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Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity

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Background/Aims: Nonalcoholic steatohepatitis is an emerging clinical problem among the obese population. However, risk factors of progression to advanced forms of liver disease in this particular group of patients remain to be defined.

Methods: The demographics and clinical and histologic features of 46 obese patients were evaluated. The intrahepatic immunological phenotype was assessed in all liver biopsy samples by immunohistochemistry.

Results: Histologic findings of nonalcoholic steatohepatitis were observed in 69.5% of the obese population studied and significant fibrosis was evident in 41% of patients with nonalcoholic steatohepatitis. Age (p=0.003), degree of steatosis (p=0.000002), and grade of inflammation (p= 0000) at liver biopsy were independent variables positively associated with fibrosis Intrahepatic expression levels of several immunologic markers of inflammation as well as nitric oxide derivatives were significantly higher in the severe forms of nonalcoholic steatohepatitis than in the mildest forms.

Conclusions: Obese persons with higher age, with greater degrees of hepatic steatosis, and specially those with increased grades of intrahepatic inflammation have the greatest risk for progression to fibrotic liver disease. An oxidative stress-triggered intrahepatic inflammatory response appears to be important in the pathogenesis of nonalcoholic steatohepatitis in obesity.

Key words: Adhesion molecules; Liver fibrosis; Nitric oxide; Nonalcoholic steatohepatitis; Obesity; Risk factors.

OBESITY is an epidemic currently recognized as a major public health problem worldwide (1,2). While it is well known that excess weight increases mortality rates (3,4), the relative contribution of excess weight to diseases associated with obesity, such as arterial hypertension and diabetes mellitus, still remains a matter of debate. Nonalcoholic steatohepatitis (NASH) is an emerging clinical problem among obese patients, and although NASH must be considered as a syndrome with a multifactorial etiology (5), obesity is the most consistently associated causal factor (6). Diagnosis of NASH is defined histologically when a combination of macrovesicular steatosis, hepatocyte injury and necrosis, mixed inflammatory infiltration and variable degrees of

fibrosis are shown in the absence of a chronic abuse of alcohol (7,8). Although traditionally described as an asymptomatic liver disease with a benign prognosis (9,10), NASH can also progress to advanced forms of liver disease, including cirrhosis (11-13). The pathogenesis of this entity remains unclear but the hypothesis that excessive intrahepatic lipid accumulation could trigger a local inflammatory response has recently been suggested (14,15). In order to better understand the relationship between steatosis, inflammation, and fibrosis, this study aimed to analyze the clinical and histological features as well as the intrahepatic immunological phenotype of 46 obese patients who were admitted to the hospital for weight-reduction surgery.

Materials and Methods

Characteristics of patients

Fifty-one obese patients who underwent surgical gastroplasty in the Department of Surgery of the Hospital Universitarlo de la Princesa from January 1996 to February 1998 were considered for inclusion in this study Patients were interviewed and physical findings (including height, weight, and waist circumference) and a blood sample for laboratory investigations were also obtained. Five patients were excluded due to alcoholism and positivity for anti-HCV Informed written consent to he included in this study was obtained from each patient.

Laboratory evaluation

Laboratory studies included glucose, creatinine, cholesterol, triglycerides, total bilirubin, aspartate aminotransferase (AST), alanine amino-transferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), albumin, and total protein levels; hepatitis B surface antigen and antibody to hepatitis C virus; autoimmune serology; studies of iron metabolism, ceruloplasmin, and al-antitrypsin levels.

Liver tissue studies

Liver histology: Diagnosis of NASH was established by the combined presence of pericentral macrovesicular steatosis, hepatocyte injury and necrosis, mixed inflammatory infiltration and variable degrees of fibrosis. Histologic grading and staging was performed using a modified scoring system based on a recently proposed classification (16). Steatosis was scored as follows: 0, none; 1, minimal; 2, mild; 3, moderate; 4, severe. Inflammation as follows: 0, none; 1, minimal; 2, mild; 3, moderate; 4, severe. Fibrosis as follows: 0, absence; 1, minimal; 2, mild; 3, moderate; 4, cirrhosis.

