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Plasma calprotectin in chronically dialyzed end-stage renal disease patients

Karin Malíčková · Helena Brodská · Jana Lachmanová · Sylvie Dusilová Sulková · Ivana Janatková · Helena Marečková · Vladimír Tesař · Tomáš Zima

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Abstract
Objective The current study aimed to evaluate plasma calprotectin levels and clearance end-stage renal disease (ESRD) patients with and without acute infection undergoing chronic hemodialysis (HD).
Materials and methods Blood samples from 54 HD patients were obtained before and after the HD and 42 healthy blood donors were examined as controls. The blood levels of calprotectin, procalcitonin, C-reactive protein (CRP), and intracellular production of interleukins 10 and 12 in monocytes were determined in both groups.
Results The concentrations of plasma calprotectin in ESRD patients were significantly higher than in healthy controls ($p < 0.05$). No differences between pre- and post-HD calprotectin plasma levels were observed ($p = 0.07$ for two-tailed test). Plasma calprotectin levels were not significantly influenced by the presence of acute infection ($p = 0.19$) or diabetes ($p = 0.42$). A significant positive correlation of plasma calprotectin to plasma beta-2 microglobulin was proven ($p < 0.05$). Procalcitonin (PCT), CRP, IL-10, and IL-12 were not correlated with plasma calprotectin before or after HD. The elevation of plasma calprotectin was correlated strongly to the hemodialysis vintage ($r = 0.55$, $p < 0.01$).
Conclusions Significantly elevated levels of plasma calprotectin in ESRD patients occur without an acute infectious cause and are not affected by the presence of diabetes. By analogy to plasma beta-2 microglobulin, a close relation of plasma calprotectin to HD vintage was shown.

Keywords Calprotectin · Polymorphonuclear leukocytes · End-stage renal disease · Hemodialysis · Infection

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K. Malíčková · H. Brodská · S. Dusilová Sulková · I. Janatková · H. Marečková · T. Zima
Institute of Clinical Biochemistry and Laboratory Diagnostics, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

J. Lachmanová · V. Tesař
Department of Nephrology, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

S. Dusilová Sulková
Department of Gerontology and Metabolism, Faculty of Medicine, Charles University, Hradec Kralove, Czech Republic

H. Marečková
Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

K. Malíčková (✉)
Institute of Clinical Biochemistry and Laboratory Diagnostics, Laboratory of Clinical Immunology and Allergology, First Faculty of Medicine, Charles University and General University Hospital, Karlovo nam. 32, 12111 Prague 2, Czech Republic
e-mail: kmali@lf1.cuni.cz

Background

Inflammation is one of the most important complications of hemodialysis (HD). In patients with end-stage renal disease (ESRD), systemic inflammation contributes to a syndrome known as the malnutrition, inflammation, and atherosclerosis (MIA) syndrome [1]. The clinical state of uremia has been reported as one cause of systemic inflammation in

ESRD due to increased oxidative stress and protein carbonylation resulting from a reduction in glomerular filtration rate causing an increase in accumulation of urea and other toxins in the blood. Furthermore, hemodialysis may induce activation of blood cells such as polymorphonuclear leukocytes, monocytes, and lymphocytes, depending on the membrane material of the dialyzer used [2–6]. Infections are prevalent in patients with underlying disease states that leave them with increased susceptibility to infection such as diabetes or glomerulonephritis treated by immunosuppression. In addition, some infections in dialyzed patients are catheter-related.

The search for the best laboratory marker of inflammation for hemodialyzed patients is still the focus of research. Currently applied laboratory parameters for inflammation can be misleading—some of these parameters may be decreased, such as white blood cell count, and others may be non-specifically increased, such as erythrocyte sedimentation rate, C-reactive protein (CRP), or procalcitonin (PCT) [7–9]. In this study, we investigated plasma calprotectin as an inflammatory marker in chronically hemodialyzed ESRD patients.

Calprotectin is the main neutrophil cytosolic protein, and its release and detection provide a sensitive marker of neutrophil turnover [10]. The plasma concentration of calprotectin increases significantly during acute-phase response to infection. Moreover, plasmatic levels of calprotectin during infection rise more rapidly than other systemic inflammatory markers since calprotectin is preformed in the leukocytes. On the contrary, unspecific calprotectin release could take place during the process of hemodialysis.

