Case Control Study

Leukocyte cell-derived chemotaxin-2 and fibroblast growth factor 21 in alcohol-induced liver cirrhosis

Jarosław Jerzy Sak, Andrzej Prystupa, Paweł Kiciński, Dorota Luchowska-Kocot, Ewa Kurys-Denis, Hanna Bis-Wencel

ORCID number: Jarosław Jerzy Sak 0000-0002-8763-0683; Andrzej Prystupa 0000-0003-4628-8911; Paweł Kiciński 0000-0002-0142-4812; Dorota Luchowska-Kocot 0000-0002-1005-6580; Ewa Kurys-Denis 0000-0002-2664-7588; Hanna Bis-Wencel 0000-0003-1425-3378.

Author contributions: Sak JJ and Prystupa A were involved in the conception of the study, data collection and analysis, drafting and revision of the manuscript; Kiciński P and Luchowska-Kocot D were involved in the data collection and analysis; Kurys-Denis E was involved in the drafting and revision of the manuscript; Bis-Wencel H contributed to the data collection and revision of the manuscript.

Institutional review board statement: The study protocol was approved by the Bioethics Committee at Medical University of Lublin, Poland.

Informed consent statement: All patients gave their written informed consent for participation in the study.

Conflict-of-interest statement: The authors declare no conflict of interest.

Abstract

BACKGROUND
The importance of early diagnosis of alcoholic liver disease underscores the need to seek better and especially non-invasive diagnostic procedures. Leukocyte cell-derived chemotaxin-2 (LECT2) has been widely studied to determine its usefulness in monitoring the course of non-alcoholic fatty liver disease but not for alcoholic liver cirrhosis (ALC).

AIM
To determine the concentration of LECT2 in the blood serum of patients in relation to progressive stages of ALC, its relation to fibroblast growth factor 1 (FGF-1) and FGF-21, and to examine the possible wider use of LECT2 in diagnosing ALC.
INTRODUCTION

Alcoholic liver disease (ALD) occurs in three stages: fatty liver, alcoholic hepatitis, and liver cirrhosis. In the present study, the role of leukocyte cell-derived chemotaxin-2 (LECT2) in the development of alcohol-induced liver cirrhosis was investigated.

In recent decades, there have been significant developments in research on the biochemical possibilities for the early diagnosis and monitoring of non-alcoholic fatty liver disease (NAFLD)[1]. Hepatokines were found to be extremely useful for NAFLD monitoring[2]. Moreover, relationships between the stages of NAFLD and fetuin-A[3, 4], selenoprotein-P[5, 6], and fibroblast growth factor 21 (FGF-21)[7] have been demonstrated. Fibroblast growth factor mimicking has been developed as a novel therapeutic option[8]. The analogues of hepatokines, such as a pegylated FGF-21 analogue[9], have been used in NAFLD therapies. However, finding similar diagnostic options for ALD remains valid[10]. ALD is among the most prevalent diseases in Western countries. It has recently been recognized as an increasingly serious epidemi-
The aim of our study was to determine the concentration of LECT2 in the blood of patients at progressive stages of alcoholic liver cirrhosis to determine the relation to FGF-1 and FGF-21, and to discuss the possible wider use of LECT2 in the diagnosis of ALC.

MATERIALS AND METHODS

The study protocol was approved by the Bioethics Committee. All patients gave their written informed consent prior to participating in the study.

Patients

The study was conducted at the Department of Internal Medicine, Medical University of Lublin, Poland, and included 69 patients from the region of Lublin (eastern Poland) with alcoholic cirrhosis. Liver cirrhosis was diagnosed based on clinical features, history of heavy alcohol consumption, laboratory tests, and abdominal ultrasonography. Heavy alcohol consumption was defined according to the guidelines of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as consuming more than four drinks on any day or more than 14 drinks per week for men and three drinks on any day or more than seven drinks per week for women. Patients with alcoholic hepatitis, hepatocellular carcinoma, or viral and autoimmune diseases were excluded from the study. Other exclusion criteria were type 2 diabetes, obesity, acute infections (e.g., pneumonia, spontaneous bacterial peritonitis), acute and chronic heart failure (> NYHA I—i.e. slight or marked limitation of physical activity, ordinary physical activity results in fatigue, palpitation, dyspnea), acute and chronic respiratory disorders resulting in respiratory insufficiency, acute kidney injury, and chronic kidney disease (> stage G2—i.e. an estimated glomerular filtration rate < 60 mL/min). Both clinical assessments and laboratory tests were used to exclude underlying liver diseases in the control group. The degree of liver cirrhosis was evaluated according to Pugh-Child criteria (the Pugh-Child score), and on that basis, patients were assigned to one of three groups: Pugh-Child (P-Ch) A (n = 21) with stage A, P-Ch B (n = 23) with stage B, and P-Ch C (n = 28) with stage C liver cirrhosis (Table 1). The control group consisted of 17 healthy individuals without liver disease who did not abuse alcohol. Detailed demographic, clinical, and biochemical characteristics of the patients are presented in Tables 1 and 2.

