Role of Neutrophil gelatinase-associated lipocalin in Decompensated Liver Cirrhosis with Spontaneous Bacterial Peritonitis Patients

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Abstract:

Spontaneous bacterial pretonitis is a very serious complication in decompansted cirrhotic patients with a high mortility rate and can precipitate complications as hepatic encephalopthy or hepato renal syndrome which may be fatal .Neutrophil gelatinase associated lipocalin in ascetic fluid may be very useful in diagnosis and follow up spontaneous bacterial pretonitis so an appropriate and early antibiotic treatment in these patients may reduce the rate of mortalilty and prevent its serious complication .

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Introduction:

Bacterial infections are prevalent complications in liver cirrhotic patients, with an incidence of about 34% during hospitalization **(1)**

A common complication of decompensated liver cirrhosis is spontaneous bacterial peritonitis (SBP), which is characterised by abdominal infection and a high recurrence rate. Once SBP occurs, inflammation may quickly promote the progress of liver and renal dysfunction,even leading to death **(2).**

Unfortunately, SBP does not have any unique clinical manifestations. Only one-third of patients have typicalabdominal symptoms and peritoneal irritation, andsome patients develop hepatic encephalopathy and intractable ascites as the firststage **(3).**Therefore, the best time for antibiotic therapy may be missed inthe early stages of SBP for some patients, affecting the prognosis. Currently, clinical diagnosis of SBP is still based on the presence of polymorphonuclear leukocytes (PMN) ≥250 cells/mm3in ascitic fluid. **(4).**

Neutrophil gelatinase-associated lipocalin (NGAL), amember of the lipocalin family, is a low molecular weight secretionprotein originally found in activated neutrophils **(5).**

There is growingevidence that urinary NGAL can reflect the kidney function damageof cirrhotic patients with acute kidney injury (AKI). Monitoring bacterial peritonitis in newly developing non-malignant ascites may be possible with ascitic N GAL **(6).**

Inflammation can affect NGAL levels, which in turn can change theinflammatory response and modulate oxidative stress.

Neutrophil gelatinase associated lipocalin (NGAL) is a novel 24 KDA glycoprotein released by human neutrophil granules and is able to bind iron-laden siderophores of different invading bacteria **(7).**

Studies on animals have shown that NGAL-deficient mice had an increased susceptibility to Escherichia coli infection because of deficiency of NGAL-sequestered iron **(8).**

Human studies have assessed the level of NGAL in the serum and urine, and this level is a helpful biomarker for differentiating acute bacterial infections from viral infections or healthy controls **(9).**Additionally, NGAL in peritoneal fluids can effectively detect bacterial ascites in ascetic patients, according to a recent report. Urinay and cerebrospinal fluid NGAL also can recognize patients with acute bacterial infection **(10).**

Since BT includes the process of bacterial invasion from intestinal lumen to blood or lymphocyte, NGAL may be elevated after encountering invasive bacteria. Therefore, it would be a useful biomarker for BT in patients with severe liver cirrhosis.

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein with a lot of functions including response to injury, as in the case of acute renal tubular damage, as well as involvement in the innate immune response to infection **(11).**

Produced by a number of tissues and cell types, NGAL is rapidly released in response to ischemia, inflammation, and metabolic disorders and has thus been investigated as a biomarker for a number of conditions,including especially urine NGAL in patients with acute kidney injury (AKI) **(9).**

NGAL measurement may also be quite helpful in patients with advanced liver disease. Urine NGAL identifies the cause of AKI in hospitalized patients with cirrhosis, a patient population in which standard diagnostic tests can be difficult to apply, and more accurately predicts in-patient mortality than MELD or serum creatinine **(12).**

Serum NGAL is also increased in the setting of liver injury. LCN-2, the gene responsible for NGAL expression, is markedly up-regulatedwhen mice are exposed to hepatotoxins**(3).**It is also believed to be important in preventing liver damage in response to injurious stimuli **(1)**