This study was aimed at (1) evaluating calprotectin plasma levels and clearance in chronically hemodialyzed ESRD patients, (2) comparing calprotectin levels in plasma before and after hemodialysis to observe the possibility of unspecific calprotectin release during the process of hemodialysis, (3) comparing plasma calprotectin levels with the severity of renal damage, hemodialysis vintage, and with other systemic inflammatory markers, namely with PCT, CRP, and intracellular IL-10 and IL-12 production in monocytes.

Materials and methods

Examined cohorts

Fifty-four patients treated for ESRD by regular dialysis treatment were considered for the study. Patients were recruited from the Department of Nephrology, General Teaching Hospital and First Faculty of Medicine in Prague, and Department of Gerontology and Metabolism, Faculty of Medicine of Charles University, Hradec Kralove, Czech Republic. The general characteristics of the ESRD cohort are shown in Table 1.

Dialysis was carried out with the Dialog+ (BBraun), AK 200 Ultra S (Gambro), and Fresenius 4008 S (Fresenius); the dialysators were from synthetic (Polyflux L, Diacap), diacetate (Dicea), and triacetate (Tricea) membranes. Blood samples were obtained just prior to the start of dialysis (T_0) and immediately after the termination of HD (T_1) and were drawn from the fistula or the permcath.

Control blood samples of 42 healthy blood donors (46.1 ± 5.25 years old; 23 men and 19 women) were used as healthy controls.

Ethical aspects

This study was approved by the Institutional Ethics Committee. The purpose and procedures of the study were explained to participants, and a signed informed consent was obtained.

Laboratory examinations

Plasma calprotectin was measured by enzyme-linked immunosorbent assay (MRP8/14, Bühlmann, Switzerland). Briefly, a monoclonal capture antibody highly specific to the calprotectin heterodimeric and polymeric complexes, respectively, was coated onto the microtiter plate. A second polyclonal detection antibody conjugated to horseradish peroxidase detected the calprotectin molecules bound to the monoclonal antibody coated onto the plate after washing. After incubation and a further washing step, tetramethylbenzidine was added, followed by a stopping reaction. Optical densities were measured at 450 nm in a Dynex MRXII Revelation double-beam spectrophotometer. A clinically relevant cut-off value of 12 μg/mL was assessed according to the manufacturer's recommendations.

Serum PCT concentrations were measured by enzyme-linked immunoabsorbent assay (Vidas Brahms, Germany). Analyses were performed according to manufacturer's

Table 1 Descriptive characteristics of end-stage renal disease patients

Characteristic	Value
Gender, female/male	25/29
Age, years (mean ± SD)	65.6 ± 11.93
HD vintage, months (mean; IQR)	51.39; 14–65
Acute infection, yes/no	10/44
Type 2 diabetes, yes/no	18/36
Residual diuresis, mL/24 h (mean ± SD)	613.9 ± 633.75

SD Standard deviation, *HD* hemodialysis, *IQR* inter-quartile range

recommendations. The concentration of 0.5 ng/mL was chosen as cut-off value. CRP was measured by turbidimetry Modular SWA (Roche Diagnostic, Switzerland). A cut-off value of greater than 5.0 mg/L was adopted.

Intracellular production of IL-10 and IL-12 in monocytes was quantified by flow cytometry after activation, fixation, permeabilization, and staining, as described previously [11]. Briefly, 500 μL of heparinized whole blood samples was used. To induce cytokine production in monocytes, whole blood cultures preincubated overnight were stimulated with 10 ng/mL LPS (LPS from *E. coli* O111:B4, Sigma–Aldrich, USA) for 4 h at 37°C in a 5% CO_2 humidified atmosphere. Subsequently, cells were exposed to 3 μM brefeldin A (Sigma–Aldrich, USA) to enhance detection of intracellular cytokines. Next, 50 μL of a pretreated blood sample was stained with anti-CD14 monoclonal antibody (CD 14 FITC; BD Biosciences, USA) for 30 min in the dark at 4°C, and cells were fixed with lysing solution (BD FACS Lysing Solution, BD Biosciences, USA) for 10 min at room temperature in the dark. After permeabilization by FACS Permeabilization Solution 2 (BD Biosciences Pharmingen, USA) and washing by phosphate buffer, 0.5 μg/10 μL of monoclonal antibodies against IL-10 (IL-10 PE, BD Biosciences Pharmingen, USA) and IL-12 (IL-12 APC, BD Biosciences, Pharmingen, USA) was added to the tube and incubated for 20 min. Finally, the blood sample was washed twice by phosphate buffer. Ten thousand cells were measured on a FACSCanto (BD Biosciences, Pharmingen, USA) flow cytometer and analyzed with FACS DiVa software. The percentage of stimulated whole blood $CD14^+$ monocytes producing the individual cytokine was the unit for assessment. Established normal values for IL-10 and IL-12 were 10–35 and 20–40%, respectively. Routine biochemical parameters such as serum urea, creatinine, total serum protein, and beta-2-microglobulin were determined with standard clinical-chemistry methods recommended by the International Federation of Clinical Chemistry (IFCC).

