Neutrophil Gelatinase–Associated Lipocalin Levels in Patients With Crohn Disease Undergoing Treatment With Infliximab

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Abstract: Increased levels of neutrophil gelatinase–associated lipocalin (NGAL), a small 25-kd stress protein released by several injured cells, have been reported in different pathological conditions such as kidney and chronic inflammatory bowel diseases. As NGAL is also emerging as a biomarker for monitoring the response to different types of treatment, the aims of this pilot study were to analyze urinary NGAL levels in a small cohort of patients affected by Crohn disease and to evaluate the eventual impact of the intravenous administration of infliximab on these levels.

Crohn disease patients presented increased NGAL values compared with controls (210.5 ng/mL [88.3–1100.0 ng/mL] vs 7.6 [4.4] ng/mL; $P = 0.001$); the infusion of a single high dose of infliximab induced an impressive reduction in these levels to 80.1 ng/mL (38.6–400.2 ng/mL; $P = 0.006$) with a mean reduction ratio of 62.1%. These findings suggest a pivotal role of NGAL in the systemic adaptations to Crohn disease, also confirming the potential usefulness of NGAL measurement in the evaluation of early responses to therapy or in predicting different clinical outcomes.

Key Words: neutrophil gelatinase–associated lipocalin, Crohn disease, infliximab, biomarker

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Neutrophil gelatinase–associated lipocalin (NGAL), a small 25-kd protein previously known as an antibacterial factor released by activated neutrophils, has recently become one of the most promising biomarkers in clinical nephrology. After damages of various nature, the renal tubular epithelium can release high quantities of NGAL in the urine as a defensive mechanism aiming to counteract the injury: a translational application of this finding has recently led to the discovery of the value of NGAL measurement in the early prediction of incoming acute renal failure in critical patients or even the worsening of renal function in subjects affected by chronic kidney diseases.[1,2] Furthermore, the dramatic decrease of urinary NGAL levels after long-term administration of angiotensin-converting enzyme inhibitors or steroids, and after the intravenous infusion of high doses of human immunoglobulins, has been seen to anticipate the following improvement of other parameters of renal damage, such as proteinuria or creatinine,[3,4] thus expressing an early positive response to these drugs. This has opened new possible scenarios of the clinical application of NGAL as a biomarker for monitoring the response or refractoriness of nephropathic subjects to different treatments. However, in parallel, other works have extended the involvement of NGAL flatly beyond the confines of nephrology, demonstrating that this protein plays a central role in the pathophysiology of chronic heart failure,[5] hematologic diseases,[6] and even human neoplasias[7]; finally, several inflammatory conditions, including those of the vascular system and the respiratory, urinary, and gastrointestinal tracts, are associated with a significant increase in the local and systemic expression of NGAL.[8] In chronic inflammatory bowel diseases, the intestinal mucosa and the immune cells recruited by inflammation produce and release high quantities of NGAL: in accordance with this phenomenon, Nielsen et al.[9] have reported elevated levels of NGAL in feces and serum of subjects affected by Crohn disease (CD), describing also a direct correlation between the extent of NGAL expression and the severity of the disease. At present, however, any study has provided data about levels of urinary NGAL in patients affected by this pathological condition and the effect of different therapies on the balance of this biomarker.

Starting from these assumptions, the aims of this pilot study were to analyze urinary NGAL levels in a small cohort of CD patients and to evaluate if a single intravenous dose of the anti–tumor necrosis factor (TNF) antibody infliximab could have an eventual impact on these levels.

MATERIALS AND METHODS

Six patients (3 women and 3 men; mean [SD] age, 24 [5] years) with an established diagnosis of CD were chosen to be treated with 5-mg/kg infliximab by intravenous infusion. Concomitant medications, budesonide (4 patients), mesalazine (5 patients), beclomethasone (1 patient), and metronidazole (1 patient), remained unchanged. Subjects with a recent diagnosis of cancer, infections, or vasculitis or showing an altered renal function were excluded from the study.

Clinical activity was assessed using the CD activity index (CDAI). Main hematological and biochemical parameters were assessed according to standard methods in the routine clinical laboratory. Level of NGAL was measured in the urine before starting the bolus of infliximab and 24 hours after the end of the infusion using a commercially available ELISA kit (BioPorto, Gentofte, Denmark). Six healthy volunteers (4 men and 2 women; mean [SD] age, 27 [9] years) served as controls.

All subjects gave their full informed approval to take part in the study. The statistical analysis of data was made using the GraphPad Prism (version 4.0; GraphPad Software, La Jolla, CA) package. The Mann-Whitney test was used to compare data from groups. A $P < 0.05$ was considered significant.

RESULTS

At baseline, CD patients showed urinary NGAL values markedly higher than controls, being 210.5 ng/mL (88.3–1100.0 ng/mL) versus 7.6 (4.4) ng/mL ($P = 0.001$; Table 1). The administration of infliximab was well tolerated in each case,

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TABLE 1. Main Biochemical Parameters Before and After Infliximab Therapy in CD Patients

Parameter	Before Infliximab	After Infliximab
Weight, kg	69 (14)	—
Body mass index, kg/m^2	24 (3)	—
CDAI score	220.5 (56.2)	—
Serum creatinine level, mg/dL	0.7 (0.1)	0.7 (0.1)
Red blood cell counts, n × 10^3	4621 (587)	4494 (502)
Hemoglobin, g/dL	12.2 (1.8)	11.9 (1.9)
White blood cell counts, n × 10^3	6.7 (1.3)	7.3 (2.3)
C-reactive protein level, mg/L	2.31 (0.13–5.95)	1.58 (0.09–3.44)
Fibrinogen level, mg/dL	429.7 (76.5)	318.5 (89.8)
Erythrocyte sedimentation rate, mm/h	26 (18)	24 (17)
Serum protein level, g/dL	7.64 (0.4)	7.46 (0.4)
Albumin level, g/dL	4.12 (0.44)	3.98 (0.55)
Urinary NGAL level, ng/mL	210.5 (88.3–1100.0)	80.1 (38.6–400.2)