We have defined mild NASH as present when the sum of steatosis and inflammation scores was equal to or lower than 4, and severe NASH when it was higher than 4. We have also defined three categories of fibrosis: absence of fibrosis was 0, focal fibrosis was 1, and significant fibrosis was equal or higher than 2.

Inmiunohistochemical staining of liver biopsy sections:

- Immunoperoxidase staining: Dehydrated acetone-fixed 5 μm cryostat liver biopsy sections from all patients were assessed using an indirect immunoperoxidase technique, as detailed elsewhere (17).
- Evaluation of inununoperoxidase-stained liver biopsy sections:

Each section was semi-quantitatively evaluated using an immuno histochemical scoring system, previously described and validated (18,19), with some minor modifications. Briefly, four distinct items were recorded on separate sheets as follows: 0, no staining; + 1, positive staining in less than 300/o of cells per high power field; +2, positive staining in more than 30% but less than 7~/o of cells per high power field, and +3, positive staining of more than 7~/o of cells per high power field. Finally, data were averaged to median values, giving a numerical score for each liver biopsy specimen, and then used for statistical analysis.

Monoclonal antibodies

The monoclonal antibodies (mAb) used in this study were D3/9 anti-CD45 (leukocytes) (20), SPV-T3b anti-CD3 (total T lymphocytes) (21), HP2/6 anti-CDI (helper T lymphocytes) (22), B9.4.2 anti-CD8 (cytotoxic T lymphocytes (23), HCl/1 anti-CDl lc (monocytes, macrophages) (24), TPl/55 anti-CD69 (activated lymphocytes) (25), *RRl/l* anti-ICAM-l (wide cell distribution) (26), and TEAl/5 antiendoglin (macrophages, endothelial cells) (27). Commercially mAb against B cells (anti-CDl9) (Dakopatts, Copenhagen, Denmark), natural killer cells (anti-CD56) Beckton Dickinson, Mountain View, CA, USA), an IgG2a mAb anti-macrophage iNOS (Transduction Laboratories, Lexington, KY, USA), and an IgG mAb anti-NTY (Upstate Biotechnology Inc., Saranac Lake, NY, USA) were also used. The P3X63 mouse myeloma supernatant was used as negative control in all immunostaining experiments.

Statistical analysis

Continuous variables were expressed as mean±standard deviation (±S.D.). Categorical data were expressed as median (range). To analyze the association between categorical variables, the Spearman rank order correlation coefficient with correction for ties was used. To determine the statistical significance or differences in the immunohistochemical scoring of the molecules studied, we first calculated the median values of staining scores from both NASH histological group (mild and severe); these values were then compared by the Mann-Whitney U test for nonparametric data. Throughout, p-values of <0.05 were considered significant.

Results

Characteristics of patients

The demographic, clinical and laboratory details of the 46 patients included in the study are shown in Table 1. The majority of patients were women *(65%)* and the mean age± S.D. of all patients was 41±11 years (range, 20±64 years). The mean body-mass index was 50.45±6.36 kg/m^2 (range, 4O-72 kg/m^2). The mean waist circumference, the best anthropometric index of visceral fat distribution (28,29), was 128.2±11.6 cm (range, 102-153 cm). Of these obese patients 45.5% had associated clinical conditions, with arterial hypertension being the most frequently observed. Diagnosis of diabetes mellitus was present in 13% of them, and evidence of hyperlipidemia was found in nine *(19.5%)* patients.

