We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,700
Open access books available

182,000
International authors and editors

195M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK CITATION INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Chapter

Nonalcoholic Fatty Liver Disease, Procalcitonin, and Gut Microbiota: Players in the Same Team

Doina Georgescu, Ana Lascu, Ioana Ionita,

Oana-Elena Ancusa, Mihai Ionita, Ciprian Rosca,

Despina Calamar-Popovici and Daniel Lighezan

Abstract

The study aimed to assess the link between procalcitonin (PCT) and gut dysbiosis in patients with nonalcoholic fatty liver disease (NAFLD). A total of 125 research participants, 100 patients with NAFLD (59% women and 41% men) age between 43 and 84 years and 25 healthy controls, joined this observational study. Patients were consecutively enrolled into two groups: 50 with gut dysbiosis and 50 without gut dysbiosis, after several conditions have been ruled out. Patients from dysbiotic group displayed significantly lesser use of biguanides and statins and elevation of fatty liver index (FLI), PCT, C-reactive protein (CRP), and alanine aminotransferase (ALT). Their gut microbiome was characterized by *Bacteroides* and *Prevotella* sp. dominant enterotype (74%) and by *Ruminococcus* sp. in only 26% of cases. The decrease of H index of biodiversity was observed in 64% of patients as well as of *Firmicutes/Bacteroidetes* (F/B) ratio and *Akkermansia muciniphila* in 60%. The increase of lipopolysaccharide positive bacteria was noted in 62% of patients. PCT strongly correlated with the level of CRP and ALT as well as to stool's H index of biodiversity and F/B ratio. Dysbiotic patients with NAFLD exhibited significant elevation of PCT that correlated well with the H index of stool's microbiota biodiversity, F/B ratio, CRP level, and severity of cytolytic syndrome.

Keywords: nonalcoholic fatty liver disease, procalcitonin, gut dysbiosis

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) represents the accumulation of fat of more than 5% of liver cells, not related to alcohol abuse. It can manifest as simple steatosis, inflammation with hepatocytes necrosis, known as nonalcoholic steatohepatitis (NASH), or in serious situations as end-stage chronic liver disease (NASH-related cirrhosis) with severe fibrosis and architectural damages [1, 2]. Over the past few decades, given the increased incidence of metabolic syndrome, NAFLD became a leading actor in liver diseases, with an exponentially upward

trend, especially in developed countries [3]. As result, NAFLD features as a problem of public health due to its evolutionary potential with propensity of development fibrosis, cirrhosis, hepatocellular carcinoma, and liver-related morbidity and mortality, not to mention the increase risk for cardiovascular diseases in conjunction to associated metabolic issues [4, 5].

The development of NAFLD/NASH could be triggered by multiple conditions such as genetic disorders, particularities of life style and diet with high intake of carbohydrates and fats, hormones imbalance and insulin resistance, host-derived features like age, ethnicity, gender, antibiotic use, and inflammatory state, as well as imbalance of gut microbiota [6].

Procalcitonin (PCT), a peptide 13-kD glycoprotein, which is a precursor of calcitonin, without hormonal activity, rises in serum as a response to proinflammatory conditions, especially related to those of bacterial origin. In this context, PCT along with C-reactive protein (CRP), interleukins (ILs), and various cytokines could be considered as an acute phase reactant [7, 8].

Interestingly, while PCT levels should decline in patients with liver diseases and hepatocytes insufficiency, however, it was observed an increase of those levels, even without a bacterial infection. Those observations shed a new light upon the relation PCT and liver conditions. In patients with acute liver failure, it seems that procalcitonin elevation is not related to bacterial infection but more to cellular injury [9, 10].

The relation between NAFLD and gut microbiota dysbiosis was observed three decades ago in rats with blind intestinal loop and small intestinal bacterial overgrowth [11]. Gut microbiota dysbiosis could intervene in NAFLD pathogenesis by modulating the energy metabolism and insulin resistance, increasing free fatty acids (FFA), decreasing choline production, increasing gut permeability, upregulating hepatic de novo lipogenesis and triglyceride synthesis, releasing hepatotoxic compounds, eliciting endogenous alcohol production, and eventually producing hepatocyte's fat accumulation as droplets of triglycerides [12].

Some studies have demonstrated that gut microbiota dysbiosis may be involved in the perturbation of the hepatic metabolism of carbohydrates and lipids that consecutively could disturb the balance between pro- and anti-inflammatory local liver cytokines, giving the possibility of development NAFLD or NASH [13].

The so-called gut-liver axis represents not only a proximity anatomical relationship but also a perfect functional link between liver and the gastrointestinal tract. Through this axis, a direct connection is made, so that many metabolites related to the gut microbiota could rapidly reach receptors located at the liver surface and consecutively trigger the activation of numerous pathogenic pathways, resulting in serious events such as insulin resistance, liver inflammation, hepatocyte destruction, and fibrosis [14, 15].

The increase of gut permeability seems to play an important role in NAFLD by releasing into the portal vein stream of several substances resulted from bacterial metabolism, such as lipopolysaccharides (LPS), bacterial components, short-chain fatty acids (SCFAs), bile acids (BAs), choline metabolites, and endogenous ethanol that reach the liver and seem to contribute to the pathogenesis of NAFLD [16].

A human study based on histology-proven fatty liver (FL) disease has demonstrated that the severity of NAFLD is related not only to gut microbiota dysbiosis per se but also to important metabolic functional modifications of the gut microbiome. It was observed that *Bacteroides* sp. were significantly increased in patients with NASH and *Ruminococcus* sp. were associated to higher stages of fibrosis: F ≥ 2. The authors attempted to make a

stratification of NAFLD related to enterotypes of gut microbiota and hypothesized that the imbalance of microbiota could be used as a possible predictor of NAFLD [17].