Biochemical measurements

Blood was drawn, and after centrifugation, serum was collected for analysis. Human LECT2, FGF-1, and FGF-21 were determined using enzyme-linked immunosorbent assay (ELISA) kits. All absorbance readings were conducted using an Epoch Microplate Spectrophotometer (BioTek Instrumentals, Inc., Winooski, VT, United States). LECT2 concentrations were determined using a BioVendor Human LECT2 ELISA kit (BioVendor, Laboratorni medicina a.s., Brno, Czech Republic). FGF-1 and FGF-21 concentrations were quantified using sandwich enzyme immunoassay kits.
Table 1 Patients’ demographics and clinical characteristics

|                         | Control group (n = 17) | Liver cirrhosis                                      | P value |
|-------------------------|------------------------|------------------------------------------------------|---------|
|                         |                        | Pugh-Child A + B (n = 37)                      | Pugh-Child C (n = 32) |         |
| Age (yr)                | 43.7 ± 14.6            | 55.7 ± 12.1                                       | 55.9 ± 10.2 | 0.021   |
| Percentage of males (%) | 64.3%                  | 73%                                                 | 72.7%     | 0.52    |
| Body weight (kg)        | 67.6 ± 8.9             | 73 ± 11.4                                          | 75.5 ± 12.8 | 0.17    |
| Height (cm)             | 173 ± 5.9              | 174 ± 8                                            | 173 ± 7.6 | 0.64    |
| Duration of alcohol abuse (yr) | -                  | 15.7 ± 8.2                                        | 18.7 ± 8.3 | 0.98    |
| Oesophageal varices (%) | -                      | 32.4%                                               | 81.8%     | < 0.0001|
| Encephalopathy (%)      | -                      | 32.4%                                               | 83.9%     | < 0.0001|
| Ascites (%)             | -                      | 40.5%                                               | 90.9%     | < 0.0001|
| Total bilirubin (mg/dL) | 0.6 ± 0.3              | 4.6 ± 6.9                                          | 10.5 ± 9.2 | < 0.0001|
| INR                     | -                      | 1.36 ± 0.35                                        | 1.95 ± 0.56 | < 0.0001|
| Albumin (g/dL)          | -                      | 3.1± 0.8                                           | 2.4± 0.4  | 0.0002  |
| Total protein (g/dL)    | 6.3 ± 0.3              | 6.4 ± 1                                            | 5.9 ± 0.9 | 0.16    |
| Alanine aminotransferase (U/L) | 17.9 ± 6          | 65.3 ± 139.9                                       | 50.6 ± 87.3 | 0.018   |
| Aspartate aminotransferase (U/L) | 18.3 ± 7          | 128.1 ± 173.5                                      | 120 ± 164.7 | < 0.0001|
| Platelets (G/L)         | 231.4 ± 29.8           | 173 ± 105.4                                        | 127.8 ± 72.3 | 0.0004  |
| Mean corpuscular volume (fL) | 84.8 ± 3.5            | 91.2 ± 9.1                                         | 95.5 ± 9  | 0.0002  |
| Urea (mg/dL)            | -                      | 27.5 ± 16.1                                        | 58.2 ± 43.7 | 0.065   |
| Sodium (mmol/l)         | 140 ± 3.3              | 138.8 ± 5                                          | 151.9 ± 6.7 | < 0.0001|
| Potassium (mmol/L)      | 4.4 ± 0.4              | 3.8 ± 0.7                                          | 3.9 ± 0.8  | 0.019   |
| C-reactive protein (mg/L) | 2.5 ± 2.3              | 19.8 ± 21                                          | 32.7 ± 27.8 | < 0.0001|
| Angiotensinogen (ng/mL) | 1006.91 ± 610.49       | 1117.04 ± 873.69                                   | 1468.7 ± 817.33 | 0.22    |

INR: International normalized ratio.