Laboratory data

Liver function tests (AST, ALT, ALP GGT, and total bilirubin) were entirely normal in 48% of the obese persons studied. In contrast, the serum ALT level was elevated in 19 (41%) patients. The median AST/ALT ratio was 0.81, and only six (13%) patients had an AST/ALT ratio equal to or greater than 1. An abnormal increase in other liver enzymes, such as ALP and GGT, was observed in six (13%) and 13(28%) patients, respectively. Total bilirubin, albumin, ceruloplasmin, and αl-antitrypsin levels were normal in all patients. Seven (15%) patients had elevated ferritin levels above 300 ng/ml, but none of them had a transferrin saturation of more than 55%.

Histological findings

Liver biopsy findings of all patients studied are shown in Table 2. Histological evidence of NASH was found in 32 (69.5%) patients, being severe in 13(41%) of them. The severe forms of this entity were more frequent in females (F/M ratio: 10/3) than the mildest ones (F/M ratio: 9/10). Interestingly, only ten (22Y%) obese persons studied has isolated steatosis and four (8.5%) had histologically normal liver. It is worth emphasizing that none of the obese individuals with hepatic steatosis alone had a severe degree (score 4) of fat infiltration. Regarding the stage of fibrosis, 28 (88%) NASH patients had increased fibrosis. It was noticeable that only four (12%) patients with NASH showed no evidence of fibrosis at liver biopsy and, more interestingly, those obese patients with severe NASH had significantly worse staging scores than

TABLE 1

Demographic, clinical and laboratory details of patients studied

Patient no	Sex	Age years	BMI (kg/m')	WC (cm)	Glucose (rng/dl)	ALT U/l	ASTI ALT	ALP U/l	GGT (U/l	Associated conditions
I	F	26	49.5	113	92	46	0.45	162	32	Nil
2	M	23	60.7	125	103	52	0.5	170	29	Nil
3	M	24	49.4	125	123	59	0.37	160	36	Nil
4	F	36	43	106	79	70	0.33	138	32	Nil
5	M	50	45.8	138	91	34	0.61	140	30	Mixed hyperlipidemia
6	F	23	42.9	102	100	25	0.84	152	42	Nil
7	M	29	50	142	88	42	1	254	49	Nil
8	F	61	53.7	N.D.	168	44	0.77	260	55	D.M., A.H., mixed hyperlipidemia
9	M	32	43	132	104	54	0.77	296	39	Nil
10	M	48	56	145	115	12	1.33	193	15	AH.
11	M	37	45	ND.	201	21	0.95	153	21	D.M., A.H.
12	F	47	64.8	131	110	26	0.88	159	42	A.H.
13	M	33	47.3	130	106	23	0.78	162	21	Nil
14	M	53	57	153	93	64	0.34	150	22	AH.
15	M	57	44.6	125	177	32	0.81	171	29	D.M., A.H.
16	F	51	40	115	102	54	0.59	186	64	AH.
17	F	35	50	ND.	106	33	0.87	162	31	A,H.
18	F	39	45.2	124	113	30	0.93	173	29	A.H.

19	F	56	49.5	137	136	56	0.58	220	67	Nil
20	F	46	52.4	118	87	14	1.35	241	81	Nil
21	M	28	59.6	139	82	33	0.54	158	35	Nil
22	F	44	48.5	138	96	15	0.73	145	12	A.H.
23	F	27	54	133	99	28	0.82	198	76	Nil
24	F	49	50.8	123	192	49	0.65	166	205	D.M., A.H., hypercholesterolemia
25	F	26	47.9	123	79	26	0.84	192	31	Nil
26	F	27	48.2	N.D.	86	20	3.55	171	100	Nil
27	M	43	49.1	142	81	69	0.68	230	121	Nil
28	F	46	48.2	117	102	29	0.86	172	31	Nil
29	F	48	58.3	131	110	21	0.95	107	29	Nil
30	F	44	53.6	N.D.	103	13	1.38	117	8	Nil
31	F	41	44.8	118	93	17	0.88	193	15	AH.
32	F	45	46	117	238	17	0.76	136	17	D.M., A.H., mixed hyperlipidemia
33	F	54	46.4	122	185	20	0.65	200	28	DM., A.H.. mixed hyperlipidemia
34	F	46	52A	115	91	32	0.68	286	38	Nil
35	M	33	41	ND.	86	47	0.42	167	20	Nil
36	F	20	47	128	74	54	0.51	174	28	Nil
37	F	27	54	133	93	12	1.41	207	17	Nil
38	F	54	49.6	147	112	21	0.85	173	13	AH., hypercholesterolemia
39	F	41	42.8	145	106	25	0.6	166	17	Nil
40	M	50	48.2	ND.	110	44	0.56	181	32	AH., mixed hyperlipidemia
41	F	45	52	128	132	38	0.65	232	75	Nil
42	~	43	55	N.D.	127	58	0.67	273	191	A.H., mixed hyperlipidemia
43	F	48	60	133	99	22	0.77	191	21	Nil
44	M	45	51.6	140	86	53	0.51	240	86	A.H.
45	F	28	45.3	112	101	53	0.75	292	113	A.H., hypercholesterolemia
46	F	64	58	ND.	112	97	0.71	286	180	AH.

