EXTRA nephron Journals

Nephron Extra 2012;2:159–168
DOI: 10.1159/000339437
Published online: June 20, 2012

Original Paper

DNA Hypermethylation and Inflammatory Markers in Incident Japanese Dialysis Patients

Sawako Kato[a] Bengt Lindholm[d] Peter Stenvinkel[d]
Tomas J. Ekström[e] Karin Luttropp[e] Yukio Yuzawa[b]
Yoshinari Yasuda[a] Yoshinari Tsuruta[c] Shoichi Maruyama[a]

[a]Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, [b]Fujita Health University School of Medicine, Toyoake, and [c]Meiyo Clinic, Toyahashi, Japan; [d]Divisions of Renal Medicine and Baxter Novum, Department of Clinical Science, Intervention and Technology, and [e]Division of Neurogenetics, Department of Molecular Medicine and Surgery, Center of Molecular Medicine, Karolinska Institutet, Stockholm, Sweden

Key Words
Chronic kidney disease · DNA methylation · Ferritin · Infection · Inflammation · Procalcitonin

Abstract
Background/Aims: Inflammation is an established mortality risk factor in chronic kidney disease (CKD) patients. Although a previous report showed that uremic Caucasian patients with inflammation had signs of global DNA hypermethylation, it is still unknown whether DNA hypermethylation is linked to inflammatory markers including a marker of bacterial infections in Japanese CKD patients. ***Methods:*** In 44 consecutive incident dialysis patients (26 males, mean age 59 ± 12 years) without clinical signs of infection, global DNA methylation was evaluated in peripheral blood DNA using the *Hpa*II/*Msp*I ratio by the luminometric methylation assay method. A lower ratio of *Hpa*II/*Msp*I indicates global DNA hypermethylation. Procalcitonin (PCT), a marker of inflammation due to bacterial infections, was measured using an immunochromatographic assay. ***Results:*** The patients were divided into hyper- and hypomethylation groups based on the median value of the *Hpa*II/*Msp*I ratio 0.31 (range 0.29–0.37). Whereas patients in the hypermethylation group had higher ferritin levels [133.0 (51.5–247.3) vs. 59.5 (40.0–119.0) ng/ml; p = 0.046], there were no significant differences in age, gender, diabetes, smoking, anemia

Sawako Kato, MD, PhD
Department of Nephrology
Nagoya University Graduate School of Medicine
65, Tsuruma-cho, Showa-ku, Nagoya, Aichi 464-8550 (Japan)
Tel. +81 52 744 2192, E-Mail sawako_riku@mac.com

KARGER

Nephron Extra 2012;2:159–168
DOI: 10.1159/000339437
Published online: June 20, 2012

www.karger.com/nne

or serum albumin levels. However, the *Hpa*II/*Msp*I ratio showed significant negative correlations with PCT ($\rho = -0.32$, $p = 0.035$) and ferritin ($\rho = -0.33$, $p = 0.027$) in Spearman's rank test. In a multiple linear regression analysis, PCT and ferritin were associated with a lower *Hpa*II/*Msp*I ratio ($R^2 = 0.24$, $p = 0.013$). ***Conclusion:*** In this study, global DNA hypermethylation was associated with ferritin and, most likely, PCT, suggesting that inflammation induced by subclinical bacterial infection promoted DNA methylation.

Introduction

Although chronic kidney disease (CKD) patients show increased mortality compared to the general population, the mechanisms for this process of accelerated ageing are not yet fully understood. Epigenetics is the study of changes in gene expression excluding changes in DNA sequence. These reversible modifications include DNA methylation, histone acetylation and RNA interference. As epigenetic changes are fundamental for the physiological processes that regulate gene activity, it can be assumed that DNA methylation is of importance not only for the development of malignant diseases [1] but also for cardiovascular disease (CVD) [2] and CKD [3]. Aberrant DNA methylation is affected by age, gender, nutritional disorders, lifestyle characteristics, infections [4] and other factors. In the uremic milieu, several features, such as inflammation, hyperhomocysteinema, oxidative stress, dyslipidemia, as well as vitamin and nutritional deficiencies, may affect the epigenome [5]. Stenvinkel et al. [6] reported that uremic patients with inflammation showed signs of global DNA hypermethylation, which was associated with CVD and mortality.

