015, 24:000–000
DOI:10.1097/MNH.0000000000000099

KEY POINTS

- The complicated pathophysiological mechanisms underlying the development of CRS in CKD include neurohumoral and haemodynamic disorders as well as CKD-related factors such as anaemia, calcium-phosphate imbalance and inflammation.
- The effects of renal venous hypertension on CRS have recently been investigated; increased tubulointerstitial pressure and decreased arterio-venous gradient can result in reduction of glomerular filtration pressure and renal blood flow, but the precise mechanisms underlying worsening of renal function secondary to renal venous hypertension remain unclear.
- Although the results of experimental studies suggest that renal sympathetic nerves play an important role in the pathophysiological mechanisms leading to CRS, it is currently unclear whether catheter-based renal denervation is useful for the treatment of CRS.
- FGF23, a newly identified phosphaturic hormone, may have both advantages and disadvantages, with a protective effect on arterial calcification in nondialyzed CKD patients and promotion of left ventricular hypertrophy in anuric patients; and alteration of FGF23 concentrations may lead to new strategies for the treatment of CRS.

ESRD [4▪]. These findings suggest that cardiac and renal injuries affect each other, and that CRS types 2 and 4 according to the Ronco classification are overlapping and coexistent.

In addition to haemodynamic changes, neurohumoral factors such as renin-angiotensin system (RAS) activation, sympathetic nerve activity (SNA) activation and nitric oxide level play important roles in the interactions between the heart and kidneys in patients with CKD and CVD [5▪,6]. In this review, we describe the interactions among these factors and their impact on the mechanisms underlying the development of CRS, and therapeutic strategies for the management of CRS.

CVD and CKD coexist in patients with CRS, and conventional risk factors for CVD and CKD such as hypertension and diabetes mellitus influence the development of CRS [7]. Understanding of the factors that cause CRS in patients with both CKD and CVD is important for determining optimal therapeutic strategies for these patients.

This study discusses the interactions among three maladaptive cycles that lead to the development of CRS: neurohumoral disorders, haemodynamic alterations and CKD-related factors (Fig. 1).

NEUROHUMORAL DISORDERS

Neurohumoral factors are essential haemodynamic regulators and strongly affect blood pressure and body fluid volume. Each of these factors interacts complicatedly with each other and also has a direct effect on organ injury in haemodynamic-independent manner.

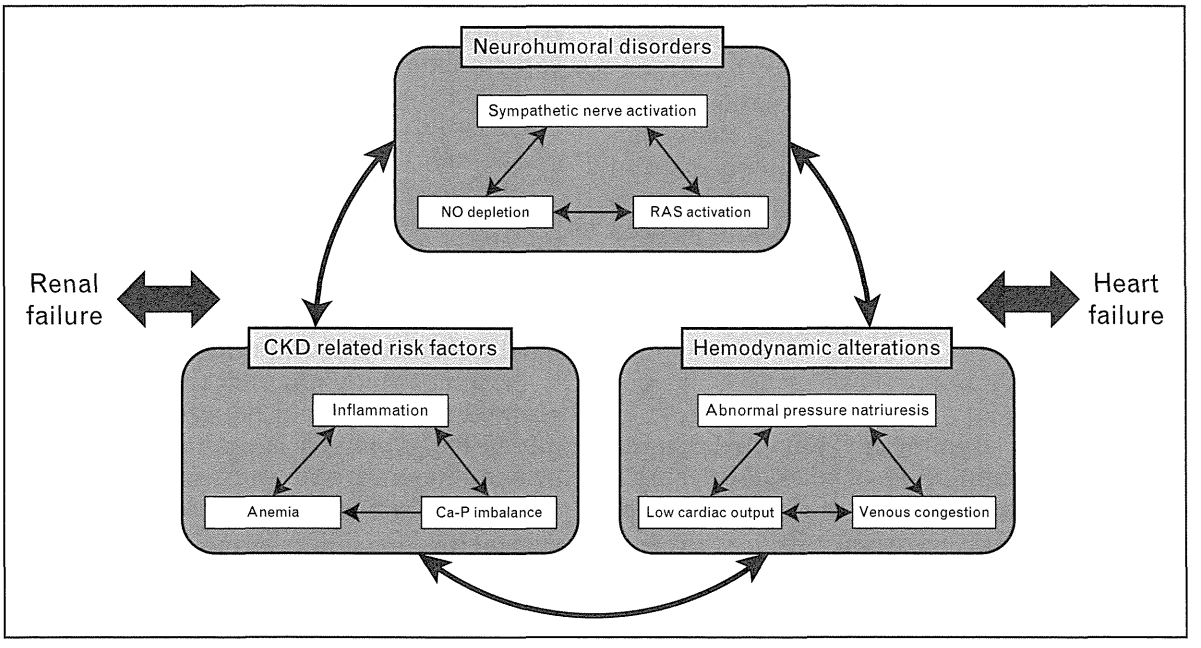

FIGURE 1. Pathophysiology of cardiorenal interactions in chronic kidney disease. The multifactorial cardiorenal interactions in patients with CKD include three positive-feedback cycles: neurohumoral disorders, haemodynamic alterations and CKD-related factors. Ca, calcium; P, phosphorus. This figure is original.

Interactions among renin-angiotensin system, sympathetic nerve activity and nitric oxide

RAS, SNA and nitric oxide interact with each other and have important roles in the neurohumoral maladaptive cycle leading to the development of CRS [6,8,9]. In an animal model, continuous intravenous injection of angiotensin II [10] or intracerebroventricular injection of angiotensin II [11] caused SNA activation, and increased secretion of renin from the juxtaglomerular apparatus after SNA-induced activation of β1 receptors caused RAS activation, resulting in a positive-feedback cycle. The RAS interacts with nitric oxide [12] and lowers the nitric oxide level in the renal cortex of rats injected with angiotensin II [13].

Conversely, inhibition of nitric oxide by chronic administration of N^{ω}-nitro-L-arginine methyl ester increases RAS activation by reducing the renal circulation [14,15▪▪], although this inhibition of nitric oxide initially lowers RAS activation [16] because of volume overload [8]. Moreover, inhibition of nitric oxide promotes a reduction in the glomerular filtration rate (GFR) by increasing the renal response to angiotensin II [17].

Inhibition of nitric oxide in rats also results in SNA activation by resetting of the baroreceptors over time, although there is an initial transient decrease in SNA activation due to the baroreceptor reflex response to increased blood pressure [18]. Blocking of the afferent baroreceptor pathways results in SNA activation immediately after inhibition of nitric oxide [18]. Several studies reported that decreased nitric oxide production in the central nervous system resulted in SNA activation [11,19].

On the contrary, activation of SNA inhibits nitric oxide production. Decreased activity of the L-arginine–nitric oxide metabolic pathway is reported in patients with CHF in whom SNA activation is thought to occur [20]. Couto *et al.* [21] found reduced nitric oxide bioavailability in the small vessels of mice that had sympathetic hyperactivity because they lacked α_{2A}/α_{2C}-adrenergic receptors.

Nitric oxide

Accumulation of asymmetric dimethylarginine results in chronic inhibition of nitric oxide [22▪]. RAS and SNA activation result in accelerated progression of CKD, and decreased nitric oxide production due to accumulation of asymmetric dimethylarginine results in further RAS and SNA activation and development of CRS [22▪]. Bongartz *et al.* [23,24] reported on the impact of nitric oxide inhibition on CRS progression using two models of CRS. These models of CRS induced by subtotal nephrectomy as well as coronary ligation, or by transient nitric oxide reduction, can be applied to clinical situations [25], and show that nitric oxide inhibition plays an important role in the development of CRS. Although these findings suggest that retrieval of nitric oxide should be an important therapeutic strategy in CRS, this strategy has not been shown to be clinically effective.

Renin-angiotensin system

RAS activation results in organ damage in patients with CKD and CVD, and RAS inhibitors are used as first-line treatment in hypertensive patients with CRS [5▪]. Albuminuria is an independent risk factor for progression of CKD and CVD even when renal function is normal [26], and randomized controlled trials of RAS inhibitors found that greater reduction in urinary protein excretion was associated with stronger protective effects against CRS [27,28]. It has also been reported that reduction in proteinuria in the early stage of CKD lowers the risk of progression of CKD [29▪]. Treatment with a RAS inhibitor is therefore required from the early stage of CKD to prevent the progression of CKD and CVD, using the degree of albuminuria as a therapeutic target.

Sympathetic nervous system

SNA activation is observed from the early stage of CKD [30] and during progression to ESRD [31], and is associated with CVD and mortality in these patients [30]. SNA activation was reported in various experimental models of renal injury [15▪▪,32,33]. Ye *et al.* [33] reported SNA activation after a limited renal injury induced by intrarenal injection of phenol.

The mechanisms underlying SNA activation in CKD include increased circulating RAS [10] and brain RAS [11], nitric oxide depletion [18], stimulation of renal baroreceptors, chemoreceptors and sensory receptors [32], reduction in renal mass [33], renal ischemia [34] and other factors [35].

A recent study reported that renal denervation resulted in reduction of albuminuria without affecting the blood pressure in a rat model of CRS induced by hemi-nephrectomy and aortic regurgitation [36]. We then investigated the effects of renal denervation on the interaction between SNA and RAS in a rat model of CRS induced by chronic nitric oxide inhibition, and found that renal denervation had protective effects against cardiac and renal dysfunction [15▪▪]. These effects were associated with decreased RAS activation and were independent of the blood pressure lowering effects (Fig. 2).