2. Aim of the study

The aim of the study was to assess whether there is a link between PCT and gut dysbiosis in noncirrhotic patients with nonalcoholic fatty liver disease (NAFLD). The study was approved by the Ethics Committee of Scientific Research of the University of Medicine and Pharmacy "Victor Babes" from Timisoara, Romania, Nr. 15/10.05.2021 and was conducted in accordance with the Declaration of Helsinki. All the participants provided written informed consent before the beginning of the study.

3. Patients and methods

3.1 Inclusion criteria

A total of 125 research participants, 100 patients with NAFLD (59% women and 41% men) having a mean age of 48.67 ± 8.66 years and 25 healthy controls, joined this observational study. Patients were consecutively enrolled, being assigned into two groups, based on the presence or absence of the gut dysbiosis (DB): 50 with DB, the study group and 50 without DB, the comparison group, after several diseases and conditions have been ruled out.

3.2 Exclusion criteria

Exclusion criteria include exposure to toxics such as alcohol abuse with heavy drinking more than 40 g/day in men and 30 g/day in women, over the past 10 years, or exposure to industrial toxic substances, as well as to several groups of drugs with liver toxicity, and other liver conditions either inherited or acquired like hemochromatosis, alpha$_1$ antitrypsin deficiency, Wilson disease, autoimmune hepatitis, primary biliary cirrhosis, infection with viral B, D, or C hepatitis. Many other conditions such as organ insufficiency (heart, lungs, liver, or kidney), cancer, recent trauma and surgery, burning, myocadial infarction and cardiogenic shock, stroke, bacterial infectious diseases and sepsis, pancreatic diseases, thyroid diseases, long-standing parenteral nutrition, inflammatory bowel disease, and other entities resulting in malnutrition syndromes, as well as recent treatment with antibiotics or probiotics have been ruled out.

3.3 Examination approach and laboratory work-up

Patients underwent measurements of waist circumference and blood pressure (BP), body mass index (BMI) assessment, as well as thoroughly clinical examination. *Laboratory work-up:* complete blood count (CBC), routine liver tests including hepatitis B surface (HBs) antigen, Delta antigen, anti-HCV antibodies, plasma iron and copper, alpha 1 antitrypsin, antinuclear antibodies (ANA), antimitochondrial antibodies (AMA), lipase, thyroid stimulating hormone, as well as C-reactive protein (CRP), procalcitonin (PCT), fasting plasma glucose (FPG), HbA$_1$c, total cholesterol, low-

density lipoprotein (LDL) and high-density lipoprotein (HDL), triglycerides, creatinine and uric acid, microproteinuria, urine and stool microbiology were run, using standardized, accredited methods.

Stool's microbiological assessment: Sterile containers with collected stool samples were frozen at −20°C and initially processed in order to determine possible aerobe, anaerobe, or microaerophiles species [18]. After identifying different types of stool species by the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) method, they were expressed as colony formatting units (CFU)/gram stool and the severity of gut microbiota DB was semiquantitative scored as follows: 0 = absent, 1 = mild, 2 = medium, 3 = severe [19]. In the case of dysbiosis, frozen stools were further processed by the 16S rRNA next-generation sequencing (NGS) method in order to assess the enterotype, H index of alpha-biodiversity, and several bioindicators of the gut microbiome [20].

3.4 Noninvasive assessment of NAFLD

3.4.1 Imaging assessment

Every study participant was performed high-resolution real-time duplex ultrasonography with semiquantitative assessment of steatosis as follows: mild, moderate, and severe [21]. Point shear wave elastography was performed in order to rule out severe fibrosis (F4) [22]. Each patient also underwent abdominal helical computed tomography (CT) with evidence of the decrease of liver density consecutive to lipid accumulation [23].

3.4.2 Fatty liver index (FLI)

The FLI was calculated based on a mathematical formula that included measurements of waist circumference (cm), BMI (kg/m^2), gamma-glutamyl-transpeptidase (GGT) (U/l), and triglycerides (mg/dl), as follows: FLI = (e 0.953*log e (triglycerides) + 0.139*BMI + 0.718*log e (GGT) + 0.053*waist circumference – 15.745)/(1 + e 0.953*log e (triglycerides) + 0.139*BMI + 0.718*log e (GGT) + 0.053*waist circumference – 15.745) × 100. The values could range between 0 and 100. If the FLI is under 30, there is a very low probability of fatty liver (FL), but an FLI over 60 substantially magnifies the risk for FL, prompting complementary examinations [24].

3.4.3 Fibromax (BioPredictive®)

Fibromax (BioPredictive®) with the calculation of SteatoTest® and NashTest® based on some biochemical blood variables, such as alpha-2 macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, gamma-glutamyl-transpeptidase (GGT), alanine—aminotransferase (ALT), aspartate—aminotransferase (AST), fasting plasma glucose (FPG), cholesterol, and triglycerides, as well as on some clinical parameters such as age, gender, weight, and height was used in this study, in order to assess NAFLD in enrolled patients [25].

3.5 Body mass index

BMI was calculated based on patients' height and weight, using the formula: BMI = weight(kg)/height(m)^2 and interpreted as underweight (≤18.5 kg/m^2), normal

(18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), obese (30.0–39.9 kg/m^2), and morbidly obese (≥40 kg/m^2).