Infectious complications contribute significantly to the increased hospitalization rate in CKD patients who progress to end-stage renal disease and to the high mortality rate among dialysis patients [7, 8]. Various factors including immune dysfunction, protein-energy wasting and comorbid conditions, such as diabetes, dental illness, vascular access devices and immunosuppression drugs, lead to an increased risk of infections in this patient group [9]. The risk of cardiovascular events increase after hospitalization related to infection [10], and cardiac complications worsen the outcomes of pneumonia in CKD patients [11]. Thus, there may be links between infection, inflammation and an increased risk of cardiovascular morbidity and mortality [12]. Indeed, it has been reported that during the 30 days following an infection-related hospitalization, the risk of cardiovascular events increases by 25% in dialysis patients [10].

It is established that inflammatory biomarkers, such as C-reactive protein (CRP), are strong predictors of poor outcome in CKD patients [13]. Ferritin levels have also shown to reflect the inflammatory status in dialysis patients [14]. Procalcitonin (PCT), a precursor of calcitonin and a polypeptide of 116 amino acids (with a molecular weight of 13 kDa), is a biomarker of inflammation induced by bacterial infection [15]. Serum PCT (sPCT) has been reported to increase during bacterial infections in CKD patients [16]. Practically, sources of persistent low-grade inflammation in CKD patients have often been vague. Central catheters [17], periodontal disease [18] and bacterial translocation from the gastrointestinal tract [19] are often verified or suspected as causes of chronic inflammation, but it is likely that many unrecognized cases of subclinical infections with opportunistic pathogens also contribute [20]. The aim of this study is to clarify if inflammation evaluated by CRP and ferritin has an impact on the DNA methylation status and if subclinical bacterial infections detected by PCT levels are involved with this mechanism in Japanese incident dialysis patients.

Nephron Extra 2012;2:159–168
DOI: 10.1159/000339437
Published online: June 20, 2012

www.karger.com/nne

Subjects and Methods

Subjects

We enrolled 44 Japanese CKD stage 5 patients (26 males and 18 females, mean age 59 ± 12 years) at the initiation of maintenance hemodialysis (HD) or peritoneal dialysis (PD) from June 2007 to August 2009 at Masuko Memorial Hospital and Meiyo Clinic in Aichi prefecture, Japan. This study is an observational study approved by the Ethics Committee of Nagoya University Graduate School of Medicine; informed consent to participate in this study was obtained from all patients. Exclusion criteria were age older than 75 years, signs of acute infectious complications, severe liver dysfunction and unwillingness to participate.

The primary causes of renal disease were glomerulonephritis (n = 10), nephrosclerosis (n = 4), diabetic nephropathy (n = 25) due to diabetes mellitus type 1 (n = 3) and type 2 (n = 22), polycystic kidney disease (n = 1) and other (n = 2) or unknown causes (n = 2). Among 39 patients starting HD, blood access was in most cases obtained by an arteriovenous fistula (n = 36); 3 patients had a graft, and only 1 patient received a double-lumen catheter into his jugular vein but showed no signs of infection. The remaining 5 patients started on PD, and all received a peritoneal catheter in advance when initiation of PD was decided. As mentioned above, patients with apparent current infection were not included in the study; however, 1 patient had hepatitis B and 5 patients had hepatitis C.

Blood Sampling and Laboratory Analysis

Blood samples were collected from all subjects before the start of maintenance dialysis therapy. Hemoglobin, leukocyte counts, platelet counts, serum albumin, total cholesterol, high-density lipid cholesterol, ferritin, creatinine, CRP and intact parathyroid hormone were measured by routine procedures at the clinical laboratory in each facility. sPCT levels were measured using an immunochromatographic assay (BRAHMS Corp, Hennigsdorf, Germany) using the samples kept frozen in –30°C. Estimated glomerular filtration rate (eGFR) was calculated from creatinine values according to the result of a Japanese study: eGFR (ml/min/1.73 m^2) = 194 × $SCr^{-1.094}$ × $Age^{-0.287}$ × 0.739 (if female) [21]. The Subjective Global Assessment (SGA) was used to evaluate the nutritional status [22]. In brief, each patient was given a score from medical history focused on weight loss, gastrointestinal symptoms and functional capacity and from physical examination focused on loss of subcutaneous fat and muscle, and presence of edema. We classified the patients into three groups based on their SGA score: A = well nourished, B = mild/moderately malnourished and C = severely malnourished.