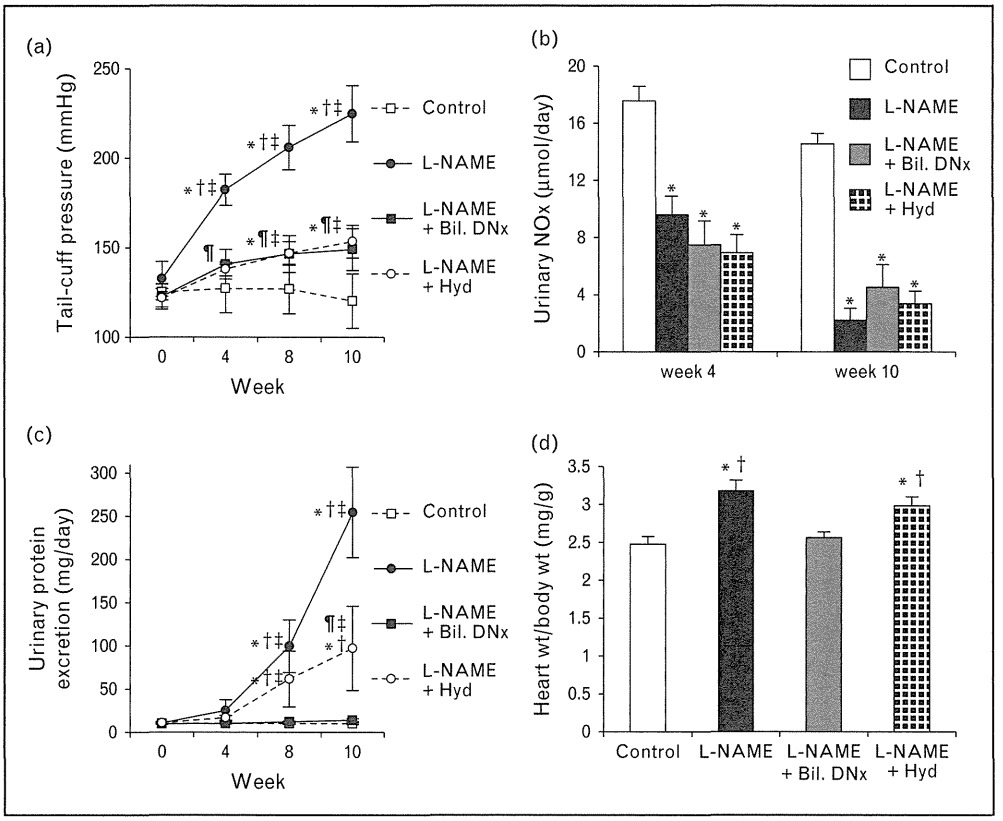

FIGURE 2. Renal denervation in an experimental study. Chronic administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME; a nitric oxide synthase inhibitor) was used to induce proteinuria and cardiac hypertrophy, similar to cardiorenal syndrome, in Wistar rats. These changes were suppressed by bilateral renal sympathetic denervation (Bil. DNx), but not by hydralazine (Hyd) treatment, even though blood pressure and nitric oxide depletion were maintained at the same levels in both groups. SBP (a), urinary nitric oxygen (NOx) (b), urinary protein excretion (c) and heart weight (d) are shown. Values are mean ± standard error of the mean. $^{*}P<0.05$ vs. control rats, $^{**}P<0.05$ vs. Bil. DNx rats, $^{***}P<0.05$ vs. L-NAME rats, $^{\dagger}P<0.05$ vs. baseline values. This figure is a direct copy of [14].

Renal denervation using catheter devices has been reported to be clinically effective for the prevention of hypertension [37], atherosclerosis [38], left ventricular hypertrophy (LVH) [39], albuminuria [40] and CKD [41], but these studies were not comparative trials. The blinded randomized controlled SYMPLICITY HTN-3 trial [42▪▪], which used a sham-operation group for comparison, did not show a significant difference in the reduction of SBP in patients with resistant hypertension (Fig. 3). As many physicians expect renal denervation to be an attractive therapeutic modality in patients with CRS, it should be determined why this was not shown to be effective in the SYMPLICITY HTN-3 trial [43]. First, it is possible that ablation using the catheter device was incomplete. We found that one-sided denervation did not prevent increase in blood pressure or progression of organ damage [15▪▪]. Second, it is possible that the patient selection process was not appropriate. In a preliminary experiment using a puromycin aminonucleoside-induced model of nephrotic syndrome, we did not find that renal denervation reduced proteinuria or hypertension. It is important to identify clinical markers that can be used to confirm adequate denervation and to ensure appropriate selection of candidates for denervation.

HAEMODYNAMIC ALTERATIONS

Haemodynamic alteration in CRS, which have been explained as the low-flow theory, is an indispensable factor in talking about cardio-renal interaction. We address the recent proposed theory about how 'renal venous hypertension' affects on the renal perfusion in this section.

Abnormal pressure natriuresis for low cardiac output (low-flow theory)

Regulation of sodium balance according to the pressure natriuresis curve and heart and kidney function is important for the maintenance of appropriate blood pressure and body fluid volume [44].

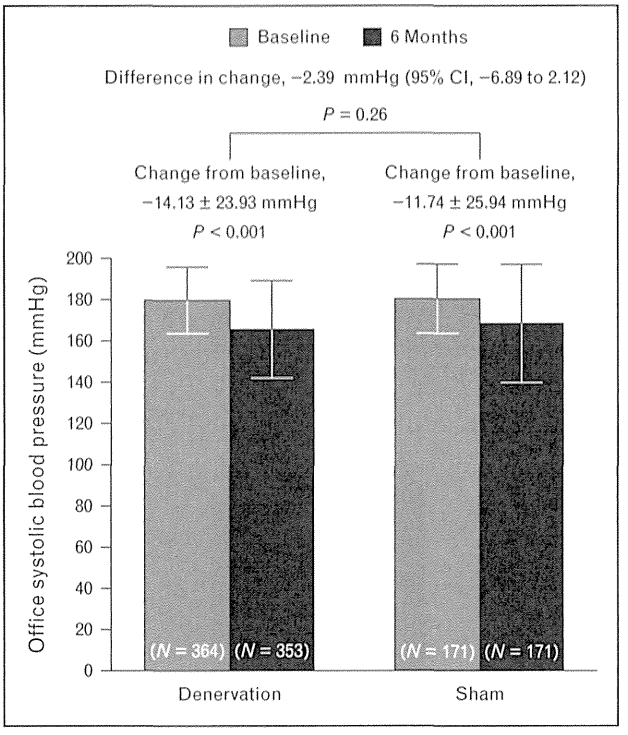

FIGURE 3. Renal denervation in a clinical study. In the SYMPLICITY HTN-3 trial, the difference in change in blood pressure between the two groups was 2.39 mmHg, which was not significant. This figure is a direct copy of [41].

Increased blood pressure resulting from a normal cardiac response to increased fluid volume, and pressure natriuresis in response to the increased blood pressure, are required for excretion of excess sodium and body fluid. In patients with CKD who have insufficient sodium excretion because of reduced GFR due to reduced numbers of functional nephrons, there is insufficient pressure natriuresis. Pressure natriuresis is also affected by neurohumoral factors, with a shift of the pressure natriuresis curve to the right after RAS and SNA activation [44].

Renal venous hypertension

It was previously thought that impaired pressure natriuresis was caused mainly by reduced renal blood flow due to low cardiac output and by arterial underfilling due to left ventricular contractile dysfunction. However, a study of 1 184 655 patients with heart failure in the ADHERE database did not find an association between left ventricular contractile dysfunction and renal dysfunction, suggesting that renal dysfunction was not attributable only to low cardiac output [45]. This finding suggests that renal venous hypertension due to venous congestion, rather than arterial underfilling, may cause renal dysfunction.

The results of recent clinical trials also suggest that renal dysfunction may be caused by renal venous hypertension due to venous congestion rather than by arterial underfilling [46,47]. A subanalysis of ESCAPE trial showed the relationship between increase in central venous pressure and decrease in estimated GFR after adjusting cardiac index (Fig. 4) [46]. GFR is considered to decrease in response to reduction in the net filtration pressure caused by increased hydrostatic pressure in Bowman's capsule secondary to increased interstitial pressure (Fig. 5) [48,49]. Other suggested causes of renal dysfunction are neurohumoral factors, myogenic responses, regulation of renal blood flow and

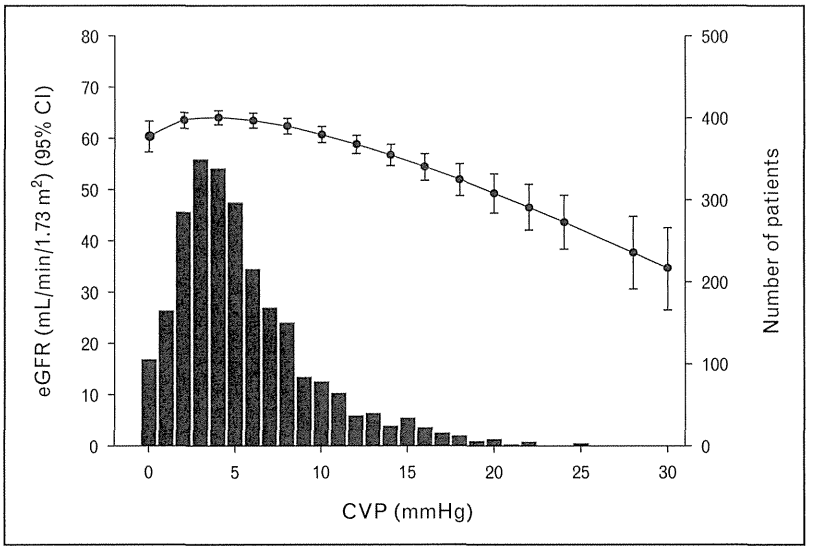

FIGURE 4. Haemodynamic impact of cardiorenal syndrome. The relationship between central venous pressure (CVP) and estimated GFR (eGFR) adjusted for age, sex and cardiac index. This figure is a direct copy of [45].

