Is Neutrophil Gelatinase–Associated Lipocalin an Optimal Marker of Renal Function and Injury in Liver Transplant Recipients?

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ABSTRACT

Background. Recently, research has focused on the association of neutrophil gelatinase–associated lipocalin (NGAL) with acute and/or active kidney injury. However, it should be remembered that NGAL is involved in iron metabolism and antimicrobial defense mechanisms.

Methods. One hundred seven consecutive liver transplant recipients were included in this study. Plasma and urine NGAL levels were measured with the use of enzyme-linked immunosorbent assay. NGAL levels were studied as plasma concentrations (pNGAL), urine concentrations (uNGAL), urinary NGAL to creatinine ratio (uNGAL/Cr), and fractional NGAL secretion (fNGAL).

Results. pNGAL was found to be inversely correlated with estimated glomerular filtration rate (eGFR) and plasma cystatine C (pCysC) ($r = -0.26$ and $P = .007$, $r = -0.38$ and $P = .00006$, respectively). uNGAL was positively correlated with urinary cystatine C to creatinine ratio (uCysC/Cr) and fractional cystatine C excretion (fCysC) ($r = 0.43$ and $P = .000004$; $r = 0.4$ and $P = .1$; respectively). uNGAL/Cr was inversely correlated with hematocrit (Htc) and hemoglobin (Hb) ($r = -0.35$ and $P = .0002$; $r = -0.39$ and $P = .00004$; respectively), and positively correlated with uCysC/Cr ($r = 0.5$ and $P < .0000001$). fNGAL was directly correlated with uCysC/Cr and fCysC ($r = 0.53$ and $P < .0000001$; $r = 0.39$ and $P = .00005$; respectively) and inversely correlated with red blood cell count (RBC; $r = -0.31$ and $P = .001$). We observed significant differences of pNGAL, uNGAL/Cr, and fNGAL between sexes, with highest values of uNGAL, uNGAL/Cr, and fNGAL in women and pNGAL in men. In multivariate regression analysis, pNGAL was positively correlated with time elapsed from liver transplantation, neutrophil count, pCysC, and sex ($\beta = 0.36$ and $P = .00001$; $\beta = 0.32$ and $P = .0001$; $\beta = 0.58$ and $P < .0000001$; $\beta = 0.17$ and $P = .025$; respectively) and inversely correlated with patient's age ($\beta = -0.18$ and $P = .02$) with $R = 0.67$ and $R^2 = 0.45$, independently from blood glucose, eGFR, RBC, white blood cell count, Hb, uCysC, uCysC/Cr, and fCysC.

Conclusions. Plasma and urine NGAL levels are strongly correlated not only with kidney function parameters, but also with red and white blood cell parameters and patient's age and sex.

NEUTROPHIL GELATINASE–ASSOCIATED LIPOCALIN (NGAL) is a small glycosylated polypeptide (25 kDa) belonging to the lipocalin family. It was originally isolated from the supernatant of activated human neutrophils [1]. Significant amounts of NGAL are located in secondary granules of human neutrophils and expressed in response to infection.

Despite the fact that many years have passed since NGAL detection, only recently has the interest in this peptide increased considerably in the context of acute kidney injury (AKI). NGAL is produced by renal tubular cells in response to different types of injury and ischemia. NGAL resistance to degradation leads to immediate excretion of intact

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peptide in the urine. Meta-analysis of several studies evaluating the diagnostic and prognostic value of NGAL in AKI revealed that both plasma and urine NGAL levels perform similarly well [2]. It was found that a significant increase of urine and plasma NGAL concentrations in a short time after a damaging event, such as administration of contrast media, cardiopulmonary bypass, or cardiac surgery, is predictive of the development of AKI [3–6]. Liver transplantation procedure could also be harmful to the kidney [7]. It is estimated that AKI occurs in 17–95% of liver transplant recipients [8]. Acute kidney injury is an unfavourable factor for future renal function and thus worsens patient survival in this population [9,10]. An open question is whether NGAL measurements can be used in the early diagnosis of AKI in this clinical setting. The objective of the present study was to evaluate the factors affecting plasma and urine NGAL levels in patients after liver transplantation.

METHODS

This was a single-center cross-sectional study assessing consecutive liver transplant recipients. The study protocol and procedures were approved by the Ethical Committee of the Medical University of Warsaw and were in accordance with the Helsinki Declaration of 1975 (as revised in 1983). Written informed consent was obtained from each patient before conducting any study procedure.

A total of 107 liver transplant recipients hospitalized in our center were included in the study. Patients were assessed 1–165 months after liver transplantation. The study population consisted of 63 (58.9%) men and 44 (41.1%) women, aged 18–66 years (median 52 y). The incidence of all types of diabetes mellitus was 35 (32.7%). Viral hepatitis (41 [38.3%] HCV, 34 [31.7%] HBV), neoplasms (20 [18.7%]), alcoholic liver disease (18 [16.8%]), and autoimmunologic liver diseases (13 [12.2%] primary sclerosing cholangitiis, 13 [12.2%] primary biliary cirrhosis, 8 [7.5%] autoimmune hepatitis) were main reasons for liver transplantation. Various constellations of these diseases were frequent. There were many different immunosuppressive regimens, including 95 patients (88.8%) receiving prednisone, 86 (80.4%) tacrolimus, 11 (10.3%) cyclosporine, 29 (27.1%) mycophenolates, and 70 (65.4%) basiliximab as an induction therapy.

