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Circulating lipocalin 2 is neither related to liver steatosis in patients with non-alcoholic fatty liver disease nor to residual liver function in cirrhosis

Elisabeth M. Meier [a], Rebekka Pohl [a], Lisa Rein-Fischboeck [a], Doris Schacherer [a], Kristina Eisinger [a], Reiner Wiest [b], Sabrina Krautbauer [a], Christa Buechler [a,*]

[a] Department of Internal Medicine I, Regensburg University Hospital, Regensburg, Germany
[b] Department of Visceral Surgery and Medicine, University Inselspital, Bern, Switzerland

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ABSTRACT

Lipocalin 2 (LCN2) is induced in the injured liver and associated with inflammation. Aim of the present study was to evaluate whether serum LCN2 is a non-invasive marker to assess hepatic steatosis in patients with non-alcoholic fatty liver disease (NAFLD) or residual liver function in patients with liver cirrhosis. Therefore, LCN2 was measured by ELISA in serum of 32 randomly selected patients without fatty liver (controls), 24 patients with ultrasound diagnosed NAFLD and 42 patients with liver cirrhosis mainly due to alcohol. Systemic LCN2 was comparable in patients with liver steatosis, those with liver cirrhosis and controls. LCN2 negatively correlated with bilirubin in both cohorts. In cirrhosis, LCN2 was not associated with more advanced liver injury defined by the CHILD-PUGH score and model for end-stage liver disease score. Resistin but not C-reactive protein or chemerin positively correlated with LCN2. LCN2 levels were not increased in patients with ascites or patients with esophageal varices. Consequently, reduction of portal pressure by transjugular intrahepatic portosystemic shunt did not affect LCN2 levels. Hepatic venous blood (HVS), portal venous blood and systemic venous blood levels of LCN2 were similar. HVS LCN2 was unchanged in patients with end-stage liver cirrhosis compared to those with well-compensated disease arguing against increased hepatic release. Current data exclude that serum LCN2 is of any value as steatosis marker in patients with NAFLD and indicator of liver function in patients with alcoholic liver cirrhosis.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a hepatic expression form of the metabolic syndrome and ranges from steatosis to non-alcoholic steatohepatitis (NASH). NASH has an increased risk to progress to liver cirrhosis and hepatocellular carcinoma [1,2].

NAFLD is associated with obesity and adipose tissue dysfunction. Adipokines are involved in all stages of liver injury including fibrosis, cirrhosis and hepatocarcinogenesis [3]. Adiponectin protects from obesity-associated metabolic diseases and reduced serum adiponectin in NAFLD is linked to hepatic inflammation and fibrosis. In advanced liver disease states adiponectin serum levels are induced with highest levels measured in patients with liver cirrhosis [2–4]. This may be related to impaired hepatic elimination of this adipokine which otherwise is rapidly cleared by the liver [5].

Lipocalin-2 (LCN2) also known as neutrophil gelatinase-associated lipocalin is an acute-phase protein [6]. LCN2 induces adiponectin expression in adipocytes [7]. LCN2 is upregulated in murine adipose tissue and liver in obesity [7,8]. LCN2 is also induced in human NASH liver when compared to normal liver [9]. Anyhow, serum LCN2 is normal in NAFLD patients and is positively associated with fasting insulin and the homeostatic model assessment of insulin resistance (HOMA-IR) [10]. Positive correlations with HOMA-IR have been identified in a second study. In this cohort LCN2 is increased in serum of NAFLD patients [11].

Acute and chronic liver injury in rodents is linked to strongly elevated hepatic and serum LCN2 [12]. LCN2 deficient mice show enhanced hepatic inflammation after lipopolysaccharide injection and increased apoptosis after bile duct ligation. Eight weeks of carbon tetrachloride application promote more severe liver fibrosis in LCN2 deficient animals compared to control animals [12].

An impaired intestinal barrier and gut microbial derived lipopolysaccharide contribute to hepatic inflammation and fibrosis in alcoholic liver disease and NAFLD [13,14]. LCN2 suppresses lipopolysaccharide induced cytokine release in macrophages and

* Corresponding author at: Department of Internal Medicine I, Regensburg University Hospital, D-93042 Regensburg, Germany.
E-mail address: christa.buechler@klinik.uni-regensburg.de (C. Buechler).

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tumor necrosis factor induced dysfunction in adipocytes [7]. LCN2 transports iron and this may be one of the mechanisms to protect against inflammation-associated oxidative stress [15]. Iron is needed for bacterial growth and the bacteriostatic effect of LCN2 is mediated by sequestering iron [16].

Injured hepatocytes have been identified as the main producers of LCN2 in the liver [17]. Inflammatory cytokines but not profibrotic factors induce hepatocyte LCN2 [9,12]. LCN2 is therefore an indicator of liver inflammation and its function is to limit tissue damage.

Acute kidney injury in patients with liver cirrhosis is associated with higher mortality [18]. LCN2 levels in urine of patients with liver cirrhosis may be used in the differential diagnosis of impaired kidney function [19]. Whether serum LCN2 will become a biomarker for renal dysfunction in cirrhosis patients has not been fully evaluated so far [20].

Here, LCN2 was measured in serum of patients with normal liver, NAFLD and patients with liver cirrhosis to identify potential associations with liver function in humans.