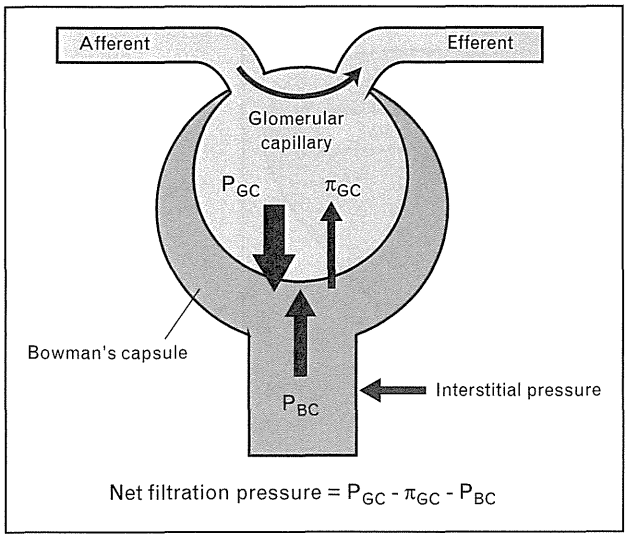

FIGURE 5. Haemodynamic impact of renal venous hypertension on glomerular capillary. Renal venous hypertension is associated with increased efferent pressure (decreased afferent–efferent gradient) and interstitial pressure (P_{BC} elevation), resulting in reduced glomerular flow and net filtration pressure. P_{BC}, hydrostatic pressure in Bowman's capsule; P_{GC}, glomerular capillary hydrostatic pressure; π_{GC}, oncotic pressure in the glomerular capillaries. This figure is original.

GFR by tubuloglomerular feedback [8], and hypoxia and inflammation of the renal parenchyma. These factors suggest that abnormal pressure natriuresis due to decreased GFR, exacerbation of venous congestion and worsening of heart failure due to low cardiac output create a positive-feedback cycle (Fig. 1).

CHRONIC KIDNEY DISEASE RELATED RISK FACTORS

In the past decade, two novel pathogenic mechanisms have been proposed for the development of CVD in patients with CKD: the cardiorenal anaemia (CRA) syndrome proposed by Silverberg *et al.* [50] and the malnutrition–inflammation–atherosclerosis (MIA) syndrome proposed by Stenvinkel *et al.* [51]. In addition, it was also recently reported that disturbances in mineral and bone metabolism are involved in the pathogenesis of CVD in patients with CKD. This mechanism has been termed CKD-related mineral and bone disorder (CKD-MBD), and includes abnormalities in bone and mineral metabolism and vascular calcification [52]. CRA syndrome, MIA syndrome and CKD-MBD are considered to interact with each other in the pathogenesis of CRS (Fig. 6).

Inflammation

Inflammation in CKD is induced by increased levels of inflammatory cytokines due to increased production of uremic toxins [53] and reduced clearance due to renal dysfunction [54]. Inflammation is a predictor of cardiovascular and total mortality in CKD [55], and is also a predictor of mortality and disease severity in patients with heart failure [56].

Venous congestion and volume overload have increasingly recognized roles in the development of inflammation in patients with CRS [57]. Edematous bowels, veins and peripheral tissues can be important sources of inflammatory mediators when exposed to high intravascular and interstitial pressures.

We recently reported that inflammation and malnutrition play important roles in the development of vascular calcification in rats with adenine-induced chronic renal failure [58▪▪], and that vascular calcification in these rats was ameliorated by antioxidant treatment [59].

Inflammation is considered to be one of the important factors regulating CRS. However, recently conducted randomized controlled trials of immune-selective anti-inflammatory derivatives such as etanercept [60] and infliximab [61] did not show any effects on the risk of death from any cause or hospitalization for heart failure. The ACCLAIM trial investigated the effects of nonspecific immunomodulation in patients with heart failure and showed no significant effects in the group overall, but was associated with reduced risk of death from any cause and first hospitalization for CVD in patients with no history of myocardial infarction and patients with NYHA class II heart failure [62].

Anaemia

Patients with heart failure may have anaemia even though they have a high plasma erythropoietin (EPO) concentration. This EPO-resistant anaemia is considered to be caused by inflammation [63]. In patients with CRS, anaemia is attributed to both EPO deficiency and inflammation-induced EPO resistance. Appropriate management of anaemia is important, because it influences mortality and renal survival in patients with CRS.

Calcium-phosphate imbalance

CRS has been reported to be associated with CKD-MBD. Activation of vitamin D exerts various effects such as reduction of RAS activation, reduction of inflammation, reduction of apoptosis, inhibition of cell proliferation and immune modulation, in addition to regulation of bone and mineral metabolism. Two studies reported that the

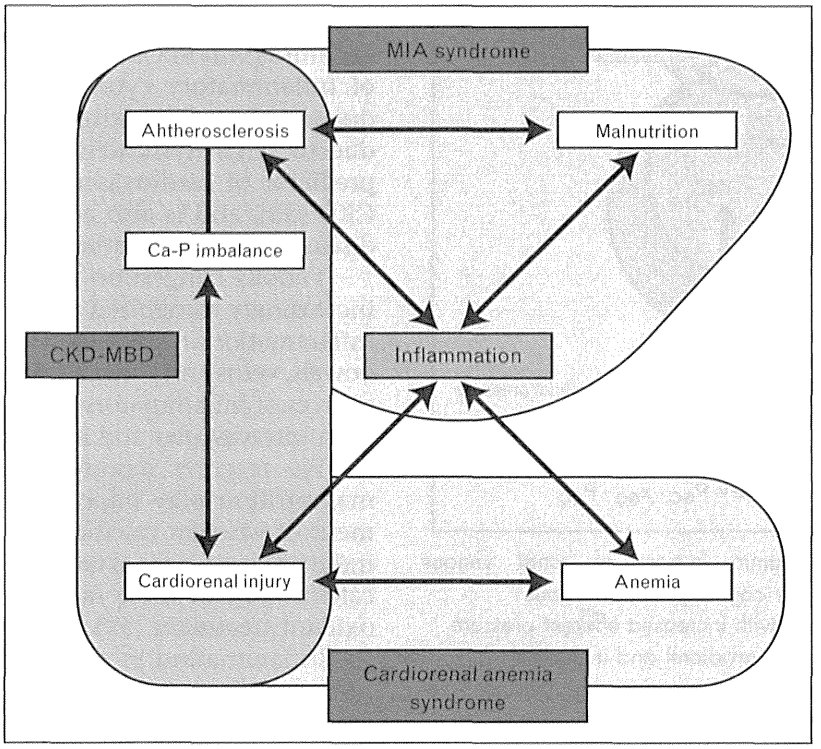

FIGURE 6. Schematic diagram of interactions among chronic kidney disease related factors. Malnutrition–inflammation–atherosclerosis (MIA) syndrome, cardiorenal-anemia (CRA) syndrome and CKD-related related mineral and bone disorder (CKD-MBD) interact with each other. Inflammation plays a central role in all three mechanisms. Ca, calcium; P, phosphorus. This figure is original.

anti-inflammatory effects of activated vitamin D provided cardiorenal protection. One study found improvements in proteinuria and renal dysfunction in a murine model of adriamycin-induced nephropathy [64], and another found improvement in LVH in rats with CKD induced by subtotal nephrectomy [65▪]. Two recent randomized controlled trials investigated the cardiorenal protection provided by paricalcitol therapy. Paricalcitol therapy reduced albuminuria in the VITAL study [66], but did not improve LVH in patients with CKD in the PRIMO trial [67]. Further accumulation of evidence of beneficial effects of vitamin D receptor activator (VDRA) on CRS is required in the clinical setting.

