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ABOUT COVER

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AIMS AND SCOPE

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

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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.

METHODS

A retrospective case-control study was conducted with 69 ALC cases and 17 controls with no ALC. Subjects were recruited from the region of Lublin (eastern Poland). Liver cirrhosis was diagnosed based on clinical features, history of heavy alcohol consumption, laboratory tests, and abdominal ultrasonography. The degree of ALC was evaluated according to Pugh-Child criteria (the Pugh-Child score). Blood was drawn and, after centrifugation, serum was collected for analysis. LECT2, FGF-1, and FGF-21 were determined using enzyme-linked immunosorbent assay kits.

RESULTS

The LECT2 Levels in the control group were 18.99 ± 5.36 ng/mL. In the study groups, they declined with the progression of cirrhosis to 11.06 ± 6.47 ng/mL in one group and to 8.06 ± 5.74 ng/mL in the other ($P < 0.0001$). Multiple comparison tests confirmed the statistically significant differences in LECT2 Levels between the control group and both test groups ($P = 0.006$ and $P < 0.0001$). FGF-21 Levels were 44.27 ± 64.19 pg/mL in the first test group, 45.4 ± 51.69 pg/mL in the second ($P = 0.008$), and 13.52 ± 7.51 pg/mL in the control group. The difference between the control group and the second test group was statistically significant ($P = 0.007$).

CONCLUSION

We suggest that LECT2 may be a non-invasive diagnostic factor for 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.

Key Words: Leukocyte cell-derived chemotaxin-2; Fibroblast growth factor 21; Fibroblast growth factor 1; Alcoholic liver cirrhosis; Pugh-Child score

Core Tip: Leukocyte cell-derived chemotaxin-2 (LECT2) was first described in 1996 as a novel chemotactic factor for neutrophils. It 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). We suggest that LECT2 may be used for the non-invasive diagnosis of 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-

ological and therapeutic problem in developing countries[11,12].

Therefore, finding new possibilities for the early diagnosis of ALD, especially novel and precise non-invasive diagnostic procedures, is a real challenge for modern hepatological practice.

LECT2 has been widely studied to determine its usefulness in monitoring the course of NAFLD. According to the available study findings, serum LECT2 concentrations increase with the advancement of NAFLD[13,14]. LECT2 was first described by Yamagoe *et al*[15] in 1996 as a novel chemotactic factor for neutrophils. Subsequent studies identified its expression in human hepatocytes and classified it as a hepatokine [16-18]. Clinical observations have demonstrated that LECT2-associated amyloidosis is a frequent cause of hepatic amyloidosis in the United States[19]. Studies in animal models have reported that LECT2 overexpression increases fibrosis, promotes sinusoid capillarization, and inhibits portal angiogenesis. LECT2 is a functional ligand of Tie1. Xu *et al*[20] suggested that serum LECT2 Levels may be a potential biomarker for the diagnosis or screening of liver fibrosis, and LECT2/Tie1 signaling may be used for the development of new drugs.

It seems that LECT2 could be of great importance in the diagnosis of fatty liver. In a cross-sectional study, Okumura *et al*[13] showed statistically significant higher levels of LECT2 in fatty liver and obesity. However, the possibility of diagnosing and monitoring the course of alcohol-induced liver cirrhosis using LECT2 has not yet been assessed.

The aim of our study was to determine the concentration of LECT2 in the blood serum 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[21]. 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	133.8 ± 5	131.9 ± 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	Pugh-Child C	
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 immunoassays: 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 χ^2 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 R^2 = 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
	FGF-1	-0.38	0.004
	FGF-21	-0.39	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	< 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: $R = 0.79$; $R^2 = 0.64$, adjusted $R^2 = 0.59$; $P < 0.0001$.
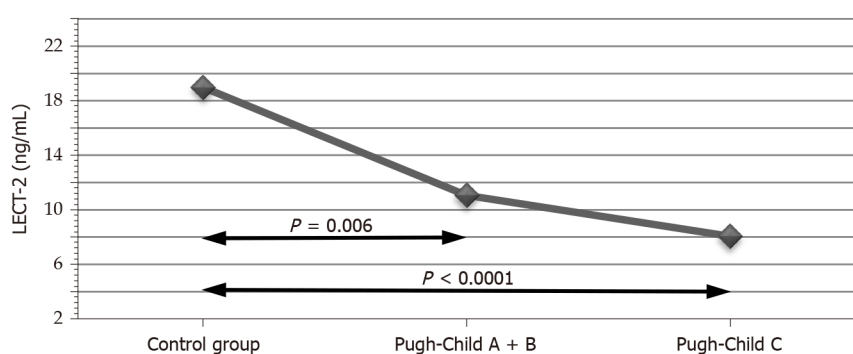
Figure 1 Concentration of leukocyte cell-derived chemotaxin-2 according to the stage of alcoholic liver cirrhosis. LECT-2: Leukocyte cell-derived chemotaxin-2.

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) during liver regeneration. The chronic activation of nonparenchymal 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.

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