2. Patients and methods

2.1. Non-alcoholic fatty liver and controls

The study was approved by the local Ethics Committee and participants signed a form of written consent. Randomly selected outdoor as well as hospitalized patients who were referred to the interdisciplinary ultrasound department of the University Hospital Regensburg for sonographic examination of the abdomen from January to June 2008 were included. Examination was performed with high-end ultrasound equipment ((Sonoline Elegra/Acuson Sequoia 512, Siemens Medical Solutions Inc., Erlangen, Germany), EUB 8500 (Hitachi Medical Corporation Inc., Tokyo, Japan)/Logiq 9 (GE Medical Systems Inc., Milwaukee, USA)). Physicians who received certification for ultrasound examinations (performed at least 400 exams under supervision of an experienced physician and passed an internal practical test) have performed the examination of the liver which was carried out in dorsal recumbency and eventually in inspiration. Using the subcostal and intercostal view both liver lobes were explored. Echo-pattern of the liver was compared with renal parenchyma. Patients were subdivided into controls (no steatosis - similar echo-pattern in the kidney and the liver) and NAFLD (increased hepatic echogenicity). Serum of patients was collected. Patients with alcohol abuse, viral infections and using drugs known to cause fatty liver were excluded. Of the 148 patients examined the remaining study population consisted of 56 patients. The cohort is described in Table 1 and samples have been used in a recent study [21].

2.2. Transjugular intrahepatic portosystemic shunt (TIPS)

The local ethical committee approved the study and all patients gave written informed consent.

The details of the 42 patients with liver cirrhosis are summarized in Table 2. Liver disease was caused by alcohol abuse (35 patients), hepatitis C infection (3 patients) and of other reasons (4 patients). Patients were electively treated by TIPS implantation because of secondary complications of liver cirrhosis which was variceal bleeding in 15 patients, hepatorenal syndrome in 1 patient, refractory ascites in 24 patients and because of other reasons in 2 patients. The procedure of TIPS implantation was performed as described and TIPS (Viatrorr-Stent, Putzbrunn, Germany) was inserted in the fasted state [22]. During TIPS implantation, samples were obtained simultaneously of one of the hepatic veins (HVS) which was not drained by the TIPS-stent, of the portal vein (PVS) and of a peripheral vein such as the superior caval vein (SVS). Patient samples analyzed herein have been used in previous studies [23–26].

Table 2
Characteristics of patient with liver cirrhosis (alanine aminotransferase, ALT; aspartate aminotransferase, AST; model for end-stage liver disease, MELD). Median and range of the values are listed. When data of less than 42 patients had been documented the respective number is shown in superscript.

	Study cohort
Number (females)	42 (9)
Type 2 diabetes	12
Age (years)	53 (26–81)
CHILD-PUGH stage A/B/C	12/14/16
MELD score	8.5 (6–20)
Ascites: no/little/modest/massive	6/11/3/22
Variceal size: no/small/large	8/7/27
C-reactive protein (mg/l)	12.9 (1.0–53.5)
Fibrinogen (mg/dl)	263 (114–520)[41]
AST (U/l)	35 (4.0–108.0)
ALT (U/l)	27.0 (2.0–84.0)
Albumin (g/l)	31.7 (1.6–44)
Bilirubin (mg/dl)	1.25 (0.3–8.2)
Quick prothrombin time (%)	73 (28–100)
Creatinine (mg/dl)	1.0 (0.5–2.7)
Creatinine clearance (ml/min)	54 (18–204)[41]

Table 1
Characteristics of the controls and patients with NAFLD (alanine aminotransferase, ALT; aspartate aminotransferase, AST; body mass index, BMI; waist-to-hip ratio, WHR). Median and range of the values are shown.

Steatosis	No	Yes	p-Value
Number (females)	32 (19)	24 (11)	
Type 2 diabetes	6	5	
Age (years)	56.5 (21.0–88.0)	62.5 (20.0–80.0)	
BMI (kg/m^2)	25.6 (18.0–38.1)	27.5 (19.3–39.7)	
WHR	0.94 (0.73–1.2)	0.97 (0.8–1.2)	
Albumin (g/l)	47.0 (34.8–56.0)	46.0 (31.6–52.8)	
AST (U/l)	28.0 (16.0–48.0)	32.0 (20.0–48.0)	
ALT (U/l)	20.0 (12.0–44.0)	24.0 (12.0–44.0)	
Ferritin (ng/ml)	78 (19–1402)	204 (3–872)	0.011
LDL (mg/dl)	112 (44–340)	108 (48–220)	
Transferrin saturation (%)	20 (6–33)	21 (4–41)	
Triglycerides (mg/dl)	116 (48–344)	130 (56–392)	
Total cholesterol (mg/dl)	214 (112–528)	212 (120–336)	
Bilirubin (mg/dl)	0.5 (0.3–1.9)	0.6 (0.2–1.7)	
Systolic blood pressure (mmHg)	130 (86–165)	139 (104–180)	
Diastolic blood pressure (mmHg)	80 (50–98)	80 (63–108)	
Adiponectin (μg/ml)	4.1 (1.5–22.6)	2.7 (1.1–8.7)	0.031