Recent studies found that an increase in the serum FGF23 level, which causes reduction of the serum phosphate level by inhibition of proximal tubular phosphate reabsorption through its own suppressive effect on the expression of type 2a and 2c sodium-phosphate cotransporter in the brush border membrane of proximal tubules, and by inhibition of intestinal phosphate absorption secondary to reduction of the 1,25-dihydroxyvitamin D level, is associated with CVD [68▪]. It is currently unclear whether FGF23 is a biomarker or a pathogenic factor in this process. Faul *et al.* [69] reported that intramyocardial or intravenous injection of FGF23 in wild-type mice resulted in LVH. However, Shalhoub *et al.* [70] reported that administration of anti-FGF23 neutralizing antibodies increased vascular calcification and mortality in a rat model of CKD. FGF23 has a preventive effect on arterial calcification because it controls the serum phosphate level via its phosphaturic action in patients with nondialyzed CKD and induces LVH by reducing the activation of vitamin D in patients with ESRD without phosphaturia. FGF23 may therefore have different effects in different patients with CRS, depending on the stage of CKD. It is expected that further elucidation of the pathophysiological impact of FGF23 will lead to the development of new strategies for the treatment of CRS.

More recently, a new phosphate-centric paradigm for pathophysiology and therapy of CKD has been proposed that extracellular phosphate exerts its cytotoxicity when it forms insoluble nanoparticles with calcium and fetuin-A, referred to as calciprotein particles (CPPs) [71▪▪]. These observations have raised the possibility that CPPs may promote progression of CKD and vascular calcification, resulting in development and progression of CRS.

CONCLUSION

Although many pathogenic factors leading to CRS have been identified, it is possible that an important underlying mechanism remains unclear. Further elucidation of the mechanisms underlying the development of CRS may lead to clinically feasible strategies for the treatment of this condition.

Acknowledgements

We would like to thank Dr Kumiko Torisu for her assistance with the study. We also thank Edanz-Editing (http://www.edanzediting.co.jp/) for the careful reading and preparation of our manuscript.

Financial support and sponsorship

None.

Conflicts of interest

The authors have no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ▪ of special interest
- ▪▪ of outstanding interest

1. Go AS, Chertow GM, Fan D, *et al.* Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 2004; 351:1296–1305.
2. Ronco C, Haapio M, House AA, *et al.* Cardiorenal syndrome. J Am Coll Cardiol 2008; 52:1527–1539.
3. ▪▪ McCullough PA, Kellum JA, Haase M, *et al.* Pathophysiology of the cardiorenal syndromes: executive summary from the eleventh consensus conference of the Acute Dialysis Quality Initiative (ADQI). Contrib Nephrol 2013; 182: 82–98.

In 2008, Ronco *et al.* [2] proposed five distinct CRS according to the temporal sequence of organ injury and failure as well as the clinical context. Later in 2008, a consensus conference was organized under the auspices of the Acute Dialysis Quality Initiative (ADQI) bringing to Venice, Italy, key opinion leaders and experts in the fields of nephrology, critical care, cardiac surgery and cardiology. In series of papers and an executive summary, this group presented the definition and classification system for the CRS, epidemiology and diagnostic criteria, prevention and therapeutic approaches, as a result of the consensus process. This study reviews and summarizes key findings from the 11th ADQI consensus conference on the spectrum of pathophysiologic mechanisms at work in the CRS.

4. ▪ Sud M, Tangri N, Pintilie M, *et al.* Risk of end-stage renal disease and death after cardiovascular events in chronic kidney disease. Circulation 2014; 130:458–465.

This retrospective cohort study involved 2964 CKD individuals and demostrated that cardiovascular events are common in patients with CKD stages 3–5 and are associated with a substantial increase in the risk of ESRD and all-cause mortality before ESRD.

5. ▪ Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, *et al.* Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. Lancet 2013; 382:339–352.

In this review, the authors discuss the epidemiology and pathophysiological mechanisms of cardiovascular risk in patients with CKD, and discuss methods of prevention.

6. Bongartz LG, Cramer MJ, Doevendans PA, *et al.* The severe cardiorenal syndrome: 'Guyton revisited'. Eur Heart J 2005; 26:11–17.
7. Schrier RW. Cardiorenal versus renocardiac syndrome: is there a difference? Nat Clin Pract Nephrol 2007; 3:637.
8. Braam B, Cupples WA, Joles JA, Gaillard C. Systemic arterial and venous determinants of renal hemodynamics in congestive heart failure. Heart Fail Rev 2012; 17:161–175.
9. Koomans HA, Blankestijn PJ, Joles JA. Sympathetic hyperactivity in chronic renal failure: a wake-up call. J Am Soc Nephrol 2004; 15:524–537.
10. Moretti JL, Burke SL, Davern PJ, *et al.* Renal sympathetic activation from long-term low-dose angiotensin II infusion in rabbits. J Hypertens 2012; 30: 551–560.
11. Campese VM, Ye S, Zhong H. Downregulation of neuronal nitric oxide synthase and interleukin-1beta mediates angiotensin II-dependent stimulation of sympathetic nerve activity. Hypertension 2002; 39 (2 Pt 2):519–524.
12. Kopkan L, Cervenka L. Renal interactions of renin-angiotensin system, nitric oxide and superoxide anion: implications in the pathophysiology of salt-sensitivity and hypertension. Physiol Res 2009; 58 (Suppl 2):S55–S67.
13. Lopez B, Salom MG, Arregui B, *et al.* Role of superoxide in modulating the renal effects of angiotensin II. Hypertension 2003; 42:1150–1156.
14. Ikeda H, Tsuruya K, Toyonaga J, *et al.* Spironolactone suppresses inflammation and prevents L-NAME-induced renal injury in rats. Kidney Int 2009; 75:147–155.
15. ▪▪ Eriguchi M, Tsuruya K, Haruyama N, *et al.* Renal denervation has blood pressure-independent protective effects on kidney and heart in a rat model of chronic kidney disease. Kidney Int 2014. [Epub ahead of print]

This experimental study examined the effect of bilateral renal denervation on CRS using rats with chronic nitric oxide inhibition and demonstrated cardio-protective and reno-protective effects of bilateral renal denervation beyond blood pressure lowering effect in rats with CRS. The authors also examined the effect of unilateral renal denervation in the same model rats and demonstrated a limited effect, despite decreases in histological renal injury and proteinuria in the denervated kidney compared with the contralateral nondenervated kidney were observed.

16. Gonzalez-Villalobos RA, Janjoulia T, Fletcher NK, *et al.* The absence of intrarenal ACE protects against hypertension. J Clin Invest 2013; 123:2011–2023.
17. Majid DS, Nishiyama A, Jackson KE, Castillo A. Superoxide scavenging attenuates renal responses to ANG II during nitric oxide synthase inhibition in anesthetized dogs. Am J Physiol Renal Physiol 2005; 288:F412–F419.
18. Sakuma I, Togashi H, Yoshioka M, *et al.* NG-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. A role for nitric oxide in the central regulation of sympathetic tone? Circ Res 1992; 70:607–611.
19. Zhou YB, Sun HJ, Chen D, *et al.* Intermedin in paraventricular nucleus attenuates sympathetic activity and blood pressure via nitric oxide in hypertensive rats. Hypertension 2014; 63:330–337.
20. Kátz SD, Khan T, Zeballos GA, *et al.* Decreased activity of the L-arginine-nitric oxide metabolic pathway in patients with congestive heart failure. Circulation 1999; 99:2113–2117.
21. Couto GK, Davel AP, Brum PC, Rossoni LV. Double disruption of alpha2A- and alpha2C-adrenoceptors induces endothelial dysfunction in mouse small arteries: role of nitric oxide synthase uncoupling. Exp Physiol 2014; 99:1427–1438.
22. ▪ Ueda S, Yamagishi S, Yokoro M, Okuda S. Role of asymmetric dimethylarginine in cardiorenal syndrome. Curr Pharm Des 2014; 20:2448–2455.

In this review, the authors hypothesize that endothelial dysfunction is one of the possible factors that could explain the pathogenesis of CRS, and discuss the roles of asymmetric dimethylarginine in the development of endothelial dysfunction, especially focusing on its roles in CRS.

23. Bongartz LG, Braam B, Verhaar MC, *et al.* Transient nitric oxide reduction induces permanent cardiac systolic dysfunction and worsens kidney damage in rats with chronic kidney disease. Am J Physiol Regul Integr Comp Physiol 2010; 298:R815–R823.
24. Bongartz LG, Joles JA, Verhaar MC, *et al.* Subtotal nephrectomy plus coronary ligation leads to more pronounced damage in both organs than either nephrectomy or coronary ligation. Am J Physiol Heart Circ Physiol 2012; 302:H845–H854.
25. Bongartz LG, Braam B, Gaillard CA, *et al.* Target organ cross talk in cardiorenal syndrome: animal models. Am J Physiol Renal Physiol 2012; 303:F1253–F1263.
26. Levey AS, de Jong PE, Coresh J, *et al.* The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int 2011; 80:17–28.
27. Asselbergs FW, Diercks GF, Hillege HL, *et al.* Effects of fosinopril and pravastatin on cardiovascular events in subjects with microalbuminuria. Circulation 2004; 110:2809–2816.
28. Appel LJ, Wright JT Jr, Greene T, *et al.* Intensive blood-pressure control in hypertensive chronic kidney disease. N Engl J Med 2010; 363:918–929.
29. ▪ Inker LA, Levey AS, Pandya K, *et al.* Early change in proteinuria as a surrogate end point for kidney disease progression: an individual patient meta-analysis. Am J Kidney Dis 2014; 64:74–85.

This meta-analysis demonstrated that early decline in proteinuria was associated with lower risk of the clinical outcome and provide new evidence supporting the use of an early reduction in proteinuria as a surrogate endpoint, in spite of insufficient evidence to establish its validity in all settings.