Measurements of DNA Methylation by LUMA

From a 5-ml EDTA sample of peripheral blood, DNA was extracted using QIAamp® DNA kit. Restriction enzymes (*Hpa*II, *Msp*I and *Eco*RI) were purchased from New England Biolabs (Beverly, Mass., USA). PSQ™ 96 SNP reagents for pyrosequencing were purchased from Biotage AB (Uppsala, Sweden). DNA quantification was performed using the RediPlate™ 96 PicoGreen®kit from Molecular Probes (Eugene, Oreg., USA). LUMA was run as described elsewhere in detail [23]. Briefly, genomic DNA (200–500 ng) was cleaved with *Hpa*II + *Eco*RI or *Msp*I + *Eco*RI in two separate reactions and was run in a 96-well format. Each reaction was performed in duplicates. The digestion reactions were run in a PSQ96™ MA system (Biotage AB). Peak heights were calculated using the PSQ96™ MA software. The *Hpa*II/*Eco*RI and *Msp*I/*Eco*RI ratios were calculated as dCTP/(dATP + dTTP) for the respective reactions. The *Hpa*II/*Msp*I ratio was defined as (*Hpa*II/*Eco*RI)/(*Msp*I/*Eco*RI).

Nephron Extra 2012;2:159–168
DOI: 10.1159/000339437
Published online: June 20, 2012

www.karger.com/nne

Table 1. Clinical characteristics of the study participants

Clinical characteristics	Total	Global DNA methylation status		p value
		*Hpa*II/*Msp*I >median	*Hpa*II/*Msp*I <median	
Patients, n	44	22	22	
Male, n (%)	26 (59.0)	12 (54.6)	14 (63.6)	0.54
Mean age ± SD, years	59 ± 12	59 ± 12	59 ± 11	0.97
Mean body mass index ± SD	21.8 ± 3.0	21.7 ± 3.1	21.9 ± 3.0	0.72
Diabetes mellitus, n (%)	26 (59.1)	11 (50.0)	15 (68.2)	0.22
Smoking, n (%)				0.28
Current smoker	9 (20.4)	3 (13.6)	6 (27.3)	
Ex-smoker	16 (36.4)	7 (31.8)	9 (40.9)	
Medication, n (%)				
ACE-I/ARBs	29 (65.9)	16 (72.7)	13 (59.1)	0.34
Statins	13 (29.5)	5 (22.7)	8 (36.4)	0.32
Vitamin D	25 (56.8)	13 (59.1)	12 (54.6)	0.76
ESA	37 (84.1)	19 (86.4)	18 (81.2)	0.68
IV iron supplementation	12 (27.3)	5 (22.7)	7 (31.8)	0.50
Oral iron supplementation	6 (14.0)	3 (13.6)	3 (13.6)	1.00
Protein energy wasting, n (%)				0.94
SGA category B (mildly–moderately)	29 (65.9)	15 (68.2)	14 (63.6)	
SGA category C (severely)	4 (9.1)	2 (9.1)	2 (9.1)	
History of CVD	12 (27.3)	5 (22.7)	7 (31.8)	0.63

ACE-I = Angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; ESA = erythropoiesis-stimulating agent; IV = intravenous.

Statistical Analysis

Data are presented as mean ± SD and/or median and interquartile range (25th–75th percentiles). A p value <0.05 was considered statistically significant. For comparisons between groups, the Wilcoxon rank sum test was used. Nominal variables were tested using the χ^2 test. Spearman's rank correlation analysis was used to determine association with *Hpa*II/*Msp*I ratio and selected laboratory biomarkers. Multivariate linear regression analysis was used to assess independent predictors of the *Hpa*II/*Msp*I ratio. All statistical analyses were performed using statistical software JMP version 8.0.1 (SAS Campus Drive, Cary, N.C., USA).