In patients with symptoms of intercurrent infection, pharyngeal and nose swabs and/or urine samples were obtained when body temperature (measured in the axilla) increased to more than 37.5°C. The same samples and blood cultures were obtained from all lumens of venous catheters and cultivated for both aerobic and anaerobic bacteria (bacT/ALERT, bioMérieux).

Statistical analysis

The distributions of laboratory test results are presented as mean ± SD or interquartile range for quantitative items as stated. The correlation among marker levels was examined with Spearman's rank correlation coefficient. To assess the accuracy of markers in groups of ESRD patients compared to controls, the Mann–Whitney *U* test was performed using the Statistica CZ program, version 7.0 (StatSoft, OK, USA). A p value smaller than 0.05 was considered significant.

Results

Inflammatory markers in ESRD patients without symptoms of acute infection compared to healthy controls

Compared to healthy controls, ESRD patients showed at the T_0 baseline time point significantly higher values of CRP (16.28 ± 9.69 and 2.05 ± 0.53 mg/L, respectively, $p < 0.01$), PCT (0.35 ± 0.50 and 0.05 ± 0.008 ng/mL, respectively, $p < 0.05$), IL-12 (32.88 ± 14.92 and 20.55 ± 6.21%, respectively, $p < 0.05$), and plasma calprotectin (27.89 ± 15.86 and 2.31 ± 0.11 μg/mL, respectively, $p < 0.01$). IL-10 production in ESRD patients' monocytes was significantly lower than healthy controls (11.08 ± 8.53 and 26.36 ± 9.47%, $p < 0.05$).

Inflammatory markers in ESRD patients before and after hemodialysis

The pre- and post-HD values of inflammatory markers for all (infectious and noninfectious) ESRD patients are included in Table 2. In contrast to serum urea and creatinine levels, the comparisons of all measured inflammatory markers between two study time points did not reveal any significant differences. Although plasma calprotectin levels tend to be higher after the hemodialysis compared to pre-HD values, this increase is not significant enough ($p = 0.07$).

Plasma levels of calprotectin in relation to other inflammatory markers and to beta-2 microglobulin as a retention rate marker

None of PCT, CRP, IL-10, and IL-12 correlated with plasma calprotectin before or after HD (Table 3). A significant positive correlation of plasma calprotectin with plasma beta-2 microglobulin in T_0 and T_1 was proven.

Plasma levels of calprotectin in relation to intercurrent infectious complications

Ten of 54 patients (18.5%) showed body temperature above 37.5°C (measured in the axilla) and/or other symptoms of intercurrent infection including positive microbiological findings in mucosal swabs, urine samples, or venous catheters. Antibiotic treatment was administered to these patients.

Table 2 Comparative characteristics of measured values before (T_0) and after (T_1) the hemodialysis

Lab examination	T_0	T_1	*P* (U)
Serum urea (mMol/L)	23.97 ± 4.95 [21, 26.4]	7.76 ± 2.18 [6.3, 9.4]	<0.001
Serum creatinine (μMol/L)	759.59 ± 221.19 [597, 913]	289.74 ± 89.90 [225, 349]	<0.0001
Total serum protein (g/L)	66.36 ± 9.35 [61, 72]	68.23 ± 8.68 [64.0, 73.0]	NS
Serum C-reactive protein (mg/L)	16.01 ± 19.38 [2, 17]	15.30 ± 18.59 [4.0, 18.0]	NS
Plasma procalcitonin (ng/mL)	0.43 ± 0.76 [0.1, 0.4]	0.42 ± 0.71 [0.1, 0.5]	NS
Intracellular IL-10 in monocytes (%)	11.11 ± 8.41 [5.0, 17.2]	10.46 ± 8.88 [3.8, 17.2]	NS
Intracellular IL-12 in monocytes (%)	33.12 ± 14.64 [21.3, 45]	31.21 ± 16.04 [19.2, 41]	NS
Plasma calprotectin (μg/mL)	24.38 ± 18.56 [11.2, 52.0]	34.54 ± 28.56 [17.5, 80.0]	NS