3.6 Blood pressure measurements

At least two measurements of blood pressure (BP) were taken in the morning, with patients at rest, in a sitting position, using the same standardized device (OMRON M2 HEM-7121E). The final value of BP represented the mean of these primary two measurements. The diagnostic of hypertension was made according to European guidelines [26].

3.7 Assessment of diabetes mellitus (DM)

According to American Diabetes Association (ADA) criteria, a fasting plasma glucose (FPG) of 126 mg% or higher, or a 2-hour plasma glucose level of 200 mg% during 75-g oral glucose tolerance test, is consistent with the diagnosis of DM [27].

3.8 Assessment of dyslipidemia

The assessment of dyslipidemia was based on the presence of abnormal concentrations of lipids or lipoproteins in the blood, resulting in low level of high-density lipoprotein (HDL), high blood levels of low-density lipoprotein (LDL), or high blood levels of triglycerides. In this study, the cutoffs were considered as follows: total cholesterol <200 mg%, LDL < 100 mg%, HDL > 50 mg%, and triglycerides <150 mg% [28].

3.9 Assessment of chronic kidney disease (CKD)

CKD diagnosis was performed using creatinine serum level and estimated glomerular filtration rate (GFR), presence of microproteinuria (30–300 mg/24 hours) and imagistic characterization of kidney [29].

4. Statistical analysis

Graph Pad Prism 9.4.1 software (Graph Pad Software, Inc., La Jolla, CA, USA) was used for statistical analysis. Given exploratory, pilot study, no sample size calculation was needed. Quantitative variables were expressed as mean values (MV) ± standard deviation (SD). Chi-squared test was used to compare the two of groups, in cases of qualitative variables expressed as percentages. The unpaired t test was calculated and $p \leq 0.05$ was considered statistically significant, with confidence interval CI = 95%. Nonparametric Pearson's correlation test was also performed in order to establish the "r" coefficient, drawing the direction and magnitude of possible links between variables.

5. Results

This is an observational, cross-sectional study concerning 125 research participants: 100 patients with NAFLD, 50 with gut dysbiosis and 50 patients without gut dysbiosis, and 25 healthy controls.

Variables	DB (+)	DB (−)	CON	P_1 DB (+) vs. CON	P_2 DB (−) vs. CON	P_3 DB(+)vs. DB(−)
Age (years)	50.31 ± 8.34	49.45 ± 8.55	50.52 ± 4.03	0.87	0.42	0.61
Gender W/ M	62%/38%	58%/42%	56%/44%	0.77	1	0.77
U/R residence	70%/30%	66%/34%	80%/20%	0.2785	0.2785	1
Hb (g/dl)	13.51 ± 1.22	13.21 ± 1.33	13.77 ± 0.91	0.34	0.06	0.25
L/mm^3	7.55x10^3 ± 2.45x10^3	7.12x10^3 ± 2.14x10^3	7.11x10^3 ± 0.8 x10^3	0.38	0.98	0.45
Plt/mm^3	310.11x10^3 ± 112.22x10^3	290.15x10^3 ± 115.16 x10^3	278.1x10^3 ± 51.40 x10^3	0.18	0.62	0.38
ALT (IU)	57.43 ± 9.32	43.81 ± 6.32	19.92 ± 4.16	**<0.0001**	**<0.0001**	**0.02**
Creat (mg/dl)	1.05 ± 0.66	1.01 ± 0.55	0.64 ± 0.1	**0.0029**	**0.0014**	0.74
FPG (mg/dl)	105.34 ± 19.97	100.54 ± 6.75	80.56 ± 7.28	**<0.0001**	**<0.0001**	0.10
HbA$_1$c	5731 ± 0,288	5255 ± 0,215	5.122 ± 0.311	**<0.0001**	**<0.0001**	**<0.0001**
LDL-C(mg/dl)	137.25 ± 25.72	124.22 ± 31.43	101 ± 21.01	**<0.0001**	**<0.0001**	**0.02**
HDL-C (mg/dl)	40.78 ± 18.45	44.77 ± 7.89	54.28 ± 13.54	**0.0018**	**0.0003**	0.1629
Tgl (mg/dl)	177.85 ± 43.12	127.89 ± 51.11	87.99 ± 11.56	**<0.0001**	**<0.0001**	**<0.0001**
PCT (ng/ml)	1.520 ± 0.045	0.512 ± 0.198	0.1364 ± 0.0418	**<0.0001**	**<0.0001**	**<0.0001**
CRP(g/dl)	2.32 ± 0.86	0.85 ± 0.52	0.2 ± 0.05	**<0.0001**	**<0.0001**	**<0.0001**
Microproteinuria	36%	32%	4%	**0.0028**	**0.0067**	0.6744
Variables	DB (+)	DB (−)	CON	P_1 DB (+) vs. CON	P_2 DB (−) vs. CON	P_3 DB(+)vs. DB(−)
Age (years)	50.31 ± 8.34	49.45 ± 8.55	50.52 ± 4.03	0.87	0.42	0.61
Gender W/ M	62%/38%	58%/42%	56%/44%	0.77	1	0.77
U/R residence	70%/30%	66%/34%	80%/20%	0.2785	0.2785	1
Hb (g/dl)	13.51 ± 1.22	13.21 ± 1.33	13.77 ± 0.91	0.34	0.06	0.25
L/mm^3	7.55x10^3 ± 2.45x10^3	7.12x10^3 ± 2.14x10^3	7.11x10^3 ± 0.8 x10^3	0.38	0.98	0.45
Plt/mm^3	310.11x10^3 ± 112.22x10^3	290.15x10^3 ± 115.16 x10^3	278.1x10^3 ± 51.40 x10^3	0.18	0.62	0.38