Results

Characteristics and Laboratory Biomarkers

The clinical characteristics are reported in table 1. The patients comprised 26 males (59%) with an average age of 59 ± 12 years (interquartile range 57–67). No patient was on steroids or any other immunosuppressive drugs. The median *Hpa*II/*Msp*I ratio was 0.31 (0.29–0.37). The patients were divided into hyper- and hypomethylation groups based on their median *Hpa*II/*Msp*I ratio. A lower ratio of *Hpa*II/*Msp*I indicates global DNA hypermethylation. There was no significant difference in age, gender, diabetes, smoking habit, nutritional status or medications between the two methylation groups (table 1).

Nephron Extra 2012;2:159–168
DOI: 10.1159/000339437
Published online: June 20, 2012

www.karger.com/nne

Table 2. Laboratory biomarkers

Laboratory biomarkers	Total	Global DNA methylation status		p value
		*Hpa*II/*Msp*I >median	*Hpa*II/*Msp*I <median	
Hemoglobin, g/dl	8.70 ± 1.3	8.86 ± 1.4	8.56 ± 1.2	0.66
White blood cells, $\times 10^3$/mm^3	5.70 ± 1.7	5.40 ± 1.5	6.00 ± 1.9	0.39
Thrombocytes, $\times 10^3$/mm^4	20.4 ± 5.8	20.4 ± 6.2	20.5 ± 5.5	0.72
Albumin, g/dl	3.41 ± 0.6	3.40 ± 0.7	3.42 ± 0.5	0.82
Total cholesterol, mg/dl	172.3 ± 38.8	178.4 ± 33.6	166.1 ± 43.3	0.10
HDL cholesterol, mg/dl	43.5 ± 12.4	44.6 ± 8.8	42.5 ± 15.3	0.16
UA, mg/dl	8.43 ± 2.2	8.57 ± 2.7	8.29 ± 1.6	0.75
eGFR, ml/min	4.86 ± 1.8	4.89 ± 1.5	4.83 ± 2.1	0.51
Intact PTH, pg/ml	419 ± 248	415 ± 251	405 ± 252	0.97
Ferritin, ng/ml	147.3 ± 178.8	92.9 ± 83.3	201.6 ± 228.8	0.046*
Median (range)	81.5 (43.3–189.5)	59.5 (40.0–119.0)	133.0 (51.5–247.3)	
CRP, mg/dl	0.349 ± 0.718	0.211 ± 0.301	0.487 ± 0.962	0.83
Median (range)	0.06 (0.02–0.33)	0.078 (0.028–0.311)	0.060 (0.023–0.635)	
sPCT, ng/ml	0.134 ± 0.166	0.107 ± 0.09	0.161 ± 0.215	0.95
Median (range)	0.080 (0.030–0.188)	0.075 (0.0357–0.153)	0.080 (0.028–0.196)	

Data presented as mean ± SD, unless otherwise indicated.
HDL = High-density lipoprotein; UA = uric acid; PTH = parathyroid hormone.
* $p < 0.05$.

Table 2 shows laboratory biomarkers. The median sPCT was 0.080 ng/ml (0.030–0.188). Although patients with apparent current infections had not been enrolled according to the exclusion criteria, sPCT levels were slightly increased in 3 patients up to the upper limit of normal level (0.50 ng/ml). Whereas patients in the hypermethylation group had higher ferritin levels [133.0 (51.5–247.3) vs. 59.5 (40.0–119.0) ng/ml; p = 0.046], there was no significant difference in anemia, serum albumin levels and other inflammatory markers between the two groups.

Correlation between Global DNA Methylation Status and Inflammatory Biomarkers

We investigated CRP, ferritin and PCT as inflammatory markers, global DNA methylation status, and albumin and SGA scores as nutritional parameters. As shown in table 3, the *Hpa*II/*Msp*I ratio showed significant negative correlations with PCT ($\rho = -0.32$, $p = 0.035$) and ferritin ($\rho = -0.33$, $p = 0.027$). CRP was positively correlated with PCT ($\rho = 0.31$, $p = 0.049$) and ferritin ($\rho = 0.37$, $p = 0.014$). Serum albumin and SGA score were not correlated with the *Hpa*II/*Msp*I ratio. Since a lower ratio of *Hpa*II/*Msp*I means global DNA hypermethylation, a more severe inflammatory status was associated with accelerated DNA methylation.