C-reactive protein level and urinary NGAL level are expressed as median (interquartile range). All other data are mean ± SD.

and no adverse effects necessitating interruption of the infusion were observed. After the treatment, no important changes in basic hematological and biochemical parameters were noted. On the contrary, an impressive decrease in NGAL levels to 80.1 ng/mL (38.6–400.2 ng/mL; $P = 0.006$) with a mean reduction ratio of 62.1% was reported. This reduction, observed in each patient, was shown to be independent from the individual duration of intravenous infusion. Figure 1 resumes these findings.

DISCUSSION

The findings reported suggest at least 2 prompts for discussion.

First, CD patients show augmented urinary levels of NGAL compared with healthy controls. As NGAL is a stress protein virtually released from every tissue after damages of various nature, it may be postulated that the inflamed intestine could represent the main biological source of increased NGAL levels in such a condition. This hypothesis is in accordance with previous studies showing that during inflammatory bowel diseases, the inflamed mucosa effectively gains the capacity to hyperexpress the gene of NGAL and to release this protein in serum and feces.[9,10] Although a direct, increased release of NGAL from the inflamed bowel could consequently lead to an augmented urinary leakage of this protein, it cannot be excluded that other peripheral cells, such as neutrophils and other elements of the innate immunity, may partly contribute to this condition. This would be supported by the notion that CD is associated with a general deregulation of the whole immune system, which is responsible for the altered balance of several cytokines found in these patients.[11]

In particular, previous studies have reported that interleukin 1 and nuclear factor-κB, 2 of the most proinflammatory factors, are strongly up-regulated in CD, probably also taking part in the pathogenesis and progression of this disease.[11,12]

As these proteins are also known as the most important inducers of NGAL expression,[13] one could speculate that the increase in NGAL levels reported in the CD patients may represent nothing but the consequence of a systemic immune cascade starting from a cytokine deregulation.

However, the renal tubular epithelium represents another tissue able to hyperrelease NGAL after damages of various nature.[14] Furthermore, recent findings have underlined that in nephropathic subjects, the increase in urinary NGAL levels sometimes precedes the alteration of other indexes of renal damage, such as serum creatinine or proteinuria. For instance, diabetic patients with albuminuria still in the reference range already present increased NGAL levels in their urine: this may reflect the presence of a tubular damage that anticipates the future manifestation of glomerular lesions.[15]

Bearing this in mind, even if all our patients showed a perfectly conserved renal function, the assumption is that the increase in urinary NGAL levels may in part express a subclinical renal condition that cannot be excluded. This last theory may be also supported by the proven susceptibility of patients affected by inflammatory bowel diseases to develop during their life particular types of nephropathies such as membranoproliferative, mesangioproliferative, membranous, and immunoglobulins A and M nephropathies, as recently described.[16]

Another important discovery of our study is certainly represented by the marked reduction of NGAL levels described in all subjects after the administration of the anti-TNF agent infliximab. This finding is in accordance with the observation that certain immunomodulatory treatments, such as intravenous immunoglobulins, are capable to dramatically reduce urinary NGAL levels.[4] Furthermore, as previously described by other

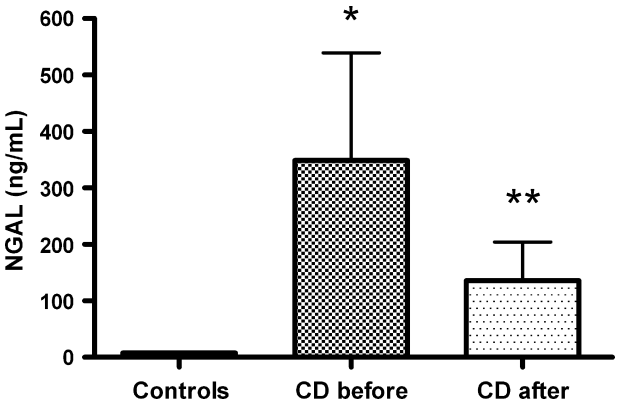

FIGURE 1. Differences in urinary NGAL values between healthy controls and patients with CD before and after the administration of infliximab (*$P = 0.001$; **$P = 0.006$).

authors, treatment with infliximab is effectively able to modify the levels of several acute stress factors such as α1-acid glycoprotein, a lipocalin belonging to the same protein superfamily of NGAL.[17]

Infliximab is known as a powerful immunosuppressor agent, thanks to its ability to counteract the proinflammatory effects of TNF, down-regulating the T_H1 response. That notwithstanding, a direct inhibitory effect of this drug on the synthesis and release of NGAL by intestinal and peripheral cells would seem to contradict previous experimental studies showing that in epithelial cells cultures, a direct stimulation of TNF-α–dependent pathways, unlike interleukin 1 and nuclear factor-κB, does not alter NGAL production.[13] Thus, at present, although fascinating, the exact relationship between infliximab administration and the following decrease in urinary NGAL levels remains unknown; further studies are awaited to throw light on this issue.

The present pilot study certainly presents evident limitations, first of all the small number of subjects analyzed. Although the totality of these patients showed increased levels of urinary NGAL and the administration of infliximab induced, in each case, an impressive decrease of these values, a validation of our findings in wider cohorts would be necessary. Finally, future studies are called to ascertain the exact biological origin of NGAL in patients affected by CD and to verify the effective potential usefulness of NGAL measurement in the evaluation of early responses to therapy or in predicting different clinical outcomes.

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