BMI: body.mass index; D.M.: diabetes mellitus; ALT: alanine aminotransferase, A.H.: arterial hypertension; AST: aspartate aminotransferase;
Glucose (normal range): 7-140; ALP: alkaline phosphatase; ALT (normal range): 1-41; GGT: gamma glutamyltransferase; ALP (normal range): 91-258; WC: waist circumference; GGT (normal range): 1149; N.D. not done.

those with mild NASH (sNASH: 2.4 (l-4) *versus* mNASH: 0.8 (0-2); p<0.00l). The results of comparing patients with normal and abnormal liver function tests with regard to histological findings showed no differences except in obese individuals with histologically normal liver (see Table 3).

Factors associated with fibrosis

The results of analyzing the association of sex, age, body-mass index, waist circumference, glucose, ALT, AST/ALT ALP, GGT; the degree of steatosis, and the grade of inflammation with the stage of fibrosis are summarized in Table 4. The age (p=O.OO3), the degree of steatosis (p=O.OOOOO2), and the grade of inflammation (p=O.OOOO) were the only independent variables positively associated with fibrosis.

Imniunohistochemica1 findings

Phenotype of inflammatory infiltrates. Liver-infiltrating inflammatory cells were predominantly macrophages

TABLE 2

Histological scoring of patients studied

Patient no.	Steatosis	Inflammation	Fibrosis
1	1	0	0
2	1	0	0
3	1	1	0
4	2	0	0
5	3	1	1
6	1	1	1

7	3	1	1
8	4	2	3
9	3	1	0
10	4	3	3
11	2	2	2
12	4	2	2
13	2	0	0
14	2	0	0
15	4	2	4
16	4	2	2
17	1	1	1
18	2	1	1
19	3	2	1
20	1	1	1
21	3	1	1
22	0	0	0
23	0	0	0
24	4	2	2
25	0	0	0
26	1	0	0
27	4	2	3
28	4	2	2
29	3	2	2
30	1	1	0
31	0	0	0
32	1	0	0
33	3	1	1
34	3	2	2
35	2	1	1
36	2	1	1
37	3	3	3
38	1	0	0
39	2	0	0
40	3	1	1
41	3	1	1
42	3	1	1
43	2	1	0
44	3	1	1
45	2	0	0
46	3	2	2

(CDllc+) and cytotoxic T lymphocytes (CD8+). It was interesting to note that the majority of CD8+ T cells coexpressed the CD69 activation molecule, and this activated cell subset was located mainly in the more severely inflamed portal and lobular areas. Comparing both histological categories of NASH (see Table 5), the percentage of CD69-expressing cells in severe NASH was significantly higher than in mild NASH (2.2 (1-3) *versus* 0.4 (O-1), $p<0.001$).