Variables	DB (+)	DB (−)	CON	P_1 DB (+) vs. CON	P_2 DB (−) vs. CON	P_3DB(+)vs. DB(−)
ALT (IU)	57.43 ± 9.32	43.81 ± 6.32	19.92 ± 4.16	**<0.0001**	**<0.0001**	**0.02**
Creat (mg/dl)	1.05 ± 0.66	1.01 ± 0.55	0.64 ± 0.1	**0.0029**	**0.0014**	0.74
FPG (mg/dl)	105.34 ± 19.97	100.54 ± 6.75	80.56 ± 7.28	**<0.0001**	**<0.0001**	0.10
HbA$_1$c	5731 ± 0,288	5255 ± 0,215	5.122 ± 0.311	**<0.0001**	**<0.0001**	**<0.0001**
LDL-C(mg/dl)	137.25 ± 25.72	124.22 ± 31.43	101 ± 21.01	**<0.0001**	**<0.0001**	**0.02**
HDL-C (mg/dl)	40.78 ± 18.45	44.77 ± 7.89	54.28 ± 13.54	**0.0018**	**0.0003**	0.1629
Tgl (mg/dl)	177.85 ± 43.12	127.89 ± 51.11	87.99 ± 11.56	**<0.0001**	**<0.0001**	**<0.0001**
PCT (ng/ml)	1.520 ± 0.045	0.512 ± 0.198	0.1364 ± 0.0418	**<0.0001**	**<0.0001**	**<0.0001**
CRP(g/dl)	2.32 ± 0.86	0.85 ± 0.52	0.2 ± 0.05	**<0.0001**	**<0.0001**	**<0.0001**
Microproteinuria	36%	32%	4%	**0.0028**	**0.0067**	0.6744

DB = dysbiosis, CON = controls, W/M = women/men, Hb = hemoglobin, L = leukocytes, Plt = platelets, ALT = alanine-aminotransferase, creat = creatinine, FPG = fasting plasma glucose, HbA1c = glycosylated hemoglobin, LDL = low-density lipoprotein, HDL = high-density lipoprotein, Tgl = triglycerides, PCT = procalcitonin, CRP = C-reactive protein, p bold = significant difference.

Table 1.
Baseline demographic and biological data in research participants.

As seen in **Table 1**, that illustrates demographic and biological baseline aspects in all research participants, patients either dysbiotic or not displayed significant differences when compared with controls, related to several variables, such as ALT, FPG, HbA1C, LDL, and HDL-cholesterol, triglycerides, creatinine, microproteinuria, CRP, and PCT. However, no significant differences were noted when compared patients' age, gender, location, and complete blood count (CBC), to those of control's group. Dysbiotic patients from the study group displayed significant elevation of PCT, C-reactive protein (CRP), and cytolytic enzymes: alanine aminotransferase (ALT), LDL-cholesterol, triglycerides, and HbA1c when compared to patients with NAFLD and no dysbiosis. No significant statistical differences were recorded between dysbiotic and normobiotic patients related to age, gender, location, CBC, creatinine, and HDL-cholesterol.

As seen in **Table 2**, that depicts the comparison of several clinical studied in patients included in this study, patients from dysbiotic group exhibited significant differences related to higher FLI, severity of fatty liver either simple steatosis or NASH, as well as less frequent treatment with biguanides and statins. The other variables, such as smoking history, sedentary life style, obesity, dyslipidemia, hypertension, G-I associated conditions, GSD, T2DM, IGT, CKD, and cardiovascular conditions, showed comparable results when compared dysbiotic patients with those with normobiosis.

As presented in **Table 3**, that displays stool's microbiota main alterations in patients with NAFLD and associated gut dysbiosis, the gut microbiome of the study

Variables	DB (+)	DB (−)	p
Smoking history	54%	38%	0.1102
Sedentary lifestyle	62%	50%	0.2291
BMI > 30 kg/m^2	58%	42	0.1114
HT	36%	28%	0.3936
FLI (units)	77.42 ± 8.44	69.23 ± 7.82	**<0.0001**
Simple steatosis	36%	58%	**0.00283**
NASH	64%	42%	**0.00283**
G-I associated conditions	56%	46%	0.3196
GSD	38%	34%	0.1567
T2DM/IGT	58%	46%	0.4005
Oral antidiabetics other than biguanides	4%	0%	0.3191
Insulin therapy	8%	0%	0.1538
Biguanides	24%	44%	**0.0357**
Statins	36%	58%	**0,0283**
Fibrates	42%	44%	0.8407
Dyslipidemia	40%	36%	0.2183
CKD	36%	32%	0.6641
C-V conditions	56%	44%	0.6897

DB = dysbiosis, BMI = body mass index, HT = hypertension, NASH = nonalcoholic steatohepatitis, G-I = gastrointestinal, GSD = gallstone disease, T2DM/IGT = type 2 diabetes mellitus/Impaired glucose tolerance, CKD = chronic kidney disease, C-V = cardiovascular, p bold = significant difference.

Table 2.
Clinical baseline aspects in patients with NAFLD.

Variables	DB +
Overall DB score	1.62 ± 0.69
Decreased F/B	64%
F/B	2.77 ± 0.68
Biodiversity Shannon-Wiener H index	2.68 ± 0.51
Decreased Shannon- Wiener H index	76%
Increased LPS (+) bacteria	60%
Decreased *Akkermansia muciniphila*	62%

DB = dysbiosis, F/B = Firmicutes/Bacteroidetes, LPS = lipopolysaccharide.

