NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN IN PERITONEAL EFFLUENT: EVALUATION IN PERITONEAL DIALYSIS PATIENTS IN BASAL CONDITION

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◆ **Purpose:** In the present study, we assessed expression of neutrophil gelatinase-associated lipocalin (NGAL) in peritoneal effluent (pNGAL) from peritoneal dialysis (PD) patients, and we evaluated factors that might affect its level in basal conditions.

◆ **Methods:** Our cross-sectional study included all 69 patients on PD at our institution. We evaluated patient history, hydration status, residual renal function, indices of dialysis adequacy, peritoneal transport type, serum C-reactive protein, ferritin, serum NGAL (sNGAL) and pNGAL. Univariate and multivariate linear regression models were used to evaluate predictors of pNGAL.

◆ **Results:** Of the study patients, 39 (56.5%) were men, and 54 (78.3%) were on continuous ambulatory PD. Median age in the group was 61 years [interquartile range (IQR): 46.5 – 71 years]. Median sNGAL was 487 ng/mL (IQR: 407 – 586 ng/mL), and median pNGAL was 35 ng/mL (IQR: 21 – 46 ng/mL). dNGAL correlated directly with weekly dialytic clearance of creatinine ($\rho = 0.291$, $p = 0.02$) and with sNGAL ($\rho = 0.269$, $p = 0.031$). The same variables were also independent predictors of pNGAL ($\beta = 0.30$ and 0.29 respectively, both $p < 0.05$) in multivariate analysis.

◆ **Conclusions:** In our analysis, basal levels of pNGAL were influenced by sNGAL and by dialytic clearance of creatinine.

siderophores (1). Its role as marker has been evaluated in several conditions, including acute and chronic kidney disease (CKD), infections, and cancers. Moreover, some studies showed that NGAL secretion can be induced in epithelial cells during inflammatory insult. Specifically, Leung and colleagues (2) demonstrated secretion of NGAL by peritoneal mesothelial cells in a small group of peritoneal dialysis (PD) patients and suggested the usefulness of NGAL in PD effluent (pNGAL) for the diagnosis of peritonitis.

Conversely, serum NGAL (sNGAL) is known to be high in patients with CKD (3), and high sNGAL might be paralleled by high pNGAL in PD patients. Moreover, certain systemic conditions such as hydration status or systemic inflammation may affect sNGAL (4) and consequently pNGAL. To date, no study assessing the possible interference of PD modality and systemic conditions on pNGAL concentration has been reported.

The aim of the present study was to evaluate the consistency of pNGAL in basal conditions for describing a peritoneal local process and to evaluate how PD and systemic conditions might affect pNGAL values.

METHODS

SUBJECTS

Our cross-sectional study, conducted at the Peritoneal Dialysis Center in San Bortolo Hospital, Vicenza, Italy, included all patients on PD who were being followed at our center. All patients provided informed consent before study enrollment. All patients underwent blood, urinary, and PD effluent examinations; a standard peritoneal equilibration test (5) using a 2-L exchange of 2.27% glucose solution; and bioimpedance analysis (performed using a multi-frequency device) to evaluate pNGAL, sNGAL, C-reactive protein, dialytic ultrafiltration,

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Neutrophil gelatinase-associated lipocalin (NGAL) is a granulocyte-secreted polypeptide that specifically blocks bacterial growth by interfering with bacterial

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glomerular filtration rate, weekly dialytic clearance of creatinine (dCCr_w), weekly dialytic Kt/V, transport type, and extracellular water.

Specifically, serum creatinine was evaluated in all fluid samples using enzymatic method on the Advia 2400 chemistry system (Siemens Diagnostics, Tarrytown, NY, USA). sNGAL was assayed within 1 hour of sample collection using a Triage NGAL test on a Triage MeterPro analyzer (Alere, Stockport, UK), and pNGAL was evaluated in an overnight effluent collection using the Architect platform (Abbott Diagnostics, Abbott Park, IL, USA). Furthermore, to understand the kinetics of NGAL in relation to the peritoneal membrane, we evaluated the ratio of pNGAL to sNGAL (NGAL D/P) at the end of a long dwell. Finally, to minimize the influence of ultrafiltration (UF) during the long dwell, we corrected the pNGAL value using the formula

$$\text{pNGAL}_{\text{UF}} = \text{pNGAL} \times (\text{effluent drainage volume} / \text{dialysate infusion volume}).$$

STATISTICAL ANALYSIS

All continuous variables are reported as medians and interquartile ranges (IQRs). Categorical variables are described as percentages. The Mann–Whitney U-test and Kruskal–Wallis test were used to compare continuous variables as appropriate. All non-normally distributed values were log-transformed to better approximate normal distribution. Linear regression analysis used the Pearson coefficient and the Spearman coefficient as appropriate. Multiple regression analysis was used to determine independent factors affecting the dependent variable. Factors showing linear correlation with pNGAL_{UF} were included in the analysis. All reported p values are two-sided, and statistical significance was set at $p < 0.05$. All analyses were performed using the SPSS software application (version 17.0: SPSS, Chicago, IL, USA).