Multivariate Regression Analysis for Global DNA Methylation Status

Next, we investigated the contributing factors to the *Hpa*II/*Msp*I ratio in a multivariate linear regression analysis. PCT and ferritin, but not CRP, were associated with a lower *Hpa*II/*Msp*I ratio ($R^2 = 0.24$; table 4).

Nephron Extra 2012;2:159–168
DOI: 10.1159/000339437
Published online: June 20, 2012

www.karger.com/nne

Table 3. Correlations between *Hpa*II/*Msp*I ratio, inflammatory markers and nutritional parameters

		WBC	Albumin	SGA	CRP	Ferritin	PCT
*Hpa*II/*Msp*I ratio	ρ	–0.1826	0.1684	–0.1103	–0.1273	–0.3343	–0.3226
	p	NS	NS	NS	NS	0.0266*	0.0348*
WBC	ρ		–0.2937	–0.087	0.1128	0.2495	0.0194
	p		NS	NS	NS	NS	NS
Albumin	ρ			0.1237	–0.1147	–0.1527	–0.0854
	p			NS	NS	NS	NS
SGA	ρ				–0.0821	–0.1394	–0.0606
	p				NS	NS	NS
CRP	ρ					0.3739	0.3059
	p					0.0135*	0.0488*
Ferritin	ρ						0.1299
	p						NS

NS = Not significant; WBC = white blood cells. * $p < 0.05$.

Table 4. Multivariate regression model predicting *Hpa*II/*Msp*I ratio in CKD stage 5 patients

Parameter	Parameter estimate	Standard error	p value
Intercept	0.450	0.079	<0.0001
Age >61 years	–0.025	0.023	0.3
Female gender	0.010	0.012	0.4
CRP	0.007	0.008	0.4
PCT	–0.025	0.011	0.034*
Ferritin	–0.030	0.013	0.029*

The adjusted R^2 of the model was 0.24. Age was dichotomized by the median value. * $p < 0.05$.

Discussion

Following death due to CVD, infection-related death is the second most common cause of death in CKD patients, accounting for about 20% of the mortality in CKD stage 5 patients [7]. Alterations of the immune system in the uremic milieu are linked to the susceptibility to infections as well as to immune activation, resulting in persistent inflammation that accelerates atherosclerosis and CVD mortality [12]. The possible factors by which a chronic subclinical inflammatory state could be related to increasing CVD risk induced by infections include endothelial dysfunction [24] and an altered coagulation system [25]. It has been reported that infections might precipitate overt CVD through activation of systemic inflammation [26].

In the present study, we investigated global DNA methylation and inflammatory biomarkers, including sPCT as a marker of asymptomatic bacterial infections, in an observational study of Japanese incident dialysis patients. Unlike other inflammatory markers, sPCT

Nephron Extra 2012;2:159–168
DOI: 10.1159/000339437
Published online: June 20, 2012

www.karger.com/nne

does not increase, or is only slightly elevated, in viral, localized infections, autoimmune diseases and during stress following surgical operations [27]. Thus, sPCT is considered to be a useful marker to distinguish bacterial infections from non-infectious inflammatory disease [28, 29]. In our study, sPCT concentrations showed a mean of 0.13 ± 0.17 ng/ml, and sPCT levels in 3 patients were slightly above the level of 0.5 ng/ml, although none of the patients had signs of current infection. As we found a positive association between sPCT and CRP, patients who showed signs of inflammation may be those who had contracted latent bacterial infections.

DNA methylation is a key mechanism for control of gene expression. The global DNA methylation level generally decreases with aging and is lower in males than in females [30]. In our study, whereas DNA hypermethylation was consistently associated with elevated ferritin levels in all there statistical methods [comparisons between the two groups divided by DNA methylation status (Wilcoxon rank sum test), Spearman's rank test and multivariate linear regression analysis], we could find statistically significant differences between DNA hypermethylation and PCT levels by only the latter two methods. We deduce this discrepancy from the findings that PCT levels were more deviated from normal distribution and more centralized in lower layers because we enrolled patients without obvious infections. We could not find any association between DNA hypermethylation and CRP levels. CRP is a common inflammatory marker but is non-specific. We speculate that various kinds of inflammatory and non-inflammatory stimuli could alter DNA methylation [31], while there perhaps might be a certain kind of inflammation that is not related to aberrant DNA methylation. Moreover, CRP is a rapidly moving target. We also speculate that DNA methylation should be altered by chronic inflammation inducing dysfunction of iron metabolism with increasing ferritin levels, and that CRP might be not enough to detect low-grade inflammations, especially in Japanese CKD patients because their CRP levels seem naturally inclined to be much lower than those in Western CKD patients [32, 33].