Table 3.
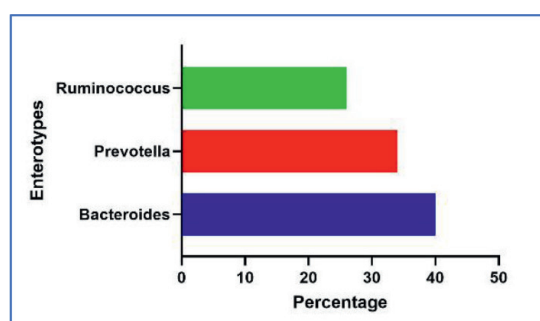*Stool's microbiota bioindicator alterations in dysbiotic patients.*

Figure 1.
Distribution of enterotypes in dysbiotic patients with NAFLD.

group was characterized by several alterations, expressed either by the decrease of various bioindicators, such as H index of biodiversity, observed in 76% of patients, *Akkermansia muciniphila* sp. in 62% of patients, and F/B ratio in 64%, or by the increase of LPS (+) bacteria in 60% of patients.

The study of the stool's microbiota enterotypes based on the mathematical analysis of the proportional relationship between *Bacteroides* sp., *Prevotella* sp., and *Ruminococcus* sp. in dysbiotic patients with NAFLD was expressed in percentages and is depicted in **Figure 1**.

As seen in **Figure 1**, patients with NAFLD and gut microbiota dysbiosis were characterized by a microbiological picture in which predominated *Bacteroides* sp. and *Prevotella* spp. dominant enterotype, observed in 74% of cases. *Ruminococcus spp.* dominant enterotype was noted in only 26% of cases.

Correlations of PCT to several blood biological variables such as CRP and ALT, as well as to stool's microbiota variables like F/B ratio, and H index of alpha biodiversity were analyzed in dysbiotic patients with NAFLD and are displayed in **Figure 2**.

As illustrated in **Figure 2**, PCT positively strong correlated ($p < 0.0001$) to the serum levels of ALT and to the F/B ratio of the gut microbiome ($p < 0.0001$). PCT also positively strong correlated with the stool's microbiota dysbiosis bioindicator, represented by the H index of alpha biodiversity ($p = 0.005$) and to the serum levels of CRP ($p = 0.0031$).

Figure 3 depicts the correlations of the gut microbiota dysbiosis intensity to Fibromax analyzed scores, such as SteatoTest, that expressed the severity of simple
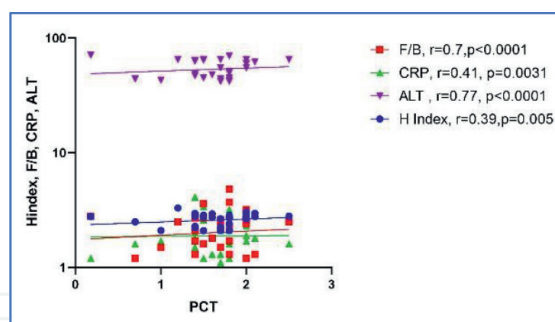
Figure 2.
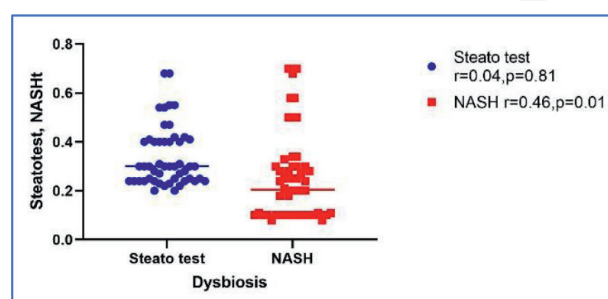*Correlations of PCT in patients with NAFLD and gut dysbiosis.*

Figure 3.
Correlations of severity to steatosis and NASH scores.

steatosis, and NashTest, that expressed the level of necro-inflammatory activity caused by the metabolic condition.

As illustrated in **Figure 3,** significantly positive correlations were noted between the severity of gut microbiota dysbiosis and the NASH scores according to Fibromax test, but no significant correlations were observed between the gut microbiota dysbiosis range and the severity of simple steatosis, represented by SteatoTest scores.

6. Discussions

In the present study, as we observed strong correlations between the levels of inflammation expressed by PCT, CRP, and the severity of cytolytic syndrome, comparable results were also reported by other researches, related to various causes of liver pathologies. Thus, the correlation of PCT and transaminases levels were noted in patients suffering from various forms of acute liver failure, where authors observed that PCT identified not the potential bacterial infections but the severity of the liver cell injury [30]. Another study published in 2019 reported that the levels of serum CRP and serum PCT were positively correlated with transaminases levels and alkaline phosphatase as well, in patients with acute pancreatitis and associated liver injury [31].