Data are expressed as mean ± standard deviation [1st quartile, 3rd quartile]

T_0 Measurements before the hemodialysis, T_1 measurements after the hemodialysis, *U* Mann–Whitney *U* test

Table 3 Plasma calprotectin levels in relation to other markers

Spearman's rank correlation coefficient (*r*)	T_0	T_1
pCAL vs. PCT	0.08 (NS)	0.08 (NS)
pCAL vs. IL-10	−0.19 (NS)	−0.25 (NS)
pCAL vs. IL-12	0.31 (NS)	0.29 (NS)
PCAL vs. beta-2-microglobulin	0.39 ($p < 0.05$)	0.41 ($p < 0.05$)

CRP C-reactive protein, *PCT* procalcitonin, *pCAL* plasma calprotectin, *IL-10* interleukin 10, *IL-12* interleukin 12

When the ESRD patients were divided into two groups—one group of hemodialyzed patients with signs of intercurrent infection and another noninfectious group—no differences were found in plasma calprotectin levels or in other measured inflammatory markers (Table 4).

Plasma levels of calprotectin in relation to type 2 diabetes

Eighteen of 54 ESRD patients (33.3%) had type 2 diabetes. Although diabetic ESRD patients tended to show higher levels of CRP, plasma calprotectin, plasma procalcitonin, and intracellular IL-12, and lower IL-10 production compared to nondiabetic ESRD patients, these differences were not statistically significant (Fig. 1).

Plasma levels of calprotectin and polymorphonuclear leukocyte (PMNLs) counts

In the ESRD cohort, mean white blood cell count ($6.95 \pm 2.6 \times 10^9$/L) and PMNL count ($4.31 \pm 2.05 \times 10^9$/L) did not differ significantly compared to healthy controls ($5.85 \pm 1.9 \times 10^9$/L and $3.65 \pm 1.95 \times 10^9$/L, respectively). However, plasma calprotectin levels were more than 12 times higher in ESRD patients compared to healthy blood donors (27.89 ± 15.86 and 2.31 ± 0.11 μg/mL, respectively; $p < 0.01$). In ESRD patients, we found only a weak correlation of PMNL count with plasma calprotectin levels (Spearman's rank correlation coefficient $r^2 = 0.29$, $r = 0.54$, $p = 0.078$). The variation of the plasma calprotectin in healthy controls was small, and most likely reflects the limited accuracy of the assay procedure.

Inflammatory markers and hemodialysis vintage

A significant positive correlation was found between plasma calprotectin levels and HD vintage (Fig. 2). However, HD vintage showed no further strong correlation with serum CRP ($p = 0.07$), PCT ($p = 0.48$), IL-10 ($p = 0.36$), or IL-12 ($p = 0.12$).

In summary, plasma calprotectin was increased several-fold in all ESRD patients and correlated strongly to beta-2 microglobulin and the hemodialysis vintage.

Table 4 Inflammatory markers in end-stage renal disease (ESRD) patients with acute infection compared to ESRD patients without clinical signs of acute infection

Lab examination	ESRD with acute infection ($n = 10$)	ESRD without acute infection ($n = 44$)	*P* (U)
Serum C-reactive protein (mg/L)	16.90 ± 3.50	16.28 ± 9.69	0.54 (NS)
Plasma procalcitonin (ng/mL)	1.02 ± 2.00	0.35 ± 0.50	0.11 (NS)
Intracellular IL-10 in monocytes (%)	5.20 ± 2.29	11.08 ± 8.53	0.09 (NS)
Intracellular IL-12 in monocytes (%)	36.10 ± 4.42	32.88 ± 14.92	0.49 (NS)
Plasma calprotectin (μg/mL)	35.98 ± 29.82	27.89 ± 15.86	0.19 (NS)