30. Grassi G, Quarti-Trevano F, Seravalle G, *et al.* Early sympathetic activation in the initial clinical stages of chronic renal failure. Hypertension 2011; 57: 846–851.
31. Zoccali C, Mallamaci F, Parlongo S, *et al.* Plasma norepinephrine predicts survival and incident cardiovascular events in patients with end-stage renal disease. Circulation 2002; 105:1354–1359.
32. Bigazzi R, Kogosov E, Campese VM. Altered norepinephrine turnover in the brain of rats with chronic renal failure. J Am Soc Nephrol 1994; 4:1901–1907.

33. Ye S, Gamburd M, Mozayeni P, *et al.* A limited renal injury may cause a permanent form of neurogenic hypertension. Am J Hypertens 1998; 11 (6 Pt 1):723–728.
34. Klein IH, Ligtenberg G, Oey PL, *et al.* Sympathetic activity is increased in polycystic kidney disease and is associated with hypertension. J Am Soc Nephrol 2001; 12:2427–2433.
35. de Beus E, de Jager R, Joles JA, *et al.* Sympathetic activation secondary to chronic kidney disease: therapeutic target for renal denervation? J Hypertens 2014; 32:1751–1761.
36. Rafiq K, Noma T, Fujisawa Y, *et al.* Renal sympathetic denervation suppresses de novo podocyte injury and albuminuria in rats with aortic regurgitation. Circulation 2012; 125:1402–1413.
37. Krum H, Schlaich M, Whitbourn R, *et al.* Catheter-based renal sympathetic denervation for resistant hypertension: a multicentre safety and proof-of-principle cohort study. Lancet 2009; 373:1275–1281.
38. Brandt MC, Reda S, Mahfoud F, *et al.* Effects of renal sympathetic denervation on arterial stiffness and central hemodynamics in patients with resistant hypertension. J Am Coll Cardiol 2012; 60:1956–1965.
39. Brandt MC, Mahfoud F, Reda S, *et al.* Renal sympathetic denervation reduces left ventricular hypertrophy and improves cardiac function in patients with resistant hypertension. J Am Coll Cardiol 2012; 59:901–909.
40. Mahfoud F, Cremers B, Janker J, *et al.* Renal hemodynamics and renal function after catheter-based renal sympathetic denervation in patients with resistant hypertension. Hypertension 2012; 60:419–424.
41. Hering D, Mahfoud F, Walton AS, *et al.* Renal denervation in moderate to severe CKD. J Am Soc Nephrol 2012; 23:1250–1257.
42. ▪▪ Bhatt DL, Kandzari DE, O'Neill WW, *et al.* A controlled trial of renal denervation for resistant hypertension. N Engl J Med 2014; 370:1393–1401.
This trial, SYMPLICITY HTN-3, is a prospective, single-blind, randomized, sham-controlled trial designed to validate the prior unblinded studies suggesting that catheter-based renal-artery denervation reduces blood pressure in patients with resistant hypertension. As a result, this study did not show a significant difference in the reduction of SBP in those patients 6 months after renal denervation as compared with a sham control.
43. Messerli FH, Bangalore S. Renal denervation for resistant hypertension? N Engl J Med 2014; 370:1454–1457.
44. Guyton AC. Renal function curve – a key to understanding the pathogenesis of hypertension. Hypertension 1987; 10:1–6.
45. Heywood JT, Fonarow GC, Costanzo MR, *et al.* High prevalence of renal dysfunction and its impact on outcome in 118,465 patients hospitalized with acute decompensated heart failure: a report from the ADHERE database. J Card Fail 2007; 13:422–430.
46. Damman K, van Deursen VM, Navis G, *et al.* Increased central venous pressure is associated with impaired renal function and mortality in a broad spectrum of patients with cardiovascular disease. J Am Coll Cardiol 2009; 53:582–588.
47. Mullens W, Abrahams Z, Francis GS, *et al.* Importance of venous congestion for worsening of renal function in advanced decompensated heart failure. J Am Coll Cardiol 2009; 53:589–596.
48. Semple SJ, De Wardener HE. Effect of increased renal venous pressure on circulatory autoregulation of isolated dog kidneys. Circ Res 1959; 7:643–648.
49. Fiksen-Olsen MJ, Strick DM, Hawley H, Romero JC. Renal effects of angiotensin II inhibition during increases in renal venous pressure. Hypertension 1992; 19 (Suppl 2):II137–141.
50. Silverberg DS, Wexler D, Blum M, *et al.* The effect of correction of anaemia in diabetics and nondiabetics with severe resistant congestive heart failure and chronic renal failure by subcutaneous erythropoietin and intravenous iron. Nephrol Dial Transplant 2003; 18:141–146.
51. Stenvinkel P, Heimburger O, Lindholm B, *et al.* Are there two types of malnutrition in chronic renal failure? Evidence for relationships between malnutrition, inflammation and atherosclerosis (MIA syndrome). Nephrol Dial Transplant 2000; 15:953–960.
52. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. *KDIGO clinical practice guideline for the diagnosis, evaluation,* prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Kidney Int Suppl 2009; 113:S1–S130.
53. Ito S, Higuchi Y, Yagi Y, *et al.* Reduction of indoxyl sulfate by AST-120 attenuates monocyte inflammation related to chronic kidney disease. J Leukoc Biol 2013; 93:837–845.
54. Armstrong EJ, Granick JL, Simon SI. Editorial: a missing link? Monocyte activation by uremic toxins in cardiorenal syndrome. J Leukoc Biol 2013; 93:821–823.
55. Barreto DV, Barreto FC, Liabeuf S, *et al.* Plasma interleukin-6 is independently associated with mortality in both hemodialysis and predialysis patients with chronic kidney disease. Kidney Int 2010; 77:550–556.
56. Torre-Amione G, Kapadia S, Benedict C, *et al.* Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). J Am Coll Cardiol 1996; 27:1201–1206.
57. Colombo PC, Ganda A, Lin J, *et al.* Inflammatory activation: cardiac, renal, and cardio-renal interactions in patients with the cardiorenal syndrome. Heart Fail Rev 2012; 17:177–190.
58. ▪▪ Yamada S, Tokumoto M, Tatsumoto N, *et al.* Phosphate overload directly induces systemic inflammation and malnutrition as well as vascular calcification in uremia. Am J Physiol Renal Physiol 2014; 306:F1418–F1428.
This study investigates the effects of dietary phosphate loading on the interactions among inflammation, malnutrition and vascular calcification in CKD using adenine-induced CKD rats fed diets with different phosphate concentrations. Dietary phosphate overload induced systemic inflammation and malnutrition, accompanied by vascular calcification and premature death in CKD, and that inhibition of phosphate loading through dietary or pharmacological interventions prevented these changes, suggesting a pivotal role of malnutrition, inflammation and atherosclerosis syndrome in phosphate-induced vascular calcification in CKD.
59. Yamada S, Taniguchi M, Tokumoto M, *et al.* The antioxidant tempol ameliorates arterial medial calcification in uremic rats: important role of oxidative stress in the pathogenesis of vascular calcification in chronic kidney disease. J Bone Miner Res 2012; 27:474–485.
60. Mann DL, McMurray JJ, Packer M, *et al.* Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). Circulation 2004; 109:1594–1602.
61. Chung ES, Packer M, Lo KH, *et al.* double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003; 107:3133–3140.
62. Torre-Amione G, Anker SD, Bourge RC, *et al.* Results of a nonspecific immunomodulation therapy in chronic heart failure (ACCLAIM trial): a placebo-controlled randomised trial. Lancet 2008; 371:228–236.
63. George J, Patal S, Wexler D, *et al.* Circulating erythropoietin levels and prognosis in patients with congestive heart failure: comparison with neurohormonal and inflammatory markers. Arch Intern Med 2005; 165: 1304–1309.
64. He W, Kang YS, Dai C, Liu Y. Blockade of Wnt/beta-catenin signaling by paricalcitol ameliorates proteinuria and kidney injury. J Am Soc Nephrol 2011; 22:90–103.
65. ▪ Panizo S, Barrio-Vazquez S, Naves-Diaz M, *et al.* Vitamin D receptor activation, left ventricular hypertrophy and myocardial fibrosis. Nephrol Dial Transplant 2013; 28:2735–2744.
In this experimental study, the authors analysed the effect of three kinds of VDRAs, which consisted of calcitriol, paricalcitol and alfacalcidol, on both LVH and myocardial fibrosis in rats with chronic renal failure induced by 7/8 nephrectomy. The results demonstrated that all VDRAs treatment prevented LVH, but only paricalcitol maintained in the normal range all parameters associated with myocardial fibrosis.
66. de Zeeuw D, Agarwal R, Amdahl M, *et al.* Selective vitamin D receptor activation with paricalcitol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomised controlled trial. Lancet 2010; 376:1543–1551.
67. Thadhani R, Appelbaum E, Pritchett Y, *et al.* Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease: the PRIMO randomized controlled trial. JAMA 2012; 307:674–684.
68. ▪ Scialla JJ, Xie H, Rahman M, *et al.* Fibroblast growth factor-23 and cardiovascular events in CKD. J Am Soc Nephrol 2014; 25:349–360.
The authors performed the survival analysis in a prospective cohort of 3860 participants with CKD stages 2–4 to determine whether elevated FGF-23 is associated with a greater risk of adjudicated CHF and atherosclerotic events. The results showed that higher FGF-23 was independently associated with a graded risk of CHF and atherosclerotic events.
69. Faul C, Amaral AP, Oskouei B, *et al.* FGF23 induces left ventricular hypertrophy. J Clin Invest 2011; 121:4393–4408.
70. Shalhoub V, Shatzen EM, Ward SC, *et al.* FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. J Clin Invest 2012; 122:2543–2553.
71. ▪▪ Kuro-o M. A phosphate-centric paradigm for pathophysiology and therapy of chronic kidney disease. Kidney Int Suppl 2013; 3:420–426.
This paper reviews the mechanism of cytotoxicity of hyperphosphatemia in CKD. The author describes that extracellular phosphate exerts its cytotoxicity when it forms insoluble nanoparticles with calcium and fetuin-A, referred to as calciprotein particles (CPPs), which are detected in the blood of animal models and patients with CKD. CPPs are highly bioactive ligands that can induce various cellular responses, including osteogenic transformation of vascular smooth muscle cells and cell death in vascular endothelium and renal tubular epithelium, and associated with adaptation of the endocrine axes mediated by fibroblast growth factor-23 (FGF23) and Klotho that regulate mineral metabolism and aging. These observations have raised the possibility that CPPs may contribute to the pathophysiology of CKD.