Few studies on DNA methylation have been published in the context of uremia. Ingrosso et al. [34] reported that global DNA methylation in a selected group of maintenance HD patients was lower than that in healthy controls, and this hypomethylation status was associated with hyperhomocysteinemia. In stage 2–4 CKD patients, the global DNA methylation level was not associated with renal function and atherosclerosis [35]. Another study of unselected incident and prevalent dialysis patients showed that inflammation was associated with global DNA hypermethylation – a feature associated with increased mortality [6]. Although the basic epigenetic status of the DNA is set, change basically refers to a heritable change and influenced maternal factor in utero and is, to a large extent, heritable. DNA methylation patterns may fluctuate in response to changes in inherited genetic polymorphisms, diet and environmental factors, such as uremic toxins, sodium and infections in the uremic milieu [3, 36]. These results imply that environmental factors have the power to alter the epigenetic code and, ultimately, the phenotype. Further indirect support for our finding is a study showing that bacterial endotoxin altered DNA methylation and gene expression in an animal model [37]. Moreover, chronic inflammation by infection of *Helicobacter pylori* induced cell proliferation via DNA hypermethylation [38], which suggests that inflammation induced by bacterial infection and/or bacterial toxins may have the potential to alter DNA methylation status.

Some limitations of this study should be acknowledged. First, the small sample size makes it difficult to draw firm conclusions. Second, although we analyzed sPCT levels, we could not define the exact infectious cause of inflammation. We checked inflammatory markers including PCT at only one time close to the start of dialysis, while serial measurements would have been more informative. Third, because we did not compare the DNA methylation status to clinical outcomes, we could not evaluate if DNA methylation predicts

outcome. Finally, we did not analyze DNA methylation status in age- and gender-matched healthy controls.

In conclusion, the present study demonstrates that global DNA hypermethylation is associated with elevated inflammatory markers including PCT in Japanese incident dialysis patients. Our results suggest that inflammation may play a role in DNA hypermethylation, and subclinical bacterial infection may be, in part, involved in this mechanism, although further studies are needed to clarify the role of aberrant DNA methylation in the premature mortality of CKD patients.

Acknowledgements

We thank Wako Pure Chemical Industries for the measurements of sPCT. Baxter Novum is the result of a grant from Baxter Healthcare Corporation to the Karolinska Institute. This study has no financial support.

Disclosure Statement

Bengt Lindholm is an employee of Baxter Healthcare Corporation. Peter Stenvinkel is a member of the Scientific Advisory Board of Gambro.