In humans, urine NGAL has also been linked to acute-on-chronicliver failure (ACLF) and mortality in a large cohort of patients with cirrhosis, and this urine NGAL elevation was directly linked to the over-expression of LCN-2 on liver biopsy **(4).**

This demonstrates that NGAL also plays a significant role in the response to acute liver injury as well. Finally, given NGAL's role in immune function, urine and serum NGAL levels have been related to a number of infections including urinary tract infections and bacteremia **(13).** However, ascites NGAL levels in patients with cirrhosis are not well characterized. Ascites NGAL has been associated with secondary peritonitis in the setting of peritoneal dialysis.???

Both NGAL and hepcidin influence the iron metabolism of bacteria through different mechanism. As a result, combination use of these twoindicators may be a more effective way to diagnose BT or bactDNA translocation**(4).**

Gene expression and regulation

Initial cloning and sequencing of the NGAL gene revealed a 3696 base-pair coding region, organized into 7 exons and 6 introns, along with a number of possible cis acting elements within the promoter region, that included a binding site for NF-kappaB (NFκB) **(14).**

NGAL gene expression is significantly induced in human epithelial cells by interleukin (IL)-1β. Its induction is dependent on the presence of the NFκB transcription factor and the de novo synthesis of the IKappaB zeta co-factor, which in turn interacts with the p50 subunit of NFκB **(15).**

Secretion of IL-17 by Th17 + CD4 lymphocytes stabilizes the IKappaB zeta transcript which may allow for a tumor necrosis

factor (TNF)-*a*-dependent up-regulation of NGAL gene expression **(2).**

This is supported by the observation that NGAL gene transcription within intestinal epithelial cells is induced by the synergistic actions of IL-17 and IL-22 **(16).**

Similarly, in a murine model of oral candidiasis, the NGAL gene was strongly induced by IL-17 within infected mucosal tissue **(17).** Additionally, NGAL gene expression has been induced in adipocytes after exposure to interferon (IFN)-γ **(18).** Moreover, Toll-like receptor(TLR)4 activation has been shownto be essential for NGAL gene induction by lipopolysaccharides (LPS)**(4).**

Neutrophil Gelatinase –Associated lipocalin and its biological function

Neutrophil gelatinase- associated lipocalin (NGAL) belongs to the lipocalin family of proteins. It consists of a single polypeptide chain with a molecular mass of 25,000 Da and was initially identified in the granules of human granulocytes **(19)**

The function the granules of human granulocytes. The function of NGAL has been extensively evaluated in the last decade. Specifically, NGAL is involved in innate immunity during bacterial infection and it modulates various eukaryotic cellular responses (apoptosis, proliferation and differentiation) **(20)**

During infection, bacteria acquire much of their iron from the host by synthesizing siderophores that scavenge iron and transport it into the pathogen. NGAL is constitutively synthesized and stored inintracellular granules of human neutrophils and it is secreted into the extracellular compartment, where NGAL binds bacterial siderophores Finally, the NGAL-siderophore complex blocks iron bacterial traffic and consequently it limits bacterial growth . Moreover, NGAL seems to act as a modulator of cell homeostasis by iron shuttling between the extracellular and intracellular space **(20)**

These inflammatory properties demonstrate why NGAL secretion has been reported in the setting of some diseases in the absence of bacterial infection, such as inflammatory conditions and kidney injury **(21)**.