J Nephrol
DOI 10.1007/s40620-014-0121-9

ORIGINAL ARTICLE

Hyporesponsiveness to erythropoiesis-stimulating agent as a prognostic factor in Japanese hemodialysis patients: the Q-Cohort study

Rieko Eriguchi · Masatomo Taniguchi · Toshiharu Ninomiya · Hideki Hirakata · Satoru Fujimi · Kazuhiko Tsuruya · Takanari Kitazono

Received: 20 April 2014 / Accepted: 30 June 2014

Abstract
Background Previous epidemiological evidence has suggested that responsiveness to erythropoiesis-stimulating agents (ESAs) is related to prognosis in hemodialysis (HD) patients. We investigated the effects of hyporesponsiveness to ESA on mortality and cardiovascular events in Japanese HD patients, taking modifying factors into account.
Methods A total of 2,905 Japanese HD patients aged ≥18 years who received ESA treatment were prospectively followed up for 4 years. Responsiveness to ESA was estimated using an erythropoietin resistance index (ERI), defined as erythropoietin dosage per week divided by post-HD weight and hemoglobin value (U/kg/week/g/dl). Patients were divided into three groups by tertiles of ERI levels: low ERI, ≤5.10; intermediate ERI, 5.11–9.43; high ERI, ≥9.44. Risk estimates were calculated by a Cox proportional hazards model, adjusting for potential confounders.
Results During follow-up, 482 patients died from any causes. The 4-year survival rate decreased linearly with higher ERI levels, being 87.5, 82.9, and 72.0 % for low, intermediate, and high ERI group (p for trend <0.001). Compared with the low ERI group, the multivariate-adjusted hazard ratio (mHR) was significantly higher in the high ERI group [mHR, 1.64 (95 % confidence interval, 1.27–2.11)]. In the high ERI group, patients with Kt/V ≥ 1.57 had a significantly lower risk of death from any causes compared with those with Kt/V ≤ 1.56 [mHR, 0.73 (0.54–0.98)].
Conclusion Our findings suggest that ESA responsiveness can be considered a significant prognostic factor in Japanese HD patients.

Keywords Hemodialysis · ESA responsiveness · Mortality · Major cardiovascular events

Introduction

Hyporesponsiveness to erythropoiesis-stimulating agents (ESAs) has received attention for its association with mortality in patients receiving maintenance hemodialysis (HD) [1–3]. Epidemiological evidence has suggested that lower hemoglobin levels are associated with poor prognosis in HD patients [4, 5]. Paradoxically, evidence from randomized control trials suggested that treatment with ESA to raise target hemoglobin levels increased the risk of all-cause death in patients on dialysis [6, 7]. A retrospective observational study also found that a higher dose of ESA was associated with increased risk of mortality irrespective of hemoglobin levels [8]. The required dose of ESA to improve anemia varies widely across HD patients, and seems to be influenced by the individual responsiveness to ESA. Therefore, hyporesponsiveness to ESA may be linked to the prognosis in HD patients.

R. Eriguchi · S. Fujimi
Fukuoka Renal Clinic, Fukuoka, Japan

R. Eriguchi · M. Taniguchi · T. Ninomiya · K. Tsuruya · T. Kitazono
Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

H. Hirakata
Division of Nephrology and Dialysis Center, Japanese Red Cross Fukuoka Hospital, Fukuoka, Japan

K. Tsuruya (✉)
Department of Integrated Therapy for Chronic Kidney Disease, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
e-mail: tsuruya@intmed2.med.kyushu-u.ac.jp

Published online: 31 July 2014

The definitions of ESA responsiveness vary among the different clinical studies [1, 2, 9]. The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (KDOQI) and European guidelines define hyporesponsiveness to ESA as failing to achieve target hemoglobin levels while receiving an ESA dosage of >500 U/kg/week [10, 11]. However, the average ESA dose and hemoglobin levels in Japan are lower than those in Western countries [12, 13], and the impact of ESA responsiveness on mortality remains uncertain in populations using relatively lower ESA dosages. In addition, the responsiveness to ESA is likely to be affected by various factors [14–17]. Therefore, it would be of great clinical value to identify the factors that modify the responsiveness to ESA.

Herein, we present some findings from a prospective cohort study, the Q-Cohort study, which was set up to explore the risk factors for comorbidities and mortality in Japanese patients receiving HD treatment. The aim of the present study was to investigate the effects of hyporesponsiveness to ESA on mortality in Japanese HD patients, taking modifying factors into account.

Materials and methods

Study population

The Q-Cohort Study is a multicenter, prospective, longitudinal, observational study conducted in Japanese HD patients [18]. Briefly, a total of 3,598 outpatients aged ≥18 years who underwent HD at 39 dialysis facilities in Fukuoka and Saga prefectures in Kyushu Island, Japan, in December 2006 and 2007 consented to participate in the study. After excluding 562 patients who did not receive ESA therapy, 34 patients who did not have demographic data, 14 patients who did not have available data for the erythropoietin resistance index (ERI), and 83 patients who did not have information regarding outcome, the remaining 2,905 patients were enrolled in the study. The study was conducted with approval from the Kyushu University Institutional Review Board for Clinical Research (Approval Number 20-31). Written informed consent was obtained from all participants. The study was registered in the University Hospital Medical Information Network (UMIN) clinical trial registry (UMIN ID: 000000556), and was performed according to the Ethics of Clinical Research (Declaration of Helsinki) requirements.

Follow-up

The patients were followed up prospectively from the date of their study registration to December 2010. Their health status was checked annually by local physicians at each dialysis facility and by mail or telephone for any patients who moved to other dialysis facilities where no collaborators of the study existed.

Definition of ESA responsiveness

Responsiveness to ESA was estimated using an ERI [2], which was calculated by the following equation:

$$\mathrm{ERI\,(U/kg/week/g/dl)} = \mathrm{weekly\,ESA\,dose\,(U/week)}/(\mathrm{post-HD\,weight\,(kg)} \times \mathrm{Hb\,(g/dl)}).$$

The ESAs used in this study were epoetin α, epoetin β, and darbepoetin α. The ESA dosage for darbepoetin α administration was obtained by multiplying the dosage (μg) of darbepoetin α by 200. The patients were divided into three groups by tertiles of ERI level: low ERI ≤5.10, intermediate ERI 5.11–9.43, high ERI ≥9.44.

Outcomes

The primary outcome was all-cause mortality rate, and the secondary outcome was major cardiovascular events, which were defined as first-ever development of cardiovascular death, stroke, myocardial infarction, hospitalization for unstable angina, coronary intervention (coronary artery bypass surgery or angioplasty), hospitalization for heart failure, and/or peripheral vascular disease. Stroke was defined as sudden onset of a non-occlusive and focal neurological deficit persisting for more than 24 h. Myocardial infarction was defined as a definitive diagnosis based on prolonged severe chest pain, abnormally elevated levels of cardiac biochemical parameters, diagnostic electrocardiographic changes, and morphological changes, including local asynergy of cardiac wall motion on electrocardiography and persistent perfusion defect on cardiac scintigraphy. Unstable angina was defined as a medical condition involving chest pain, abnormally elevated levels of cardiac biomarkers, and diagnostic electrocardiographic changes, without meeting the criteria for myocardial infarction. Heart failure was defined as unplanned presentation to an acute care setting with signs and symptoms that required active treatment for fluid removal. Peripheral vascular disease was defined as gangrene/tissue necrosis, lower limb amputation, and/or revascularization procedure (bypass surgery or angioplasty) for the peripheral vasculature. All events were adjudicated on the basis of patient medical records and imaging performed by the study members.