Blood and urine for cystatin C (CysC) and NGAL were collected from patients. These samples were centrifuged (1,000*g*, 15 min) and frozen at −40°C. CysC and NGAL levels were determined with the use of enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (R&D Systems, Minneapolis, Minnesota). CysC and NGAL levels were studied as plasma concentrations (pCysC and pNGAL), urine concentrations (uCysC and uNGAL), urine concentration to creatinine ratios (uCysC/Cr and uNGAL/Cr), and fractional excretion (fCysC and fNGAL). fCysC was determined as the result of the following equation: (pCr × uCysC)/(pCysC × uCr). Fractional excretion of NGAL was determined as the result of the following equation: (pCr × uNGAL)/(pNGAL × uCr). Anemia was diagnosed based on hemoglobin levels <13.0 g/dL in men and <12.0 g/dL in women.

The analyses of clinical data were performed with the use of Statistica 9.0 version. Unless specified otherwise, continuous data were described as mean ± SD for normal distribution, and median (range) for data with a distribution different from normal. Comparisons between subgroups were made with the use of *t* test for normally distributed data and Mann-Whitney *U* test for nonnormally distributed data. *P* values of <.05 were considered to be significant.

RESULTS

Plasma NGAL levels were found to be inversely correlated with estimated glomerular filtration rate (eGFR) as calculated with the use of Chronic Kidney Disease–Epidemiology Collaboration (CKD-EPI) equation and pCysC ($r = -0.26$ and $P = .007$; $r = -0.38$ and $P = .00006$; respectively). Urinary NGAL levels were positively correlated with urinary C-reactive protein (CRP), uCysC/Cr ratio, fCysC, and lymphocyte count ($r = 0.2$ and $P = .036$; $r = 0.43$ and $P = .000004$; $r = 0.4$ and $P = .01$; $r = -0.29$ and $P = .003$; respectively). uNGAL/Cr was inversely correlated with hematocrit (Htc), hemoglobin (Hb), white blood cell count (WBC), and lymphocyte count ($r = -0.35$ and $P = .0002$; $r = -0.39$ and $P = .00004$; $r = -0.24$ and $P = .01$; $r = -0.37$ and $P = .00008$; respectively) and positively correlated with N-terminal pro–B-type natriuretic peptide (NT-proBNP), CRP, and uCysC/Cr ($r = 0.3$ and $P = .006$; $r = 0.2$ and $P = .208$; $r = 0.5$ and $P < .0000001$; respectively). fNGAL was directly correlated with NT-proBNP, uCysC/Cr and fCysC ($r = 0.31$ and $P = .004$; $r = 0.53$ and $P < .0000001$; $r = 0.39$ and $P = .00005$; respectively) and inversely correlated with Htc, Hb, red blood cell count (RBC), WBC, and neutrophil and lymphocyte counts ($r = -0.3$ and $P = .002$; $r = -0.3$ and $P = .001$; $r = -0.31$ and $P = .001$; $r = -0.29$ and $P = .003$; $r = -0.2$ and $P = .028$; $r = -0.32$ and $P = .008$; respectively).

Significant differences between subgroups are presented in Table 1. There were no statistically significant differences in plasma and urine NGAL parameters when patients were divided into subgroups according to the presence of diabetes, cigarette smoking, arterial hypertension, and type of calcineurin inhibitor administered (cyclosporine or tacrolimus). In multivariate regression analysis, pNGAL was positively correlated with time from liver transplantation, neutrophil count, pCysC, and sex ($\beta = 0.36$ and $P = .00001$; $\beta = 0.32$ and $P = .0001$; $\beta = 0.58$ and $P < .0000001$; $\beta = 0.17$ and $P = .025$; respectively) and inversely correlated with patient's age ($\beta = -0.18$ and $P = .02$) with $R = 0.67$ and $R^2 = 0.45$, independently from blood glucose, eGFR, RBC, WBC, Hb, uCysC, uCysC/Cr, and fCysC.

DISCUSSION

We have demonstrated that after liver transplantation, plasma and urinary levels of NGAL are associated not only with renal function, but also with inflammation parameters, complete blood count parameters, iron balance, and patient's sex and age. Plasma NGAL is a product of many sources, including activated neutrophils, injured epithelial cells, and neoplasms. However, it was found to be one of the most up-regulated genes in the kidney following ischemic injury [3]. We also observed higher plasma and urine NGAL parameters in patients with impaired renal function and anemia (Table 1). The usefulness of NGAL application in