Numerous studies have shown its usefulness in the diagnosis of acute kidney disease, and currently NGAL concentration in the urineis considered a valid tool to detect renal tubular injury **(18).**

Role of NGAL during infection. Key events during infectionwithout and with NGAL: In absence of NGAL, the siderophore- iron complex delivers iron into bacterial cell and permits bacterial proliferation. The NGAL secreted from neutrophils in response to infection binds to the siderophore- iron complex.This siderophore- iron- NGAL complex blocks the iron delivery into bacterial cell and limits bacterial proliferation.**(18).**

NGAL has been shown to be constitutively present in human fetal trophoblast cells but absent from cells in the maternal decidua. Furthermore, elevated NGAL levels were observed in placental tissues from women with an intraamniotic infection **(22)**

The in vitro stimulation of trophoblast cells with IL-1β or TNF-α also enhanced NGAL production. Thus, it appears that fetal NGAL participates in antimicrobial immunity during gestation **(23)**

There is also evidence that alterations in NGAL concentrations are characteristic of other non-infectious disturbances of pregnancysuch as preeclampsia and gestational diabetes **(18).**

Viral infections

The role of NGAL in defense against viral infections has not been clearly defined, with only a small number of studies being published. Serum NGAL was able to discriminate between viral and bacterial infections with a higher sensitivity and specificity than CRP in a sample of patients with acute infections **(24)**

NGAL did not have a protective effect against West Nile encephalitis even though its gene expression was induced in brain tissue **(25).**

Other viruses, such as rotavirus and SV40 virus, induce NGALgene expression in human intestinal epithelial cells and murine kidney cells, respectively **(26)**

NGAL gene up-regulation has been detected in human papillomavirus (HPV)-positive cervical lesions, as well as in HPV-infected keratinocytes **(19)**

Human immunodeficiency virus(HIV)-infected patients display significantly decreased serum NGAL levels when compared to healthy controls, which rise after initiation of highly active antiretroviral therapy (HAART), especially in the case of good responders **(24)**

These findings may be linked to the neutrophil count since a strong positive correlation existed with NGAL levels Furthermore, NGAL is a promising marker in the diagnosis of HIV-associated nephropathy, a progressive form of chronic kidney disease that is currently diagnosed by an invasive renal biopsy **(27)**

Genital tract bacterial infections

Plasma levels of NGAL and NGAL/MMP-9 complexes were shown to be elevated in women with pelvic inflammatory disease(PID) when compared to healthy controls **(28)**

These values dropped significantly following a 3-day antibiotic treatment However, plasma NGAL levels in PID patients did not further increase in the presence of a tubo-ovarian abscess **(24)**

NGAL is present in vaginal fluid, and its concentration is reduced in women with bacterial vaginosis, a common disorder in which *Lactobacilli* are replaced by large numbers of anaerobic bacilli and facultative bacteria **(19)**

Vaginal NGAL levels were strongly correlated with the vaginal l-lactic acid concentration, suggesting that vaginal *Lactobacilli* may enhance production of NGAL **(25)**

NGAL, also known as lipocalin-2, is a 25-kDa protein covalently bound to matrix metalloproteinase 9 (MMP-9) and chiefly secreted by neutrophils. Like other lipocalins, NGAL forms a barrel-shaped tertiary structure with a hydrophobic calyx that binds several lipophilic molecules **(5).**

It is expressed only at very low levels in several human tissues but it is markedly increased in injured respiratory cells, intestinal and colon epithelial cells , hepatocytes endothelial cells , and tubuli cells of the kidneys **(19)**

NGAL exerts bacteriostatic effects, attributed to its ability to capture and deplete siderophores secreted by certain bacteria, thereby reducing available iron for bacterial growth **(18)** Furthermore, NGAL has been suggested to modulate various cellular responses, such as proliferation, apoptosis, and differentiation, by playing a pivotal role in NGAL-mediated iron shuttling between extracellular and intracellular spaces **(15).**

It has been also demonstrated that NGAL, by binding to MMP-9, inhibits inactivation of this metalloproteinase, leading to prolonged effects on collagen degradation**(4).**

Over the last few years, several studies have identified induction of NGAL in acute and chronic kidney injury, cardiovascular disease, transplantation, sepsis, chronic obstructive pulmonary disease, pancreatitis, and various cancers **(21)**.

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