Risk factor measurements

The demographic information (e.g. age, sex, time on dialysis therapy) and clinical data [e.g. hemoglobin, serum

albumin, serum calcium, serum phosphorus, serum total cholesterol, serum C-reactive protein (CRP), serum ferritin, body mass index (BMI), and Kt/V] were collected at baseline. The physicians at each dialysis facility checked the current use of antihypertensive agents and history of diabetes or cardiovascular disease. Body height and weight were measured in light clothing without shoes, and the BMI (kg/m^2) was calculated. All available data on blood pressure were gathered from the medical records. Blood samples were collected from a vascular access before the initiation of dialysis. Hemoglobin levels were determined using sodium lauryl sulfate. Serum albumin and total cholesterol levels were determined enzymatically. Serum CRP levels were determined by a latex immunity nephelometry measurement method. Serum ferritin levels were measured by chemiluminescent enzyme immunoassay. Dialysis doses were measured by single-pool Kt/V by the Daugirdas method [19].

Statistical analysis

Baseline data are presented as mean (standard deviation), median (interquartile range), or percentage for categorical measures in patients. The linear trends in mean values and frequencies of risk factors across ERI levels were tested by linear regression analysis and logistic regression analysis, respectively. Using baseline data, the risk factors associated with high ERI were explored by multivariate logistic regression analysis with a backward selection procedure ($p < 0.1$), with binary outcomes for high ERI vs. low or intermediate ERI. The event-free survival probabilities for all-cause mortality and major cardiovascular events according to the ERI levels were depicted by the Kaplan–Meier method and compared using a log-rank test. The incidence rates of outcomes in each ERI level were calculated using person-years methods. The hazard ratios (HRs) and 95 % confidence intervals (CIs) of all-cause mortality and major cardiovascular events according to the ERI levels were estimated by a Cox proportional hazards model. In the multivariate-adjusted model, adjustments were made for the following clinically or biologically plausible risk factors for the outcomes: age; sex; dialysis duration; predialysis systolic blood pressure; antihypertensive agent use; diabetes; history of cardiovascular disease; serum albumin; serum calcium; serum phosphorus; serum total cholesterol; serum CRP; serum ferritin; BMI; Kt/V. We also investigated the effects of dialysis dosage on mortality according to ERI subgroups (low or intermediate ERI vs. high ERI). The cutoff points for dialysis dosage were defined according to the median values of 1.56. All statistical analyses were performed using PASW Statistics version 17 software (IBM SPSS, USA). Two-sided values of $p < 0.05$ were considered statistically significant in all analyses.

Results

Study participants and baseline characteristics

Patients' baseline characteristics based on the categories of ERI levels are listed in Table 1. The patients with higher ERI levels were older, more likely to be female and have longer dialysis duration, and less likely to have diabetes. The mean values for serum albumin, serum phosphorus, serum cholesterol, and BMI, and the median values of serum ferritin decreased with increasing ERI levels. In contrast, there were upward trends in the mean values of serum calcium and Kt/V, median values of serum CRP, and frequency of history of cardiovascular disease with higher ERI levels. Considering the definition of ERI, subjects with higher ERI levels clearly had lower hemoglobin levels and higher ESA dosages.

Risk factors for ESA hyporesponsiveness

We examined the risk factors associated with ESA hyporesponsiveness defined as ERI $\geq$ 9.44 U/kg/week/g/dl (i.e. high ERI levels) at baseline (Table 2). As a consequence, the multivariate logistic regression analysis showed that female sex, longer dialysis duration, lower levels of serum albumin, serum total cholesterol, serum ferritin, BMI, and higher CRP level were significantly associated with ESA hyporesponsiveness.

Effects of ESA responsiveness on risk of mortality and major cardiovascular events

During the 4-year follow-up period (median 3.9 years), 482 patients (16.6 %) died of all causes and 500 patients (17.2 %) experienced major cardiovascular events. The survival rates according to the ERI levels are shown in Fig. 1. The 4-year survival rate decreased with higher ERI levels (log rank = 74.0, $p < 0.001$), being 87.5, 82.9, and 72.0 % for low, intermediate and high ERI, respectively. Patients with high ERI levels had a 2.23 times (95 % CI, 1.76–2.81) increased risk of all-cause death than those with low ERI levels after adjustment for age and sex (Table 3). This relationship remained largely unchanged after adjustment for potential confounding factors [HR 1.64 (95 % CI, 1.27–2.11)]. With regard to the major cardiovascular events, higher ERI levels were significantly associated with a lower event-free survival rate for major cardiovascular events (log-rank = 16.6, $p < 0.001$), being 81.2, 81.1, and 74.6 % for low, intermediate, and high ERI, respectively (Fig. 2). The multivariate-adjusted risk of major cardiovascular events increased significantly by 1.38 times (95 % CI, 1.10–1.73) in patients with high ERI levels compared to those with low ERI levels (Table 3).

Table 1 Baseline characteristics according to erythropoietin resistance index levels

Variables (unit)	Erythropoietin resistance index levels			*p* for trend
	Low (≤5.10) ($n = 970$)	Intermediate (5.11–9.43) ($n = 967$)	High (≥9.44) ($n = 968$)	
Age (years)	62.3 (12.5)	64.2 (12.8)	65.9 (13.0)	<0.001
Women (%)	36.2	43.3	50.1	<0.001
Dialysis duration (years)	4.8 (1.9–10.0)	5.1 (2.0–10.0)	5.8 (2.0–12.6)	<0.001
Predialysis systolic blood pressure (mmHg)	153.7 (22.4)	154.5 (22.9)	152.3 (24.5)	0.18
Predialysis diastolic blood pressure (mmHg)	76.8 (12.2)	76.0 (12.6)	75.8 (12.9)	0.07
Antihypertensive agent use (%)	62.0	69.3	65.5	0.11
Diabetes (%)	32.4	30.9	26.8	0.007
History of cardiovascular disease (%)	29.3	32.7	34.8	0.01
Hemoglobin (g/dl)	10.8 (0.9)	10.5 (0.9)	9.9 (1.2)	<0.001
Serum albumin (g/dl)	3.9 (0.4)	3.8 (0.4)	3.6 (0.5)	<0.001
Serum calcium[a] (mg/dl)	9.4 (0.7)	9.4 (0.7)	9.5 (0.8)	<0.001
Serum phosphorus (mg/dl)	5.0 (1.2)	4.9 (1.2)	4.8 (1.3)	<0.001
Serum total cholesterol (mg/dl)	159.8 (37.5)	155.7 (34.2)	149.4 (38.2)	<0.001
Serum c-reactive protein (mg/dl)	0.11 (0.05–0.25)	0.13 (0.05–0.30)	0.15 (0.08–0.47)	<0.001
Serum ferritin (ng/mL)	193 (89-335)	163 (82–304)	163 (69–284)	0.001
Body mass index (kg/m^2)	21.8 (3.1)	21.0 (2.8)	20.1 (2.8)	<0.001
Kt/V (single pool)	1.55 (0.26)	1.59 (0.27)	1.61 (0.30)	<0.001
Dosage of erythropoiesis-stimulating agent (U/week)	2,000 (1,500–2,250)	4,500 (3,000–4,500)	9,000 (6,000–9,000)	<0.001

Values are represented as mean (standard deviation), median (interquartile range), or percentage

[a] Values are corrected by serum albumin

Subgroup analysis

Finally, we estimated the effects of high doses of dialysis on all-cause death according to the ERI levels (Fig. 3). In the high ERI group, patients with Kt/V ≥1.57 had a significantly lower risk of death from any causes than those with Kt/V ≤1.56 in both the age- and sex-adjusted model [HR, 0.73 (95 % CI, 0.55–0.97)] and multivariate-adjusted model [HR, 0.73 (95 % CI, 0.54–0.98)].

Discussion

In the present prospective cohort study of HD patients, we found that hyporesponsiveness to ESA was associated with a higher risk of all-cause mortality and of major cardiovascular events. These associations remained largely unchanged even after adjustment for the potential confounding factors of age, sex, dialysis duration, predialysis systolic blood pressure, antihypertensive agent use, diabetes, history of cardiovascular disease, serum albumin, serum calcium, serum phosphorus, serum total cholesterol, serum CRP, serum ferritin, BMI, and Kt/V. In particular, the high ERI group had a higher risk of mortality and of cardiovascular events. In addition, a higher dose of dialysis was significantly associated with a lower risk of death from any causes. The results suggest that hyporesponsiveness to ESA could be considered as a significant prognostic factor in HD patients, and imply that a higher dose of dialysis could improve the prognosis in patients with ESA hyporesponsiveness.