Table 2 Levels of selected biochemical parameters according to the stage of liver cirrhosis

|                             | Control group         | Liver cirrhosis                                      | P value |
|-----------------------------|-----------------------|------------------------------------------------------|---------|
|                             |                        | Pugh-Child A + B (n = 37)                      | Pugh-Child C (n = 32) |         |
| LECT2 (ng/mL)               | 18.99 ± 5.36          | 11.06 ± 6.47                                       | 8.06 ± 5.74 | < 0.0001|
| FGF-1 (pg/mL)               | 37.94 ± 40.4          | 144.77 ± 14.42                                     | 164.52 ± 169.46 | 0.01    |
| FGF-21 (pg/mL)              | 13.52 ± 7.51          | 44.27 ± 64.19                                       | 45.4 ± 51.69 | 0.008   |

LECT2: Leukocyte cell-derived chemotaxin-2; FGF-1: Fibroblast growth factor 1; FGF-21: Fibroblast growth factor 21.

produced by Cloud-Clone Corp. (Katy, TX, United States). Serum samples had been suitably diluted (20-fold dilution for LECT2) or used without dilution (FGF-1 and FGF 21) prior to testing, in accordance with the manufacturers’ recommendations. Testing was carried out in accordance with the typical standard applicable for enzyme-linked immunosassays: samples, standards, and blanks were applied to a plate pre-coated with a factor-specific antibody. Subsequently, horseradish peroxidase conjugated avidin was added to each well, and the plate was incubated for one hour at room temperature (LECT2) or at 37°C (FGF-1 and FGF-21). Next, TMB substrate was added; the wells containing biotin-conjugated antibody and enzyme-conjugated avidin exhibited a change in color. The enzyme-substrate reaction was terminated by adding acidic solution, and the absorbance of the complex formed was measured at a
wavelength of 450 nm. The concentrations of the study parameters were determined using a standard curve. Results were multiplied by the dilution factor, when necessary.

**Statistical analysis**
Statistica 13.3 (TIBCO Software, Inc.) was used for data analysis. Continuous variables were expressed as mean ± SD. Before calculations, variables were checked for normality using the Shapiro-Wilk test. To compare the results between more than two groups, one-way ANOVA and the Kruskal-Wallis test were used, depending on distribution. Correlations among variables were tested using Pearson’s and Spearman’s correlation tests, depending on distribution. Qualitative variables were shown as indicators of structure (percentage). For intergroup comparisons, the χ² test was used. For all tests, P < 0.05 was considered statistically significant.

**RESULTS**
The study group consisted of 69 patients (50 men), including 37 with P-Ch A or P-Ch B cirrhosis and 32 with P-Ch C. The control group included 17 gender-matched individuals (P = 0.52). The age of patients in the control group was lower than that of patients with cirrhosis (P = 0.021). The duration of alcohol abuse in the study group was, on average, 15.7 ± 8.2 years in the P-Ch A + B subgroup and 18.7 ± 8.3 years in the P-Ch C subgroup.

As expected, patients with liver cirrhosis were characterized by significantly lower albumin levels and higher total bilirubin (TB), alanine aminotransferase, aspartate aminotransferase (AST), international normalized ratio, and C-reactive protein levels (Table 1).

Angiotensinogen levels increased with the progression of cirrhosis, reaching the highest in the P-Ch C group of 1468.7 ± 817.33 ng/mL. However, the differences observed were not statistically significant (P = 0.22).

The LECT2 Levels in the control group were 18.99 ± 5.36 ng/mL. With the progression of cirrhosis in the P-Ch A + B group, this value dropped to 11.06 ± 6.47 ng/mL and to 8.06 ± 5.74 ng/mL in the P-Ch C group (P < 0.0001) (Table 2). Multiple comparisons confirmed the statistically significant differences in LECT2 Levels between the control group and the P-Ch A + B (P = 0.006) and between the control group and P-Ch C (P < 0.0001) (Figure 1).

Otherwise, the lowest FGF-1 Level was found in the control group — 37.94 ± 40.4 pg/mL — and was higher in patients with cirrhosis, increasing to 144.77 ± 1 in the P-Ch A + B group and to 164.52 ± 169.46 pg/mL in the P-Ch C group (P < 0.01). The difference between the control group and P-Ch C was statistically significant (P = 0.002) (Table 2).

A similar trend was observed for FGF-21. Its concentration in the control group was 13.52 ± 7.51 pg/mL, 44.27 ± 64.19 pg/mL in the P-Ch A + B group, and 45.4 ± 51.69 pg/mL in the P-Ch C group (P = 0.008). The difference between the control group and the P-Ch C group was statistically significant (P = 0.007) (Table 2).

The strongest correlations were observed between LECT2 and TB (r = -0.59; P < 0.0001) and angiotensinogen (r = -0.51; P < 0.0001) (Table 3).

In the multiple regression model, angiotensinogen, AST, TB, and age were observed to be independent LECT2-related variables (Table 4). This model was statistically significant (P < 0.0001) and explained less than two-thirds of variability (adjusted R² = 0.59).