RESULTS

The study enrolled 69 patients [39 men (56.5%), 54 on continuous ambulatory PD (78.3%)]. Table 1 shows baseline characteristics for the study patients.

Median sNGAL was 487 ng/mL (IQR: 407 – 586 ng/mL), and median pNGAL_{UF} was 38 ng/mL (IQR: 22.1 – 49.5 ng/mL). sNGAL was normally distributed (Shapiro–Wilks $p = 0.113$), but the pNGAL_{UF} distribution was asymmetric (Shapiro–Wilks $p < 0.001$). The NGAL D/P showed an asymmetric distribution (Shapiro–Wilks $p < 0.001$; median: 0.065; IQR: 0.042 – 0.0937) and no correlation with dwell time (Spearman $\rho = 0.02$, $p = 0.985$). No significant

differences in pNGAL_{UF} were found with respect to sex, PD modality, transport type, and presence of residual renal function (all $p > 0.05$).

In univariable analysis, $\log(\text{pNGAL}_{\text{UF}})$ was directly correlated with sNGAL (Pearson $\rho = 0.28$, $p = 0.013$) and with dCCr_w (Pearson $\rho = 0.314$, $p = 0.009$). No significant correlations were found for other variables (all $p > 0.05$). In multiple regression analysis, sNGAL and dCCr_w were independent predictors of $\log(\text{pNGAL}_{\text{UF}})$ in PD patients (adjusted $R^2 = 0.095$, $F = 4.570$, $p = 0.014$; Table 2).

DISCUSSION AND CONCLUSIONS

Our study demonstrates that, in basal conditions, pNGAL_{UF} is poorly represented in peritoneal effluent and that sNGAL and dCCr_w weakly predict its value. Those parameters explained just 9.5% of total model variance, probably because pNGAL_{UF} is related to NGAL secretion by activated peritoneum, even in basal conditions when stimuli to NGAL secretion in the peritoneal space are limited. Moreover, it is not surprising that dCCr_w poorly

TABLE 1
Baseline Demographic and Clinical Data

Variable	Median	IQR	p Value ^a
Age (years)	61	47–71	<0.001
PD vintage (months)	30	11–51	< 0.001
CRP (mg/dL)	0.55	0.1–1.27	<0.001
Daily dialytic UF (cm^3)	750	165–1145	0.786
GFR (mL/min)	2.3	0.9–3.8	<0.001
Weekly dialytic CCr (L)	41.7	35.65–47.9	0.763
Weekly dialytic Kt/V	2.1	1.9–2.45	0.064
Extracellular water (L)	17.9	14.85–20.85	0.601

IQR = interquartile range; PD = peritoneal dialysis; CRP = C-reactive protein; UF = ultrafiltration; GFR = glomerular filtration rate; CCr = creatinine clearance.

^a By Shapiro–Wilks test.

TABLE 2
Multivariable Regression of Logarithm of Neutrophil Gelatinase–Associated Lipocalin (NGAL) in Peritoneal Dialysis Effluent Normalized by Ultrafiltration in a Long Dwell

Variable	Standardized β	T	p Value
Serum NGAL	0.29	2.477	0.016
Weekly dialytic CCr	0.31	2.652	0.010

CCr = creatinine clearance.

predicted the concentration of pNGAL_{UF}. The different molecular weights of NGAL and creatinine (25000 Da and 113 Da respectively) can strongly influence their diffusive transport through the peritoneal membrane. As shown with other molecules of similar molecular weight, NGAL has poor peritoneal diffusive capacity (6). For all those reasons, it is also not surprising that pNGAL_{UF} was poorly predicted by sNGAL and that NGAL D/P was relatively low and not influenced by dwell time.

The present study has some limitations. First, we did not assess other factors such as markers of local inflammation [interleukin 6, interleukin 8, cancer antigen 125, hyaluronan (7)], which could better explain the model variance of pNGAL. Second, the kit used to evaluate pNGAL was not specifically created to assay PD effluent, but currently no specific kit is available for pNGAL.

DISCLOSURES

All authors made a substantial contribution to the information or material submitted for publication. All read and approved the final manuscript. The study received no funding of any kind. CR has received speaking honoraria from Abbott Laboratories, Alere, and Gambro Lundia. All other authors have no competing interests to declare.

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