Data are expressed as mean ± standard deviation

U Mann–Whitney *U* test

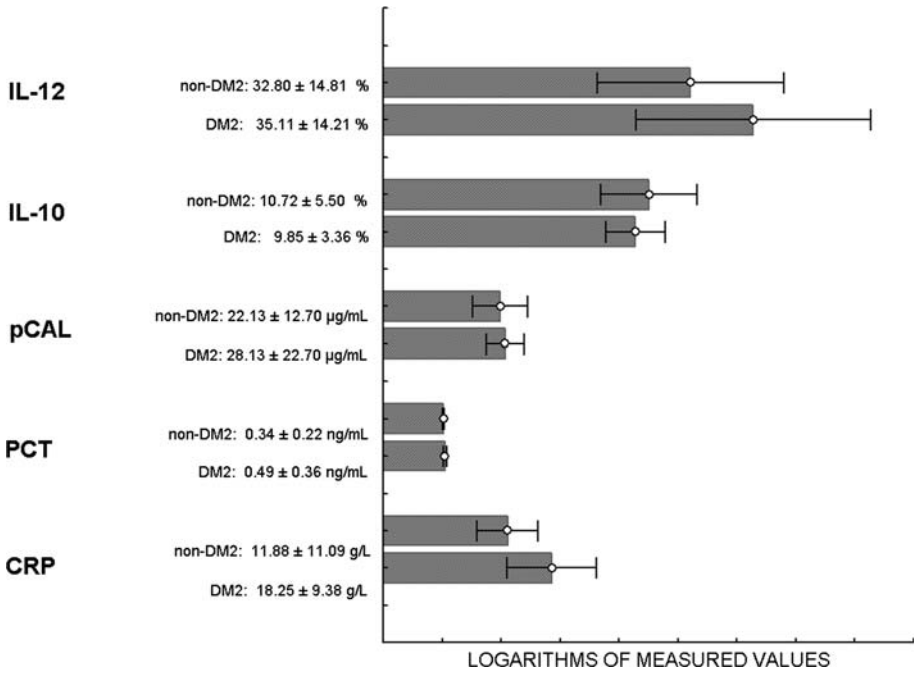

Fig. 1 Plasma levels of calprotectin in relation to type 2 diabetes. Data are expressed as mean ± standard deviation. *H* Healthy controls, *ESRD* end-stage renal disease patients, *CRP* C-reactive protein, *PCT* procalcitonin, *pCAL* plasma calprotectin, *IL-10* interleukin 10, *IL-12* interleukin 12

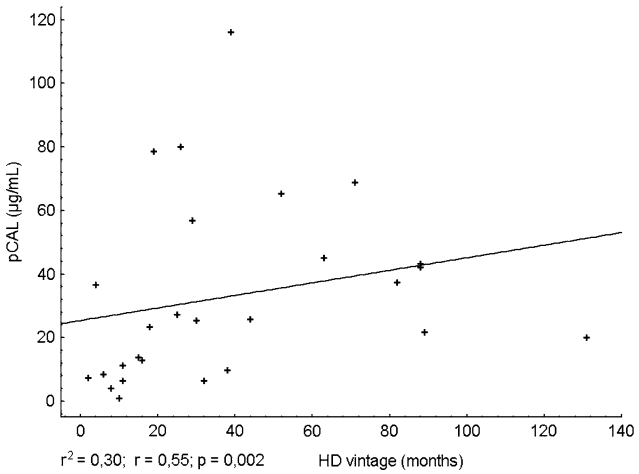

Fig. 2 Correlation between plasma calprotectin and HD vintage

Discussion

To our knowledge, this is the first report arising from the investigation of plasma levels of calprotectin in ESRD patients. It shows high levels of plasma calprotectin in all examined ESRD patients before and after the hemodialysis in spite of the presence of acute infection or diabetes, as well as a strong correlation of plasma calprotectin levels with the hemodialysis vintage.

It is known that inflammation is a major risk factor for mortality in patients with ESRD [12]. Inflammation is a complex process that reflects the local and systemic responses to different immunological and nonimmunological stimuli. Inflammation can be initiated in different ways and is characterized by the activation of acute phase response and release of specific markers. The causes of the ongoing inflammatory process in ESRD patients are not well understood, but it is accepted that continuous exposure to an inflammatory stimulus (e.g., uremia, oxidative stress, and endotoxin exposure) results in local and systemic cell activation. In addition to these proinflammatory conditions, patients on hemodialysis are exposed to different external stimuli such as high/low flux dialysis, bioincompatible membranes, dialysate contamination, and access site infections.