References

1 Boumber Y, Issa JP: Epigenetics in cancer: what's the future? Oncology 2011;25:220–226, 228.
2 Ordovas JM, Smith CE: Epigenetics and cardiovascular disease. Nat Rev Cardiol 2010;7:510–519.
3 Dwivedi RS, Herman JG, McCaffrey TA, Raj DS: Beyond genetics: epigenetic code in chronic kidney disease. Kidney Int 2011;79:23–32.
4 Lambert MP, Paliwal A, Vaissiere T, Chemin I, Zoulim F, Tommasino M, Hainaut P, Sylla B, Scoazec JY, Tost J, Herceg Z: Aberrant DNA methylation distinguishes hepatocellular carcinoma associated with HBV and HCV infection and alcohol intake. J Hepatol 2011;54:705–715.
5 Stenvinkel P, Ekstrom TJ: Epigenetics – a helpful tool to better understand processes in clinical nephrology? Nephrol Dial Transplant 2008;23:1493–1496.
6 Stenvinkel P, Karimi M, Johansson S, Axelsson J, Suliman M, Lindholm B, Heimburger O, Barany P, Alvestrand A, Nordfors L, Qureshi AR, Ekstrom TJ, Schalling M: Impact of inflammation on epigenetic DNA methylation – a novel risk factor for cardiovascular disease? J Intern Med 2007;261:488–499.
7 Sarnak MJ, Jaber BL: Mortality caused by sepsis in patients with end-stage renal disease compared with the general population. Kidney Int 2000;58:1758–1764.
8 Mix TC, St Peter WL, Ebben J, Xue J, Pereira BJ, Kausz AT, Collins AJ: Hospitalization during advancing chronic kidney disease. Am J Kidney Dis 2003;42:972–981.
9 Quori A, Baamonde-Laborda E, Garcia-Canton C, Lago-Alonso MM, Toledo-Gonzalez A, Monzon-Jimenez E, Jimenez-Diaz D, Checa-de-Andres M, Molina-Cabrillana J: Surveillance for infections and other adverse events in dialysis patients in southern Gran Canaria (in Spanish). Nefrologia 2011;31:457–463.
10 Dalrymple LS, Mohammed SM, Mu Y, Johansen KL, Chertow GM, Grimes B, Kaysen GA, Nguyen DV: Risk of cardiovascular events after infection-related hospitalizations in older patients on dialysis. Clin J Am Soc Nephrol 2011;6:1708–1713.
11 Viasus D, Garcia-Vidal C, Cruzado JM, Adamuz J, Verdaguer R, Manresa F, Dorca J, Gudiol F, Carratala J: Epidemiology, clinical features and outcomes of pneumonia in patients with chronic kidney disease. Nephrol Dial Transplant 2011;26:2899–2906.