**DISCUSSION**
ALD is a serious health consequence of excessive alcohol consumption. The spectrum of clinical-histologic ALD changes includes fatty liver, alcoholic hepatitis, and cirrhosis [22]. It is estimated that over 90% of all heavy drinkers have fatty liver; about 25% of them have alcoholic hepatitis, and 15% have cirrhosis. According to a meta-analysis conducted by Askgaard et al [23], the probability of alcoholic liver cirrhosis reaches 16% after 8–12 years of alcoholization; 45% of patients with cirrhosis had been consuming more than 110 g of alcohol daily. The above results correspond to our observations based on a relatively small sample. Alcohol-induced liver cirrhosis accounts for half of all cirrhosis cases in the United States. In recent years, the importance of finding new non-invasive methods to diagnose more severe forms of
### Table 3 Correlations between leukocyte cell-derived chemotaxin-2 and other clinical and laboratory parameters (only those statistically significant were included)

| Pair of variables | Correlation coefficient | R   | P value |
|------------------|-------------------------|------|---------|
| LECT2            | Age                     | -0.29| 0.048   |
|                  | Total bilirubin         | -0.59| < 0.0001|
|                  | Platelets               | 0.34 | 0.02    |
|                  | Alanine transaminase    | -0.43| 0.003   |
|                  | C-reactive protein      | -0.4 | 0.008   |
|                  | Angiotensinogen         | -0.51| < 0.0001|
| FG1              | FGF-21                  | -0.38| 0.004   |

LECT2: Leukocyte cell-derived chemotaxin-2; FGF-1: Fibroblast growth factor 1; FGF-21: Fibroblast growth factor 21.

### Table 4 Independent factors associated with leukocyte cell-derived chemotaxin-2 concentration (multiple regression)

| Effect                  | B*  | SE with B* | B   | SE with B | P value |
|-------------------------|-----|------------|-----|-----------|---------|
| Constant                | 30.64| 3.68       | 3.68|           | < 0.0001|
| Angiotensinogen         | -0.423| 0.114      | -0.004| 0.001     | 0.001   |
| Alanine aminotransferase| -0.341| 0.115      | -0.02 | 0.005     | 0.005   |
| Total bilirubin         | -0.279| 0.108      | -0.25 | 0.099     | 0.014   |
| Age                     | -0.275| 0.109      | -0.16 | 0.064     | 0.016   |

B*: Standardized coefficient (Beta). Model: R = 0.79; R² = 0.64, adjusted R² = 0.59; P < 0.0001.

**Figure 1** Concentration of leukocyte cell-derived chemotaxin-2 according to the stage of alcoholic liver cirrhosis. LECT-2: Leukocyte cell-derived chemotaxin-2.

ALD and predict prognosis has been strongly emphasized[24,25].

In our study, the serum levels of FGF-1 and FGF-21 in the study groups and control group were determined to obtain biochemical reference points for levels of LECT2. FGF-1 is an angiogenic factor that modifies the migration and proliferation of endothelial cells and regulates the metabolism of lipids and carbohydrates. FGF-1 is involved in response to injury and fibrosis. The highest expression of FGF has been observed in the late stages of hepatic morphogenesis in animal models, as well as during hepatic differentiation in the adult liver. FGF-1 is present in perisinusoidal hepatic stellate cells (HSCs) (also called Ito cells and fat-storing cells) is the major contributor to liver fibrogenesis resulting from chronic toxic insult primarily through
its production of extracellular matrix components.

FGF-1 reduces hepatic lipid accumulation independently of insulin and is important in the pathogenesis of NAFLD. Moreover, it has therapeutic potential for the treatment of ischemic disease[26]. Previous studies have demonstrated an inverse relationship between this factor and portal pressure in patients after liver transplantation[27]. In animal model studies, the protective effect of FGF-1 on liver cells was confirmed, as it prevented acute inflammation and apoptosis induced by acetaminophen[28]. The main source of FGF-1 in the human body is liver cells. However, this protein is also expressed in the pancreas, testes, duodenum, and adipose tissue. For this reason, its use as an indicator of liver function is clearly limited, and in recent years this problem has not been studied. Among fibroblast growth factors, FGF-21 has been tested as a marker of liver function[29,30]. According to a Chinese prospective study, this protein is an independent predictor of NAFLD[31]. The possible use of FGF-21 as an NAFLD marker has also been described in an American study conducted in children[32]. However, the above study demonstrated significant relationships between the level of this marker and the prevalence of obesity, with or without insulin resistance. In a study on ALD, Yang et al[33] suggested that FGF-21 may indicate a progression from heavy drinking to alcoholic cirrhosis. In their latest study, Willis et al[34] indicated that acute high-fat overfeeding augments circulating concentrations of FGF-21, LECT2, and fetuin-A in healthy men. Perhaps a slightly opposite effect than in this subgroup occurs in patients with cirrhosis with regard to correlation of LECT2 and FGF-21. The results of our study showed that LECT2 Levels correlated inversely with FGF-1 and FGF-21 in ALD. However, based on our results, it is not possible to state whether this is specific to ALD. Previous studies have shown that LECT2 could be of great importance in the diagnosis of NAFLD[13,14]. We suggest the need for further, more extensive, including prospective, studies.