As far as we know, at the moment, there are not so many studies addressing the relationship between NAFLD/NASH, procalcitonin, and gut dysbiosis. One case control study that included 50 patients with NAFLD proven by histology did not reveal significant increase of PCT when compared with healthy controls. However, CRP was considered useful in the diagnosis of NAFLD being significant augmented in patients by comparing to controls, but was not capable to discriminate between NASH and simple steatosis [32]. An important relationship between inflammation and NAFLD

was observed by others, as we also noted in the present study. One cross-sectional study that included 55 patients over 30 years old, diagnosed with NAFLD, demonstrated a relationship between fatty liver and CRP levels, bringing additional proof regarding the role of inflammation in NAFLD. Of the proinflammatory cytokines, it seems that tumor necrosis factor-alpha (TNF-alpha) may play a pivotal role in liver inflammation. Also, proinflammatory cytokines and several interleukins (ILs) as well as LPS could trigger reactive oxygen species (ROS). As a consequence, the augmentation of the hepatocyte damage will develop, accompanied by activation of Kupffer cells and further increase of expression of TNF-α and IL-6 that will increase the levels of local and systemic proinflammatory cytokines [33]. A recent review starting from the known phenomenon of persistent inflammation in NAFLD discussed the relationship between continuing subclinical inflammation in NAFLD and the risk for developing hepatocellular carcinoma [34].

According to the Pearson's parametric correlation analysis, the present paper revealed that the levels of PCT correlated strong with certain characteristics of the bioindicators of the intestinal microbiota, namely, the Shannon-Wiener index of alpha biodiversity and the F/B ratio. We have not found in the literature similar studies that analyze this relationship, PCT-gut DB in patients with NAFLD. Regarding this particular relationship between PCT and DB, recently, literature studies have been especially focused on the DB-PCT relationship mostly in COVID-19-infected patients. Thus, in patients suffering from COVID-19 infection and associated hyperinflammatory reaction with augmentation of CRP ≥ 10 mg/dl, PCT ≥ 5 ng/ml, and WBC ≥ 15 G/l, alterations of the gut microbiota finger print were reported, with modification characterized by increase of *Parabacteroides* sp. and *Lachnoclostridium* sp., and reduction of *Blautia* sp., *Faecalibacterium* sp., and *Ruminococcus* sp. [35]. Other studies reported that the so-called triad in patients infected by COVID-19, expressed by the dysbiosis of the gut microbiota, augmented immune response, and high inflammatory state could make the difference between patients, regarding the way they can cope, either being resilient or being fragile and developing the "cytokine storm" with its consecutive severe outcome [36]. Understanding the changes in the intestinal microbiome in COVID-infected patients that could associate a particular host response could explain the unfavorable evolution of those with severe inflammation and increase of CRP and PCT, as well as the persistence of some symptoms as a consequence of remnant dysbiosis [37]. If situations that result in more or less expressed inflammatory syndrome, in which it was demonstrated the increase of the level of CRP and PCT, that were associated with some specific changes in the intestinal microbiota, we could hypothesize that the dysbiosis associated with NAFLD would generate an inflammation and would result in the growth of inflammatory proteins of the acute phase, such as CRP and PCT.

As others and we previously reported, several alterations of the gut microbiome were observed in the present study regarding dysbiotic patients with NAFLD [38, 39]. These modifications were characterized by the decrease of biodiversity of the F/B ratio and of *Akkermansia muciniphila* sp. and by the increase of the LPS (+) bacteria. Modifications of the enterotypes of the microbiota in patients with NAFLD and associated dysbiosis were also seen; thus, *Bacteroides* sp. and *Prevotella* sp. were the dominant enterotypes (enterotypes I and II) in three-fourth of patients, only one-fourth expressing *Ruminococcus* sp. (enterotype III) [40, 41].

Many studies advocated the anti-inflammatory role of statins, but only recently researchers have reported a relationship between statins and gut microbiome. We also observed that patients with NAFLD, obesity, and associated metabolic issues exhibited

alterations of gut microbiota and were less treated with statins for their dyslipidemia. Recent studies reported that patients with obesity presented gut dysbiosis that was negatively associated with statin treatment. Thus, patients displayed alterations of gut microbiota with modifications of the enterotypes of study participants [42]. Others also hypothesize the possibility of statins to even modulate the gut microbiome [43, 44].

Results of the present study showed that patients with NAFLD and dysbiosis with increase of LPS positive bacteria and decrease of *Akkermansia* sp. were less treated with biguanides, by comparing with those without dysbiosis. Alteration of gut microbiota and antidiabetic drugs especially biguanides is a subject to recent debates. Researchers reported that metformin could associate an increase of small chain fatty acid (SCFA)-producing bacteria and may favor some species such as *Proteobacteria phylum*, *Allobaculum Lactobacillus* genera, and *Verrucomicrobia phylum*. The mucin-degrading bacteria are also abundant, such as *Akkermansia* sp. It was also observed that metformin increases *Escherichia* sp. and decreases *Intestinibacter* sp. in human gut microbiota and some species such as *Bifidobacterium adolescentis* were negatively correlated with HbA1c. From this point of view, the lowering effect of the glucose level can also be mediated by the microbiome modifications induced by metformin [45].

7. Conclusion

Dysbiotic patients having NAFLD displayed significant elevation of inflammatory acute phase reactant proteins such as PCT and CRP. Significant increase of the cytolytic enzymes like alanine aminotransferase (ALT) and other biological variables like LDL-cholesterol, triglycerides, and HbA1c was also noted. Patients with NAFLD from the dysbiotic group exhibited significant differences related to higher FLI and severity of fatty liver either simple steatosis or NASH. Less often treatment with biguanides and statins was recorded in patients with fatty liver and gut dysbiosis. The gut microbiome of the patients with NAFLD was characterized by various alterations. The decrease of some bioindicators, such as H index of biodiversity, *A. muciniphila* sp., and F/B ratio, was frequently observed. However, other species, namely, LPS (+), were often found abundant. The enterotypes of patients with NAFLD and dysbiosis were characterized mostly by *Bacteroides* sp. and *Prevotella* spp. and rarely by *Ruminococcus spp.* Strong positive correlations were observed between PCT and some blood biological variables, such as ALT and CRP, as well as between PCT and some stool's microbiota bioindicators, such as F/B ratio and stool's H index of alpha biodiversity. Gut dysbiosis of patients with NAFLD was significantly positively correlated with the severity of NASH scores. All these correlations between PCT and various bioindicators of the gut microbiome and also between dysbiosis and NASH severity suggest that these three entities, namely, PCT, dysbiosis, and NAFLD, are closely related..