12 Kato S, Chmielewski M, Honda H, Pecoits-Filho R, Matsuo S, Yuzawa Y, Tranaeus A, Stenvinkel P, Lindholm B: Aspects of immune dysfunction in end-stage renal disease. Clin J Am Soc Nephrol 2008; 3:1526–1533.
13 Stenvinkel P: C-reactive protein – does it promote vascular disease? Nephrol Dial Transplant 2006; 21:2718–2720.
14 Akdag I, Yilmaz Y, Kahvecioglu S, Bolca N, Ercan I, Ersoy A, Gullulu M: Clinical value of the malnutrition-inflammation-atherosclerosis syndrome for long-term prediction of cardiovascular mortality in patients with end-stage renal disease: a 5-year prospective study. Nephron Clin Pract 2008; 108:c99–c105.
15 Weglohner W, Struck J, Fischer-Schulz C, Morgenthaler NG, Otto A, Bohuon C, Bergmann A: Isolation and characterization of serum procalcitonin from patients with sepsis. Peptides 2001;22:2099–2103.
16 Steinbach G, Bölke E, Grünert A, Störck M, Orth K: Procalcitonin in patients with acute and chronic renal insufficiency. Wien Klin Wochenschr 2004;116:849–853.
17 Yao Q, Axelsson J, Heimburger O, Stenvinkel P, Lindholm B: Systemic inflammation in dialysis patients with end-stage renal disease: causes and consequences. Minerva Urol Nefrol 2004;56:237–248.
18 Borawski J, Wilczynska-Borawska M, Stokowska W, Mysliwiec M: The periodontal status of predialysis chronic kidney disease and maintenance dialysis patients. Nephrol Dial Transplant 2007;22: 457–464.
19 Kotanko P, Carter M, Levin NW: Intestinal bacterial microflora – a potential source of chronic inflammation in patients with chronic kidney disease. Nephrol Dial Transplant 2006;21:2057–2060.
20 Cazzavillan S, Ratanarat R, Segala C, Corradi V, de Cal M, Cruz D, Ocampo C, Polanco N, Rassu M, Levin N, Ronco C: Inflammation and subclinical infection in chronic kidney disease: a molecular approach. Blood Purif 2007;25:69–76.
21 Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A: Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982–992.
22 Detsky AS, McLaughlin JR, Baker JP, Johnston N, Whittaker S, Mendelson RA, Jeejeebhoy KN: What is subjective global assessment of nutritional status? JPEN J Parenter Enteral Nutr 1987;11:8–13.
23 Karimi M, Johansson S, Ekström TJ: Using LUMA: a Luminometric-based assay for global DNA-methylation. Epigenetics 2006;1:45–48.
24 Ait-Oufella H, Maury E, Lehoux S, Guidet B, Offenstadt G: The endothelium: physiological functions and role in microcirculatory failure during severe sepsis. Intensive Care Med 2010;36:1286–1298.
25 Herzberg MC: Coagulation and thrombosis in cardiovascular disease: plausible contributions of infectious agents. Ann Periodont 2001;6:16–19.
26 Sims JB, de Lemos JA, Maewal P, Warner JJ, Peterson GE, McGuire DK: Urinary tract infection in patients with acute coronary syndrome: a potential systemic inflammatory connection. Am Heart J 2005;149:1062–1065.
27 Becker KL, Snider R, Nylen ES: Procalcitonin assay in systemic inflammation, infection, and sepsis: clinical utility and limitations. Crit Care Med 2008;36:941–952.
28 Uzzan B, Cohen R, Nicolas P, Cucherat M, Perret GY: Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med 2006;34:1996–2003.
29 Monneret G, Doche C, Durand DV, Lepape A, Bienvenu J: Procalcitonin as a specific marker of bacterial infection in adults. Clin Chem Lab Med 1998;36:67–68.
30 Zhu ZZ, Hou L, Bollati V, Tarantini L, Marinelli B, Cantone L, Yang AS, Vokonas P, Lissowska J, Fustinoni S, Pesatori AC, Bonzini M, Apostoli P, Costa G, Bertazzi PA, Chow WH, Schwartz J, Baccarelli A: Predictors of global methylation levels in blood DNA of healthy subjects: a combined analysis. Int J Epidemiol 2010;41:126–139.
31 Schlinzig T, Johansson S, Gunnar A, Ekstrom TJ, Norman M: Epigenetic modulation at birth – altered DNA-methylation in white blood cells after caesarean section. Acta Paediatr 2009;98:1096–1099.
32 Kawaguchi T, Tong L, Robinson BM, Sen A, Fukuhara S, Kurokawa K, Canaud B, Lameire N, Port FK, Pisoni RL: C-reactive protein and mortality in hemodialysis patients: the Dialysis Outcomes and Practice Patterns Study (DOPPS). Nephron Clin Pract 2011;117:c167–c178.
33 Crews DC, Sozio SM, Liu Y, Coresh J, Powe NR: Inflammation and the paradox of racial differences in dialysis survival. J Am Soc Nephrol 2011;22:2279–2286.

Nephron Extra 2012;2:159–168
DOI: 10.1159/000339437
Published online: June 20, 2012

www.karger.com/nne

34 Ingrosso D, Cimmino A, Perna AF, Masella L, De Santo NG, De Bonis ML, Vacca M, D'Esposito M, D'Urso M, Galletti P, Zappia V: Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. Lancet 2003;361:1693–1699.

35 Nanayakkara PW, Kiefte-de Jong JC, Stehouwer CD, van Ittersum FJ, Olthof MR, Kok RM, Blom HJ, van Guldener C, ter Wee PM, Smulders YM: Association between global leukocyte DNA methylation, renal function, carotid intima-media thickness and plasma homocysteine in patients with stage 2–4 chronic kidney disease. Nephrol Dial Transplant 2008;23:2586–2592.

36 Mu S, Shimosawa T, Ogura S, Wang H, Uetake Y, Kawakami-Mori F, Marumo T, Yatomi Y, Geller DS, Tanaka H, Fujita T: Epigenetic modulation of the renal β-adrenergic-WNK4 pathway in salt-sensitive hypertension. Nat Med 2011;17:573–580.

37 McClure EA, North CM, Kaminski NE, Goodman JI: Changes in DNA methylation and gene expression during 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced suppression of the lipopolysaccharide-stimulated IgM response in splenocytes. Toxicol Sci 2011;120:339–348.

38 Hur K, Niwa T, Toyoda T, Tsukamoto T, Tatematsu M, Yang HK, Ushijima T: Insufficient role of cell proliferation in aberrant DNA methylation induction and involvement of specific types of inflammation. Carcinogenesis 2011;32:35–41.