Our study is the first attempt to assess the usefulness of LECT2 in the non-invasive diagnosis of alcohol-induced liver cirrhosis. Therefore, the points of reference are scarce. However, considering the above-mentioned studies on the marker function of FGF-21, it is worth noting that our results are compatible with those reported by Yang et al[33] In our study, the concentration of FGF-21 in the control group, that is, patients without cirrhosis, was significantly lower compared to both subgroups of the study group. However, the differences in FGF-21 concentrations between the two subgroups (P-Ch A + B and P-Ch C) were not statistically significant. FGF-21 may play an important role in supporting non-invasive diagnostics of alcohol-induced liver cirrhosis and in monitoring the course of NAFLD. We did not find it useful in non-invasive monitoring of alcohol-induced liver cirrhosis, contrary to the level of serum taurine/glycine-conjugated bile acids as a non-invasive marker to predict the severity of alcohol-induced liver cirrhosis, as tested by Yang et al[33]. Our results suggest that LECT2 might be used as a diagnostic and monitoring marker to determine the severity of alcohol-induced liver cirrhosis. Its highest statistically significant concentration was observed in the control group. In the study groups, as cirrhosis progressed, the plasma levels of LECT2 dropped. The lowest values of LECT2 were observed in P-Ch C stage patients, that is, in the most advanced stage of the disease.

LECT2 Levels correlated inversely with TB, AST, and angiotensinogen (AGT). Although strong correlations were identified between LECT2 and cirrhosis progression, and between AGT and LECT2, we did not observe an analogous relationship between AGT and cirrhosis progression. We suggest that this may be caused by low sample size and decreased power. The liver’s renin-angiotensin system plays an important role in the development of liver cirrhosis. The levels of total bilirubin, AST, and AGT increase as alcohol-induced liver cirrhosis progresses. Higher serum concentration of AGT indicates unfavorable histological remodeling of the liver parenchyma closely related to liver dysfunction. Previous studies on animal models have indicated that AGT plays an important role in NAFLD[35-37]. AGT is an important precursor of hepatic fibrogenesis, which has been confirmed in animal studies[38]. According to the reported data, AGT inhibition could be an effective anti-liver fibrosis strategy.

**CONCLUSION**

Our research suggests that LECT2 may be used for the non-invasive diagnosis of alcohol-induced liver cirrhosis. The usefulness of LECT2 for non-invasive monitoring of alcohol-induced liver cirrhosis was indirectly confirmed by the multiple regression model developed on the basis of our statistical analysis.
ARTICLE HIGHLIGHTS

Research background
Leukocyte cell-derived chemotaxin-2 (LECT2) has been widely studied to determine its usefulness for monitoring the course of non-alcoholic fatty liver disease but not for alcoholic liver cirrhosis (ALC).

Research motivation
The aim of our study was to assess and discuss LECT2’s possible wider use in the diagnosis of ALC.

Research objectives
The purpose of this study was to determine the concentration of LECT2 in the blood serum of patients in accordance with progressive stages of ALC and its relation to fibroblast growth factor 1 (FGF-1) and FGF-21.

Research methods
A study was conducted with an ALC group and a control group with no ALC. The extent of ALC was evaluated according to Pugh-Child criteria (the Pugh-Child score). LECT2, FGF-1, and FGF-21 were determined using enzyme-linked immunosorbent assay kits.

Research results
Our study showed strong correlations between LECT2 and cirrhosis progression. LECT2 levels correlated inversely with FGF-1 and FGF-21.

Research conclusions
LECT2 may be used for the non-invasive diagnosis of alcohol-induced liver cirrhosis.

Research perspectives
Further prospective studies should be conducted to explore whether the inverse correlation of LECT2 and FGF-21 is specific to ALD.

ACKNOWLEDGEMENTS
This study was performed at the Medical University of Lublin, Poland. The authors thank Anna Misiuna, who provided medical writing services on behalf of the Medical University of Lublin, Poland.