Polymorphonuclear leukocytes (PMNLs) play a crucial role in inflammatory cascade in ESRD patients [13–17]. Impaired chemotaxis, phagocytic capacity, bactericidal activity, and metabolic dysfunction of these cells in dialyzed patients have been reported [18–20]. Uremia could be a cause of some of these deviations. Stenvinkel [12] hypothesized that factors in the uremic milieu prime PMNLs, leading to oxidative stress and low-grade inflammation in ESRD. Once primed, PMNLs become fully activated when exposed to a second stimulus such as blood-membrane interaction, leading to the release of reactive oxygen species, destructive proteases and inflammatory mediators.

One of the main inflammatory mediators of PMNs is calprotectin. Calprotectin (synonyms: MRP8/14, calgranulin A/B, leukocyte L1 antigen complex) is the main antigenic glycoprotein in the cytoplasm of PMNLs; it contributes to approximately 50% of the soluble cytosolic content of PMNLs [21]. Calprotectin is a 24-kDa heterodimer composed of light (S100A8) and heavy (S100A9)

chains; it belongs to the S100 protein calcium binding superfamily. Released after neutrophil activation, calprotectin is involved in innate immunity processes, particularly in leukocyte chemotaxis and adhesion, and has potent antimicrobial properties. In addition, it also emerges as an important mediator of diverse processes within chronic inflammation due to its participation in the cytokine cascade [9]. It has been shown that increased plasma concentration of calprotectin is a useful biomarker of disease activity in inflammatory disorders, such as inflammatory bowel disease [21–23], multiple sclerosis [24], sarcoidosis [25], or transplant rejection [21, 26].

In our findings, plasma calprotectin was significantly higher in ESRD patients than in healthy controls (24.38 ± 18.56 vs. 1.23 ± 0.21 μg/mL, $p < 0.01$). Excessive activation of PMNLs evidently induces pathophysiologically unfavorable conditions in ESRD. The released serum calprotectin may play a role in the propagation of inflammation by recruiting neutrophils and monocytes and enhancing their migration, as well as by production of proinflammatory cytokines by mononuclear cells. Therefore, it could serve as an effector and amplifier of inflammation with the wide range of activities. Since no significant difference was found in calprotectin levels before and after the HD (24.38 ± 18.56 vs. 34.54 ± 28.56 μg/mL), unspecific calprotectin release from PMNLs probably does not have great importance during the process of hemodialysis. However, the direct effect of HD on calprotectin release from PMNLs cannot be definitely excluded by a single measurement of a sample taken immediately after the procedure. A prospective study with a time course of calprotectin level measurement during the first day following hemodialysis will be useful for resolution of this item.

Ward and McLeish [28, 29] reported that PMNLs from patients with ESRD are in a primed state. It is apparent that primed peripheral PMNLs contribute to chronic systemic inflammatory processes.

Since PMNLs are the major source of calprotectin, their numbers might be important for the final concentrations of this mediator. In our ESRD cohort, the mean PMNL count did not differ significantly from healthy controls; however, plasma calprotectin levels were more than 12 times higher in ESRD patients than healthy blood donors. Moreover, we found only a weak correlation of PMNL count with plasma calprotectin levels. Therefore we assume that the leukocyte crushing could have an importance in hemodialyzed ESRD patients.

Our results agree with Bodlaj et al. [27] because type 2 diabetes in our ESRD patients was not associated with further increased serum inflammatory parameters including plasma calprotectin. These observations suggest that the worsened prognosis of diabetic ESRD patients is probably not explainable by superimposing inflammatory processes.

We have noted a strong positive correlation of plasma calprotectin concentrations with HD vintage ($r = 0.55$, $p < 0.01$). Since calprotectin is the main cytosolic inflammatory protein of PMNLs, we suggest that the elevation of plasma calprotectin is a marker of chronic systemic inflammation derived from PMNL priming and contributes to maintaining the inflammatory state by the potentiation of oxidative stress and stimulation of antigen-presenting cells.

Considering the substantial inflammatory properties of calprotectin, there seems to be a discrepancy between high concentrations of plasma calprotectin and lack of correlation with other systemic inflammatory markers such as PCT, CRP, IL-10, and IL-12, before or after HD, suggesting that these inflammatory markers occur independently of each other.