Several epidemiological studies have examined the association between ESA responsiveness and mortality [1–3]. López-Gómez et al. [2] showed that hyporesponsiveness to ESA estimated by the same indicator used in our study was associated with comorbidities and 1-year survival: the mean ERI was 10.2 ± 7.3 U/kg/week/g/dl and ERI >15 U/kg/week/g/dl was related to poor outcomes. These findings are in accord with our results, although the ERI levels in their study were much higher than our levels. Higher levels of ERI have also been reported in another study from the United States, in which the mean ERI was 15.0 ± 14.1 U/kg/week/g/dl [20]. On the contrary, a study conducted in Japanese HD patients demonstrated that patients with ESA dosage of ≥6,000 U/week, even with a lower dose defined as ESA hyporesponsiveness in the guidelines [10, 11], had significantly higher 1-year mortality from any causes and cardiovascular events among those with hemoglobin levels of <10 g/dl [3]. Furthermore, the results from the Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT) conducted in patients with

Table 2 Risk factors associated with high levels of erythropoietin resistance index

Variables (Unit)	Unadjusted		Multivariate-adjusted	
	OR (95 % CI)	*p* value	OR (95 % CI)	*p* value
Age (per 10 year increment)	1.19 (1.11–1.26)	<0.001	ns	
Women (vs. men)	1.52 (1.30–1.78)	<0.001	1.77 (1.46–2.14)	<0.001
Dialysis duration (per 1 year increment)	1.02 (1.01–1.03)	<0.001	1.02 (1.01–1.03)	0.003
Predialysis systolic blood pressure (per 10 mmHg decrement)	1.04 (1.00–1.07)	0.04	ns	
Antihypertensive agent use (vs. no)	0.99 (0.85–1.17)	0.94	ns	
Diabetes (vs. no)	0.79 (0.66–0.94)	0.007	ns	
History of cardiovascular disease (vs. no)	1.19 (1.01–1.40)	0.04	ns	
Serum albumin (per 0.1 g/dl decrement)	1.15 (1.13–1.18)	<0.001	1.13 (1.11–1.16)	<0.001
Serum calcium (per 0.1 g/dl increment)	1.02 (1.01–1.03)	<0.001	ns	
Serum phosphorus (per 0.1 g/dl decrement)	1.01 (1.00–1.02)	0.001	ns	
Serum total cholesterol (per 10 mg/dl decrement)	1.07 (1.04–1.09)	<0.001	1.07 (1.04–1.10)	<0.001
Log-transformed serum C-reactive protein (per 1 log [mg/dl] increment)	1.37 (1.23–1.52)	<0.001	1.24 (1.10–1.39)	<0.001
Log-transformed serum ferritin (per 1 log [mg/dl] decrement)	1.10 (1.03–1.18)	0.005	1.18 (1.09–1.28)	<0.001
Body mass index (per 1 kg/m^2 decrement)	1.18 (1.14–1.21)	<0.001	1.17 (1.13–1.21)	<0.001
Kt/V (single pool) ($\leq$1.56) (vs. Kt/V $\geq$1.57)	0.96 (0.82–1.12)	0.60	1.20 (1.00–1.45)	0.055

The risk estimates were computed using a multivariate logistic regression model with a backward selection procedure ($p < 0.1$), with binary outcomes for high level vs. low or intermediate level of erythropoietin resistance index

ns not selected, *OR* odds ratio, *CI* confidence interval

type 2 diabetes and chronic kidney disease who were not receiving dialysis showed that the patients who poorly responded to high doses of darbepoetin alfa had the highest risk of cardiovascular events and death [21]. These findings suggest that ESA hyporesponsiveness is associated with a greater risk of mortality and cardiovascular events.

Considering the mechanism underlying the association between ESA responsiveness and mortality, one potential notion is that ESA responsiveness is affected by diverse comorbidity factors [2] and malnutrition-inflammation-atherosclerosis syndrome [16, 17] which is linked to prognosis among HD patients. Malnutrition is closely related to inflammation and arteriosclerosis [22], and through common mediators such as interleukin-6 or tumor necrosis factor-α it may play an important role in ESA resistance [23]. In addition, various clinical factors are considered to influence ESA responsiveness [15, 20, 24]. We found that female sex, longer duration of dialysis, lower serum albumin level, lower serum total cholesterol, lower BMI, higher CRP level, and lower serum ferritin level were significantly associated with hyporesponsiveness to ESA. Lower levels of serum total cholesterol, BMI, and serum albumin may well reflect some aspects of a patient's nutritional status. The serum CRP level is a biomarker of inflammation. Serum ferritin is a marker of both iron stores and inflammation. Therefore, it may be reasonable to suppose that optimal management of nutritional status, chronic inflammation, and iron metabolism would improve hyporesponsiveness to ESA.

In this study, we demonstrated that a higher dose of dialysis was significantly associated with lower mortality in the high ERI group, even after correction for differences in the underlying conditions at baseline. Recent reports described that Kt/V $\geq$1.6 was associated with a survival advantage in HD patients [25], due to improved medium molecule clearance, rather than only small molecule clearance [26]. In addition, longer HD treatment time could more effectively remove larger molecules, such as beta-2 microglobulin, the accumulation of which is implicated as a cause of adverse outcomes in dialysis patients [27]. Therefore, it may be reasonable to suppose that a higher dose of dialysis could be effective in improving the prognosis of HD patients with hyporesponsiveness to ESA. Nevertheless, we cannot exclude the possibility that this finding from our observational study is affected by residual confounding. An ongoing randomized control trial addressing the effect of intensive dialysis on the prognosis of HD patients, A Clinical Trial of IntensiVE dialysis (ACTIVE) (Clinical Trials gov number, NCT00649298), may elucidate this issue, if relevant subgroup analyses are performed.

The strength of this study is that it is a prospective cohort study with a longer duration of follow-up than previous reports. Several limitations of the present study should be noted. First, the generalizability of our findings may be limited. Although most (97 %) of the patients at the participating facilities were recruited for this study, the participating facilities may not be representative of all Japanese HD centers. On the contrary, as the participating

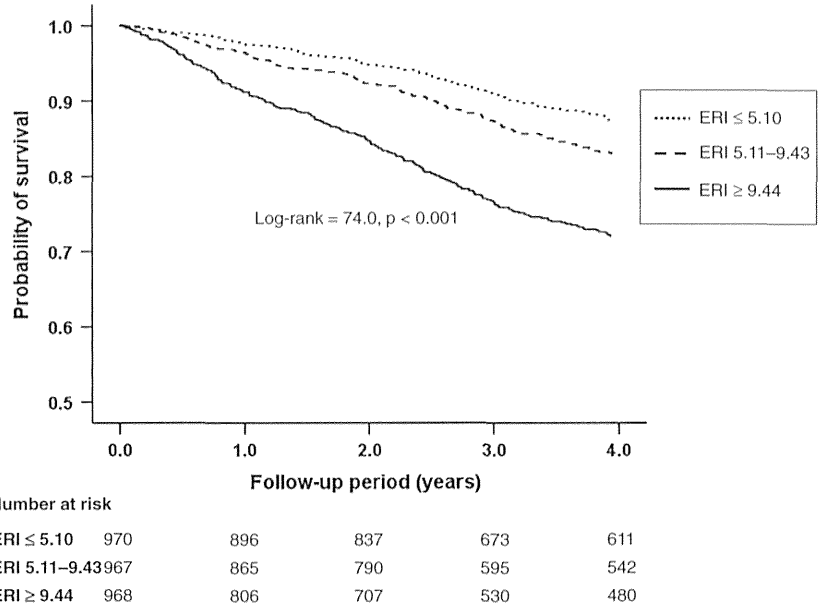

Fig. 1 Survival rates according to the erythropoietin resistance index levels during the 4-year follow-up period. *ERI* erythropoietin resistance index

Table 3 Associations between erythropoietin resistance index levels and risks of all-cause mortality and cardiovascular events

ERI levels	Median ERI	No. of events/ patients	Incidence rate	Age- and sex-adjusted				Multivariate-adjusted[a]			
				HR	95 % CI	*p*	*p* for trend	HR	95 % CI	*p*	*p* for trend
All-cause death											
ERI (per 1 increment)	8.68	482/2,905	0.053	1.06	1.05–1.07	<0.001		1.03	1.01–1.04	<0.001	
Low (≤5.10)	3.35	106/970	0.033	1.00	reference		<0.001	1.00	reference		<0.001
Intermediate (5.11–9.43)	7.07	141/967	0.046	1.29	1.00–1.67	0.047		1.21	0.94–1.56	0.15	
High (≥9.44)	14.4	235/968	0.084	2.23	1.76–2.81	<0.001		1.64	1.27–2.11	<0.001	
Major cardiovascular event											
ERI (per 1 increment)	8.68	500/2,905	0.059	1.03	1.01–1.04	<0.001		1.03	1.01–1.04	0.001	
Low (≤5.10)	3.35	157/970	0.051	1.00	reference		0.001	1.00	reference		0.002
Intermediate (5.11–9.43)	7.07	151/967	0.052	0.98	0.79–1.23	0.89		0.95	0.76–1.20	0.67	
High (≥9.44)	14.4	192/968	0.075	1.41	1.14–1.75	0.002		1.38	1.10–1.73	0.006	

ERI erythropoietin resistance index, *HR* hazard ratio, *CI* confidence interval

[a] Adjusted for age, sex, dialysis duration, predialysis systolic blood pressure, antihypertensive agent use, diabetes, history of cardiovascular disease, serum albumin, serum calcium, serum phosphorus, serum total cholesterol, log-transformed serum C-reactive protein, log-transformed serum ferritin, body mass index, and Kt/V

facilities share similar treatment strategies for HD patients, the management of patients (e.g. blood pressure lowering, condition of HD) would be less heterogeneous than that of other reports. Second, the ERI data were obtained at a single time point (baseline examination). This may have caused misclassification of study participants into different categories. Such misclassification, if present, would weaken the association found in this study, biasing the results toward the null hypothesis. Finally, we were unable to obtain information about the risk factors and medical treatments prescribed during the follow-up period. The lack of this information may have reduced the accuracy of our findings to some extent. However, we believe that our findings provide useful information toward a better understanding of the association between ESA hyporesponsiveness and mortality.