REFERENCES
1 Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. World J Hepatol 2017; 9: 715-732 [PMID: 28652891 DOI: 10.4254/wjh.v9.i16.715]
2 Ke Y, Xu C, Lin J, Li Y. Role of Hepatokines in Non-alcoholic Fatty Liver Disease. J Transl Int Med 2019; 7: 143-148 [PMID: 32010600 DOI: 10.2478/jtim-2019-0029]
3 Sato M, Kamada Y, Takeda Y, Kida S, Ohara Y, Fujii H, Akita M, Mizutani K, Yoshida Y, Yamada M, Hougaku H, Takehara T, Miyoshi E. Fetuin-A negatively correlates with liver and vascular fibrosis in nonalcoholic fatty liver disease subjects. Liver Int 2015; 35: 925-935 [PMID: 25627311 DOI: 10.1111/liv.12478]
4 Celеби G, Genc H, Gurel H, Sertoglu E, Kara M, Tapan S, Acikel C, Karsioglu Y, Er cin CN, Dogru T. The relationship of circulating fetuin-a with liver histology and biomarkers of systemic inflammation in nondiabetic subjects with nonalcoholic fatty liver disease. Saudi J Gastroenterol 2015; 21: 139-145 [PMID: 26021772 DOI: 10.4103/1319-3767.157556]
5 Choi HY, Hwang SY, Lee CH, Hong HC, Yang SJ, Yoo HI, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Increased selenoprotein p levels in subjects with visceral obesity and nonalcoholic fatty liver disease. Diabetes Metab J 2013; 37: 63-71 [PMID: 23439771 DOI: 10.4093/dmj.2013.37.1.63]
6 Polyzos SA, Kountouras J, Mavrouli M, Katsinelos P, Doulberis M, Gavana E, Duntas L. Selenoprotein P in Patients with Nonalcoholic Fatty Liver Disease. Exp Clin Endocrinol Diabetes 2019; 127: 598-602 [PMID: 30625508 DOI: 10.1055/a-0811-9136]
7 Praktiknjo M, Djayadi N, Mohr R, Schierwagen R, Bischoff J, Dold L, Pohlmann A, Schwarze-Zander C, Wasmuth JC, Boesecke C, Rockstroh JK, Trebicka J. Fibroblast growth factor 21 is
independently associated with severe hepatic steatosis in non-obese HIV–infected patients. *Liver Int* 2019; 39: 1514-1520 [PMID: 30916873 DOI: 10.1111/liv.14107]

8 Ocker M. Fibroblast growth factor signaling in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Paving the way to hepatocellular carcinoma. *World J Gastroenterol* 2020; 26: 279-290 [PMID: 31988569 DOI: 10.3748/wjg.v26.i3.279]

9 Sanyal A, Charles ED, Neuschwander-Tetri BA, Loomba R, Harrison SA, Abdelmalek MF, Lawitz EJ, Haregona-De Marzio D, Kundra S, Noviello S, Luo Y, Christian R. Pegbelfermin (BMS-986036), a pegylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. *Lancet* 2019; 392: 2705-2717 [PMID: 30554783 DOI: 10.1016/S0140-6736(18)31785-9]

10 Mueller S, Seitz HK, Rausch V. Non-invasive diagnosis of alcoholic liver disease. *World J Gastroenterol* 2014; 20: 14626-14641 [PMID: 25356026 DOI: 10.3748/wjg.v20.i40.14626]

11 Addolorato G, Mirijello A, Barrio P, Gual A. Treatment of alcohol use disorders in patients with alcoholic liver disease. *J Hepatol* 2016; 65: 618-630 [PMID: 27155530 DOI: 10.1016/j.jhep.2016.04.029]

12 Arab JP, Roblero JP, Altamirano J, Bessone F, Chaves Araujo R, Higuera-De la Tijera F, Restrepo JC, Torre A, Urzua A, Simonetto DA, Abraldes JG, Méndez-Sánchez N, Contreras F, Lucey MR, Shah VH, Cortez-Pinto H, Bataller R. Alcohol-related liver disease: Clinical practice guidelines by the Latin American Association for the Study of the Liver (ALEH). *Ann Hepatol* 2019; 18: 518-535 [PMID: 31055346 DOI: 10.1016/j.ajohep.2019.04.005]

13 Okamura A, Unoki-Kubota H, Matsumiya Y, Shiga T, Moriyoshi Y, Yamagoe S, Kaburagi Y. Increased serum leukocyte cell-derived chemotaxin 2 (LECT2) levels in obesity and fatty liver. *Biosci Trends* 2013; 7: 276-283 [PMID: 24390366]