It is known that chronic retention of proteins is connected with some of the observed problems of ESRD patients such as immune dysfunction, amyloidosis, and hormone imbalance. The concentration of beta-2 microglobulin in plasma and other body fluids increases in proportion to lost kidney function, as it is normally eliminated by the process of glomerular filtration followed by reabsorption and catabolism by the proximal tubular cells. Blumberg et al. [30] reported correlation of beta-2 microglobulin with dialysis vintage, whereas beta-2 microglobulin production and tissue accumulation enhance the rate of progression of dialysis-related amyloidosis [31]. In our study, the significant correlation of plasmatic calprotectin with beta-2 microglobulin and with dialysis vintage could be explained by the increased time-dependent production and secretion of cytokines due to blood contact with bio-incompatible parts of HD membranes and the relevant impact of infections that activate immunocompetent cells.

A potential limitation of our study is the sample size. In particular, we found no differences in inflammatory markers of ESRD patients with and without clinical signs of acute infection. The association of ESRD with inflammatory markers could be caused either by their increased production, decreased clearance, or a combination of both mechanisms. Our "infectious" cohort was small ($n = 10$, 18.5%), and increased production of inflammatory markers such as CRP was probably overbalanced with other immunopathological mechanisms.

In conclusion, we described the promising possibility of calprotectin serving as a marker for inflammatory status in ESRD. In future studies, the dynamics of calprotectin during ESRD and HD should be investigated.

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Conflict of interest statement The authors declare that they have no competing interests.