Conflict of interest

The authors declare no conflict of interest.

Author details

Doina Georgescu[1*], Ana Lascu[2], Ioana Ionita[1], Oana-Elena Ancusa[1], Mihai Ionita[1], Ciprian Rosca[1], Despina Calamar-Popovici[1] and Daniel Lighezan[1]

1 Department of Internal Medicine I, "V Babes" University of Medicine and Pharmacy, Timisoara, Romania

2 Department of Functional Sciences, "V Babes" University of Medicine and Pharmacy, Timisoara, Romania

*Address all correspondence to: doina.georgescu@umft.ro

IntechOpen

References

[1] Williams CD, Stengel J, Asike MI, et al. Prevalence of NAFLD and NASH among a largely middle –aged population utilizing ultrasound and liver biopsy: A prospective study. Gastroenterology. 2011;**140**:124

[2] Chasalani N, Younossi Z, Lanvine JE, et al. The diagnostic and management of NAFLD: Practice guidance from AASLD. Hepatology. 2018;**67**:328

[3] Perumpail BJ, Khan MA, Yoo ER, et al. Clinical epidemiology and disease burden of NAFLD. World Journal of Gastroenterology. 2017;**23**(47):8263

[4] Marjot T, Moolla A, Cobbold JF, Hodson L, Tomlinson JW. Nonalcoholic fatty liver disease in adults: Current concepts inetiology, outcomes, and management. Endocrine Reviews. 2020;**41**(1):66-117. DOI: 10.1210/endrev/bnz009

[5] Younossi ZM, Paik JM, Al Shabeeb R, Golabi P, Younossi I, Henry L. Are there outcome differences between NAFLD and metabolic-associated fatty liver disease? Hepatology. 2022;**76**(5):1423-1437. DOI: 10.1002/hep.32499

[6] Ancusa OE, Georgescu D, Iurciuc S, et al. Acute phase inflammatory proteins, uut microbiota dysbiosis and severity of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. Review Chimica. 2020;**71**(5):290-298. DOI: 10.37358/Rev

[7] Gomez-Cerquera JM, Daroca-Perez R, Baeza-Trinida R, et al. Validity of procalcitonin on the diagnosis of bacterial infection in elderly patients. Enfermedades Infecciosas y Microbiología Clínica. 2015;**33**:52

[8] Lu J, Cl C, Jin JD, et al. Continuous elevation of procalcitonin in cirrhosis combined with hepatic carcinoma: A case report. BMC Infectious Diseases. 2021;**21**:29. DOI: 10.1186/s12879-020-05684- 2

[9] Dong R, Wan B, Lin S, et al. Procalcitonin and liver disease; a literature review. Journal of Clinical and Translational Hepatology. 2019;**7**(1):51

[10] Rule JA, Hynan LS, Attar N, Sanders C, Korzun WJ, Lee WM, et al. Procalcitonin identifies cell injury, not bacterial infection, in acute liver failure. PLoS One. 2015;**10**(9):e0138566. DOI: 10.1371/journal.pone.0138566

[11] Lichtman SN, Keku J, Schwab JH, Sartor RB. Hepatic injury associated with small bowel bacterial overgrowth in rats is prevented by metronidazole and tetracycline. Gastroenterology. 1991;**100**:513-519

[12] Auguet T, Bertran L, Binetti J. Intestinal dysbiosis and non-alcoholic fatty liver disease. In: Beloborodova NV, Grechko AV, editors. Human Microbiome. London: IntechOpen; 2020. DOI: 10.5772/intechopen.92972

[13] Kolodziejczyk AA, Zheng D, Shibolet O, Elinav E. The role of the microbiome in NAFLD and NASH. EMBO Molecular Medicine. 2019;**11**:e9302

[14] Brandl K, Kumar V, Eckmann L. Gut-liver axis at the frontier of host-microbial interactions. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2017;**312**:G413-G419

[15] Khan A, Ding Z, Ishaq M, et al. Understanding the effects of gut microbiota dysbiosis on nonalcoholic

fatty liver disease and the possible probiotics role: Recent updates. International Journal of Biological Science. 2021;**17**(3):818-833. DOI: 10.7150/ijbs.56214

[16] Janssen S, Depoortere I. Nutrient sensing in the gut: New roads to therapeutics? Trends in Endocrinology and Metabolism. 2013;**24**(2):92-100. DOI: 10.1016/j.tem.2012.11.006

[17] Boursier J, Mueller O, Barret M, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatology. 2016;**63**(3):764-775

[18] Brown R, Poxton IR, Wilkinson JF. Centrifuges, colorimeters and bacterial counts. In: Collee JG, Duguid JP, Fraser AG, Marmion BP, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. Edinburgh, Churchill Livingstone; 1999. pp. 200-247

[19] Sandrin TR, Goldstein JE, Shoemaker S. MALDI TOF MS profiling of bacteria at the strain level: A review. Mass Spectrometry Reviews. 2013;**32**(3):188

[20] Ji B, Nielsen J. From next generation sequencing to systemic modeling of the gut microbiome. Frontiers in Genetics. 2015;**6**:219