14 Lee HY, Hwang SY, Choi JH, Lee HJ, Chung HS, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Association of leukocyte cell-derived chemotaxin 2 (LECT2) with NAFLD, metabolic syndrome, and atherosclerosis. *PLoS One* 2017; 12: e0174717 [PMID: 28376109 DOI: 10.1371/journal.pone.0174717]

15 Yamagoe S, Yamakawa Y, Matsuo Y, Minowada J, Mizuno S, Suzuki K. Purification and primary amino acid sequence of a novel neutrophil chemotactic factor LECT2. *Immunol Lett* 1996; 52: 9-13 [PMID: 8877413 DOI: 10.1016/0165-2478(96)00257-2]

16 Lebenthal DM, Filsak-Jackiewicz M, Bialokoz-Kalinowska I, Bobrus-Chociej A, Kowalska I. Hepatokines and non-alcoholic fatty liver disease. *Acta Biochim Pol* 2016; 63: 459-467 [PMID: 27262842 DOI: 10.18388/abp.2016_1252]

17 Yamagoe S, Mizuno S, Suzuki K. Molecular cloning of human and bovine LECT2 having a neutrophil chemotactic activity and its specific expression in the liver. *Biochem Biophys Acta* 1998; 1407: 109-113 [PMID: 9524238 DOI: 10.1016/s0167-4781(97)00181-4]

18 Lan F, Misu H, Chikamoto K, Takayama H, Kikuchi A, Mohri K, Takata N, Hayashi H, Matsuzawa-Nagata N, Takeshita Y, Noda H, Matsumoto Y, Ota T, Nagano T, Nakagen M, Miyamoto K, Takatsuki K, Sato T, Iwayama K, Tokuyama M, Matsuso S, Sato T, Saito Y, Yamagoe S, Kaneko S, Takamura T. LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance. *Diabetes* 2014; 63: 1649-1664 [PMID: 24747839 DOI: 10.2337/db13-0728]

19 Mercere OM, Thies JD, Vraja JA, Law ME, Grogg KL, Dasari S, Chandas VS, Wu TT, Jimenez-Zepeda VH, Fonseca R, Dispensieri A, Kurtin PJ, Dogan A. Leukocyte cell-derived chemotaxin 2 (LECT2)-associated amyloidosis is a frequent cause of hepatic amyloidosis in the United States. *Blood* 2014; 123: 1479-1482 [PMID: 24415538 DOI: 10.1182/blood-2013-07-517938]

20 Xu M, Xu HH, Lin Y, Sun X, Wang LJ, Fang ZP, Su X, Liang XJ, Hu Y, Liu ZM, Cheng Y, Wei Y, Li J, Li J, Li HJ, Chen Z, Tang N, Peng C, Li T, Liu T, Qiao L, Wu D, Ding YQ, Zhou WJ. LECT2, a Ligand for Tiel1, Plays a Crucial Role in Liver Fibrogenesis. *Cell* 2019; 178: 1478-1492. e20 [PMID: 31474362 DOI: 10.1016/j.cell.2019.07.021]

21 National Institute on Alcohol Abuse and Alcoholism (NIAAA). Drinking Levels Defined. [cited 4 Jan 2021]. Available from: https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/moderate-binge-drinking

22 Singal AK, Bataller R, Ahn J, Kamath PS, Shah VH. ACG Clinical Guideline: Alcohol-Related Liver Disease. *Am J Gastroenterol* 2018; 113: 175-194 [PMID: 29363454 DOI: 10.1038/ajg.2017.469]

23 Askgaard G, Kjer MS, Tolstrup JS. Opportunities to Prevent Alcoholic Liver Cirrhosis in High-Risk Populations: A Systematic Review With Meta-Analysis. *Am J Gastroenterol* 2019; 114: 221-232 [PMID: 30353053 DOI: 10.1038/s41395-018-0282-6]

24 Moreno C, Mueller S, Szabo G. Non-invasive diagnosis and biomarkers in alcohol-related liver disease. *J Hepatol* 2019; 70: 273-283 [PMID: 30655728 DOI: 10.1016/j.jhep.2018.11.023]

25 Lucey MR. Alcohol-Associated Cirrhosis. *Clin Liver Dis* 2019; 23: 115-126 [PMID: 30454826 DOI: 10.1016/j.cld.2018.09.013]

26 Xia X, Babcock JP, Blaber SL, Harper KM, Blaber M. Pharmacokinetic properties of third-generation fibroblast growth factor-1 mutants for therapeutic application. *PLoS One* 2012; 7: e48210 [PMID: 23133616 DOI: 10.1371/journal.pone.0048210]