References

1. Stenvinkel P, Heimbürger O, Paultre F, Diczfalusy U, Wang T, Berglund L, et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney Int. 1999;55(5):1899–911.
2. Weiner DE, Tighiouart H, Elsayed EF, Griffith JL, Salem DN, Levey AS, et al. Inflammation and cardiovascular events in individuals with and without chronic kidney disease. Kidney Int. 2008;73(12):1406–12.
3. Alexiewicz JM, Smogorzewski M, Fadda GZ, Massry SG. Impaired phagocytosis in dialysis patients: studies on mechanisms. Am J Nephrol. 1991;11(2):102–11.
4. Bohler J, Donauer J, Birmelin M, Schollmeyer PJ, Hörl WH. Mediators of complement-independent granulocyte activation during haemodialysis: role of calcium, prostaglandins and leukotrienes. Nephrol Dial Transplant. 1993;8(12):1359–65.
5. Girndt M, Sester M, Sester U, Kaul H, Köhler H. Molecular aspects of T- and B-cell function in uremia. Kidney Int Suppl. 2001;78:S206–11.
6. Haag-Weber M, Horl WH. Dysfunction of polymorphonuclear leukocytes in uremia. Semin Nephrol. 1996;16(3):192–201.
7. Teruel JL, Marcen R, Ocaña J, Fernández-Lucas M, Rivera M, Tabernero G, et al. Clinical significance of C-reactive protein in patients on hemodialysis: a longitudinal study. Nephron Clin Pract. 2005;100(4):140–5.
8. Lorton F, Veinberg F, Ielsch D, Deschenes G, Bensman A, Ulinski T. Procalcitonin serum levels in children undergoing chronic haemodialysis. Pediatr Nephrol. 2007;22:430–5.
9. Steinbach G, Bolke E, Grunert A, Storck M, Orth K. Procalcitonin in patients with acute and chronic renal insufficiency. Wien Klin Wochenschr. 2004;116:849–53.
10. Kerkhoff C, Klempt M, Sorg C. Novel insights into structure and function of MRP8 (S100A8) and MRP14 (S100A9). Biochim Biophys Acta. 1998;1448(2):200–11.
11. Maino VC, Picker LJ. Identification of functional subsets by flow cytometry: intracellular detection of cytokine expression. Cytometry. 1998;34(5):207–15.
12. Stenvinkel P. New insights on inflammation in chronic kidney disease-genetic and non-genetic factors. Nephrol Ther. 2006;2(3):111–9.
13. Daniels I, Fletcher J, Haynes APOD. Role of p38 in the priming of human neutrophils by peritoneal dialysis effluent. Clin Diagn Lab Immunol. 1999;6(6):878–84.
14. Dobos GJ, Traynor-Kaplan AE, Ward D, Schollmeyer PJ. Neutrophil dysfunction in end-stage renal failure: reduced response to priming by C5a. Clin Investig. 1994;72(5):353–7.
15. Haag-Weber M, Mai B, Horl WH. Effect of hemodialysis on intracellular calcium in human polymorphonuclear neutrophils. Miner Electrolyte Metab. 1992;18(2–5):151–5.
16. Heidland A, Hörl WH, Heller N, Heine H, Neumann S, Heidbreder E. Proteolytic enzymes and catabolism: enhanced release of granulocyte proteinases in uremic intoxication and during hemodialysis. Kidney Int Suppl. 1983;16:S27–36.
17. Karpati I, Seres I, Mátyus J, Ben T, Paragh G, Varga Z, et al. Which parameters affect cytosolic free calcium in polymorphonuclear leukocytes of haemodialysis patients? Nephrol Dial Transplant. 2001;16(7):1409–15.
18. Hansch GM, Karnaoukhova S, Chang SH, Rus H, Nicolescu F, Deppisch R, et al. Activation of human neutrophils after contact with cellulose-based haemodialysis membranes: intracellular calcium signalling in single cells. Nephrol Dial Transplant. 1996;11(12):2453–60.
19. Hörl WH, Haag-Weber M, Mai B, Massry SG. Verapamil reverses abnormal [Ca2+]i and carbohydrate metabolism of PMNL of dialysis patients. Kidney Int. 1995;47(6):1741–5.
20. Massry S, Smogorzewski M. Dysfunction of polymorphonuclear leukocytes in uremia: role of parathyroid hormone. Kidney Int Suppl. 2001;78:S195–6.
21. Ikemoto M, Tanaka T, Takai Y, Murayama H, Tanaka K, Fujita M, et al. New ELISA system for myeloid-related protein complex (MRP8/14) and its clinical significance as a sensitive marker for inflammatory responses associated with transplant rejection. Clin Chem. 2003;49(4):594–600.
22. Lugering N, Stoll R, Kucharzik T, Schmid KW, Rohlmann G, Burmeister G, et al. Immunohistochemical distribution and serum levels of the Ca(2+)-binding proteins MRP8, MRP14 and their heterodimeric form MRP8/14 in Crohn's disease. Digestion. 1995;56(5):406–14.
23. Lugering N, Stoll R, Schmid KW, Kucharzik T, Stein H, Burmeister G, et al. The myeloic related protein MRP8/14 (27E10 antigen)–usefulness as a potential marker for disease activity in ulcerative colitis and putative biological function. Eur J Clin Invest. 1995;25(9):659–64.
24. Floris S, van der Goes A, Killestein J, Knol DL, Barkhof F, Polman CH. Monocyte activation and disease activity in multiple sclerosis. A longitudinal analysis of serum MRP8/14 levels. J Neuroimmunol. 2004;148(1–2):172–7.
25. Terasaki F, Fujita M, Shimomura H, Tsukada B, Otsuka K, Otsuka K, et al. Enhanced expression of myeloid-related protein complex (MRP8/14) in macrophages and multinucleated giant cells in granulomas of patients with active cardiac sarcoidosis. Circ J. 2007;71(10):1545–50.
26. Striz I, Jaresová M, Lácha J, Sedlácek J, Vítko S. MRP 8/14 and procalcitonin serum levels in organ transplantations. Ann Transplant. 2001;6(2):6–9.
27. Bodlaj G, Berg J, Pichler R, Sailer T, Biesenbach G. Type 2 diabetes in patients with end-stage renal disease is not associated with further increased serum inflammatory parameters. J Nephrol. 2004;17(1):112–7.
28. Ward RA. Phagocytic cell function as an index of biocompatibility. Nephrol Dial Transplant. 1994;9:46–56.
29. Ward RA, McLeish KR. Oxidant stress in hemodialysis patients: what are the determining factors? Artif Organs. 2003;27:230–6.
30. Blumberg A, Burgi W. Behaviour of beta 2-microglobulin in patients with chronic renal failure undergoing hemodialysis, hemodiafiltration and continuous ambulatory peritoneal dialysis (CAPD). Clin Nephrol. 1987;27(5):245–9.
31. Kalocheretis P, Revela I, Spanou E, Drouzas A, Makriniotou I, Iatrou C. Strong correlation of B2-microglobulin (B2-m) with procalcitonin (PCT) in the serum of chronic hemodialysis patients: a role for infections in the dialysis-related amyloidosis? Ren Fail. 2008;30(3):261–5.