[21] Li Q, Dhyani M, Grajo JR, et al. Current status of imaging in NAFLD. World Journal of Hepatology. 2018;**10**(8):530

[22] Sirli R, Sporea I, Popescu A, Danila M. Ultrasound-based elastography for the diagnosis of portal hypertension in cirrhotics. World Journal of Gastroenterology. 2015;**21**(41):11542-11551

[23] Zhang YN, Fowler KJ, Hamilton G. Liver fat imaging—A clinical overview of ultrasound, CT, and MR imaging. The British Journal of Radiology. 2018;**91**(1089):20170959

[24] Begogni G, Bellentani S, Miglioli L, et al. The fatty liver index: A simple and accurate prediction of hepatic steatosis in general population. BMJ Gastroenterology. 2006;**6**(1):33

[25] Morra R, Munteanu M, Imbert-Bismut F, et al. Fibromax: Towards a new universal biomarker of liver disease? Expert Review of Molecular Diagnostics. 2007;7:5

[26] Mancia G, Fagard R, Narkiewicz K, et al. ESH/ESC Guidelines for the management of arterial hypertension. In: 23rd European Meeting on Hypertension & Cardiovascular Protection. 2013. http://www.esh2013.org/wordpress/wp-content/uploads/2013/06/ESC-ESH-Guidelines-2013

[27] American Diabetes Association. Diagnostic and classification of diabetes mellitus. Diabetes Care. 2014;**37**(suppl1):S81-S90

[28] Grundy SM, Cleeman JL, Merz CN, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. Circulation. 2004;**110**:227

[29] Johnson CA, Levey AS, Coresh J, et al. Clinical practice guidelines for chronic kidney disease in adults: Part I. definition, disease stages, evaluation, treatment, and risk factors. American Family Physician. 2004;**70**(5):869

[30] Sugihara T, Koda M, Okamoto T, et al. Serum procalcitonin in patients with acute liver failure. Yonago Acta

Medica. 2017;**60**(1):40-46. PMID: 28331420; PMCID: PMC5355843

[31] Li XC, Y, Liu Z, Chen, H Mao H. The relationship between liver injury and serum levels of C-reactive protein and procalcitonin in patients with acute pancreatitis. Journal of the College of Physicians and Surgeons Pakistan. 2019;**29**(3):287-289

[32] Oruc N, Ozutemiz O, Yuce G, et al. Serum procalcitonin and CRP levels in non-alcoholic fatty liver disease: A case control study. BMC Gastroenterology. 2009;**9**:16. DOI: 10.1186/1471-230X-9-16

[33] Foroughi M, Maghsoudi Z, Khayyatzadeh S, Ghiasvand R, Askari G, Iraj B. Relationship between non-alcoholic fatty liver disease and inflammation in patients with non-alcoholic fatty liver. Advanced Biomedical Research. 2016;**5**:28

[34] Peiseler M, Tacke F. Inflammatory mechanisms underlying nonalcoholic teatohepatitis and the transition to hepatocellular carcinoma. Cancers (Basel). 2021;**13**(4):730. DOI: 10.3390/cancers13040730

[35] Schult D, Reitmeier S, Koyumdzhieva P, et al. Gut bacterial dysbiosis and instability is associated with the onset of complications and mortality in COVID-19. Gut Microbes. 2022;**14**(1):2031840. DOI: 10.1080/19490976.2022.2031840

[36] Ferreira C, Viana SD, Reis F. Is gut microbiota dysbiosis a predictor of increased susceptibility to poor outcome of COVID-19 patients? An update. Microorganisms. 2020;**9**(1):53. DOI: 10.3390/microorganisms9010053

[37] Yeoh YK, Zuo T, Lui GC, et al. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. Gut. 2021;**70**:698-706

[38] Georgescu D, Iurciuc M, Ionita I, et al. Portal vein thrombosis and gut microbiota: understanding the burden. Revista de Chimie. 2019;**70**(6):2181-2185. DOI: 10.37358/RC.70.19

[39] Georgescu D, Ionita I, Lascu A, et al. Gallstone disease and bacterial performance of gut microbiota in middle aged and older people. International Journal of General Medicine. 2022;**15**:5513. DOI: 10.2147/IJGM.S350104

[40] Salzman ET, Palacios T, Thomsen M, Vitetta L. Intestinal microbiome shifts, dysbiosis, inflammation and nonalcoholic fatty liver disease. Frontiers in Microbiology. 2018;**9**:61

[41] Grabherr F, Grander C, Effenberger M, Adolph TE, Tilg H. Gut dysfunction and non-alcoholic fatty liver disease. Frontiers in Endocrinology. 2019;**10**:611

[42] Vieira- Silva S, Falony G, Belda E, et al. Statin therapy is associated with lower prevalence of gut microbiota dysbiosis. Nature. 2020;**581**:310-315. DOI: 10.1038/s41586-020-2269-x

[43] Dias AM, Cordeiro G, Estevinho MM, et al. Gut bacterial microbiome composition and statin intake - a systematic review. Pharmacology Research & Perspectives. 2020;**8**:e00601. DOI: 10.1002/prp2.601

[44] Lim GB. Improved gut microbiota profile in individuals with obesity taking statins. Nature Reviews. Cardiology. 2020;**17**:385https. DOI: 10.1038/s41569-020-0396-6

[45] Montandon SA, Jornayvaz FR. Effects of antidiabetic drugs on gut microbiota composition. Genes. 2017;**8**:250. DOI: 10.3390/genes810025