27 Serrano CA, Ling SC, Verdaguer S, León M, Jarufe N, Guerra JF, Pattillo JC, Benitez C, Villagran A, Torres J, Concha M, Villarreal L, Dellepiane P, Domínguez P, Martinez J, Gana JC. Portal Angiogenesis in Chronic Liver Disease Patients Correlates with Portal Pressure and Collateral Formation. *Dig Dis* 2019; 37: 498-508 [PMID: 31067534 DOI: 10.1159/000500115]

28 Wang X, Zhang X, Wang F, Pang L, Xu Z, Li X, Wu J, Song Y, Xiao J, Lin H, Liu Y. FGFl protects
against APAP-induced hepatotoxicity via suppression of oxidative and endoplasmic reticulum stress. *Clin Res Hepatol Gastroenterol* 2019; 43: 707-714 [PMID: 31029643 DOI: 10.1016/j.clinre.2019.03.006]

29 **Yang M**, Xu D, Liu Y, Guo X, Li W, Guo C, Zhang H, Gao Y, Mao Y, Zhao J. Combined Serum Biomarkers in Non-Invasive Diagnosis of Non-Alcoholic Steatohepatitis. *PLoS One* 2015; 10: e0131664 [PMID: 26121037 DOI: 10.1371/journal.pone.0131664]

30 **Gong Z**, Tan E, Yakar S, Muzumdar R. Hepatic lipid metabolism and non-alcoholic fatty liver disease in aging. *Mod Cell Endocrinol* 2017; 455: 115-130 [PMID: 28017785 DOI: 10.1016/j.mce.2016.12.022]

31 **Li H**, Dong K, Fang Q, Hou X, Zhou M, Bao Y, Xiang K, Xu A, Jia W. High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China. *J Hepatol* 2013; 58: 557-563 [PMID: 23142063 DOI: 10.1016/j.jhep.2012.10.029]

32 **Sodhi K**, Bracero L, Feyh A, Nichols A, Srikanthan K, Latif T, Preston D, Shapiro JI, Elitsur Y. Role of Serum Biomarkers in Early Detection of Non-Alcoholic Steatohepatitis and Fibrosis in West Virginian Children. *J Clin Cell Immunol* 2016; 7 [PMID: 27182456 DOI: 10.4172/2155-9899.1000393]

33 **Yang Z**, Kusumanchi P, Ross RA, Heathers L, Chandler K, Oshodi A, Thoudam T, Li F, Wang L, Liangpunsakul S. Serum Metabolomic Profiling Identifies Key Metabolic Signatures Associated With Pathogenesis of Alcoholic Liver Disease in Humans. *Hepatol Commun* 2019; 3: 542-557 [PMID: 30976744 DOI: 10.1002/hepc.1322]

34 **Willis SA**, Sargeant JA, Yates T, Takamura T, Takayama H, Gupta V, Brittain E, Crawford J, Parry SA, Thackray AE, Varela-Mato V, Stensel DJ, Woods RM, Hulston CJ, Aithal GP, King JA. Acute Hyperenergetic, High-Fat Feeding Increases Circulating FGF21, LECT2, and Fetuin-A in Healthy Men. *J Nutr* 2020; 150: 1076-1085 [PMID: 31919514 DOI: 10.1093/jn/nxz333]

35 **Takahashi N**, Li F, Hua K, Deng J, Wang CH, Bowers RR, Bartness TJ, Kim HS, Harp JB. Increased energy expenditure, dietary fat wasting, and resistance to diet-induced obesity in mice lacking renin. *Cell Metab* 2007; 6: 506-512 [PMID: 18054319 DOI: 10.1016/j.cmet.2007.10.011]

36 **Frantz ED**, Penna-de-Carvalho A, Batista Tde M, Aguila MB, Mandarinin-de-Lacerda CA. Comparative effects of the renin-angiotensin system blockers on nonalcoholic fatty liver disease and insulin resistance in C57BL/6 mice. *Metab Syndr Relat Disord* 2014; 12: 191-201 [PMID: 24517411 DOI: 10.1089/met.2013.0129]

37 **Tao XR**, Rong JB, Lu HS, Daugherty A, Shi P, Ke CL, Zhang ZC, Xu YC, Wang JA. Angiotensinogen in hepatocytes contributes to Western diet-induced liver steatosis. *J Lipid Res* 2019; 60: 1983-1995 [PMID: 31604805 DOI: 10.1194/jlr.M093252]

38 **Lu P**, Liu H, Yin H, Yang L. Expression of angiotensinogen during hepatic fibrogenesis and its effect on hepatic stellate cells. *Med Sci Monit* 2011; 17: BR248-BR256 [PMID: 21873937 DOI: 10.12659/msm.881928]