Expression of ICAM-1. In severe NASH, hepatocytes showed an intense membranous ICAM- I

TABLE 3

Comparison of patients with normal and abnormal liver function tests with regard to histological findings

	Normal liver tests	Abnormal liver tests
Severe NASH *n*= 13	6 (46%)	7 (54%)
Mild NASH n= 19	9 (47%)	10 (50'A)
Isolated steatosis n= 10	4 (~/o)	6 (60%)
Normal liver n=4	3 (75%)	1(24%)

NASH: nonalcoholic steatohepatitis.
Liver tests refer to ALT and/or ALP and/or GGT.

TABLE 4

Test of association between the presence of fibrosis and the demographics, laboratory, and histological variables studied

	Fibrosis (stage)		*p*
	Absence (0)	*Presence (IA)*	
Patients	18	28	
Sex(MIF)	5/13	11/17	ns
Age (years)	36.2±10.2	43.5±l1:1	0.003
BMI (kg/m^2)	49.3±5.6	50.4±5.8	ns
WC (cm)	128.1±13.3	128.3±1l.l	ns
Glucose (mg/dl)	106.2±34.6	115.9±36.2	ns
ALT (U/I)	35±l9A	38.5±18.8	ns
ASTIALT	0.86±0.71	0.78±0.25	ns
GGT (U/I)	35.2±30.1	59.6±52.6	ns
ALP (U/I)	178.4±47.2	197.9±52.6	ns
Steatosis			
0	4	0	0.000002
1	7	3	
2	6	4	
3	1	13	
4	0	8	
Inflammation			
0	14	0	0.0000
1	4	14	
2	0	12	
3	0	2	
4	0	0	

BMI: Body-mass index; WC: Waist circumference; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyltransferase.

expression, always in close relation with either lobular or portal areas of inflammatory infiltration and hepatocellular necrosis. ICAM-l was markedly expressed on sinusoidal-lining cells (SLC) as well. In contrast, in mild NASH, a weak ICAM-l reactivity was detected on some hepatocytes located close to focal lobular areas with mild mononuclear cell infiltration and on scattered SLC. In liver samples with steatosis alone or histologically normal, ICAM-1 expression was restricted to SLC. Comparing both types of NASH (see Table 5), the ICAM- 1 staining score was higher in severe NASH (2.4 (1-3)) than in mild NASH (0.6 (0-1), *p<0.00l).*

Expression of endoglin. A significant relationship between the intrahepatic endoglin expression and the presence of fibrosis in NASH patients was observed (see Table 6) The endoglin immunoreactivity was significantly stronger and showed a more diffuse distribution pattern in NASH with fibrosis (1.9 (1-3)) than in those without (0.3 (0-l)), *p<0.001.* In liver biopsies with significant fibrosis the endoglin staining was observed on SLC throughout the lobules as well as on fibroblast-like cells located in portal

TABLE 5

Comparative immunohistochemical scoring between severe and mild histological forms of NASH

Molecule	Mild NASH *(n=19)*	Severe NASH *(n=13)*	*p*
CD69 median (range)	0.4 (0-l)	2.2 (1-3)	<0.001
[CAM-I median (range)	0.6 (0-1)	2.4 (1-3)	<0.001
iNOS median (range)	0.9 (~1)	2.6 (1-3)	<0.001
NTY median (range)	0.4 (O~1)	1.8(1-3)	<0.001

NASH: Nonalcoholic steatohepatitis: ICAM-I: Intercellular adhesion molecule; iNOS: Inducible nitric oxide synthase: NTY: Nitrotyrosine.

tracts and in fibrous septa, whereas in cases with focal fibrosis the endoglin expression was mainly restricted to SLC located in perivenular areas and to some mildly expanded portal tracts. In liver sections without fibrosis, positivity for endoglin was found only on scattered SLC.

Detection of nitric oxide Markers. The intrahepatic iNOS expression was different between severe

TABLE 6

Relationship between immunohistochemical endoglin staining and the presence of fibrosis

Molecule	Absence of fibrosis *(n=18)*	Presence of fibrosis *(n=28)*	*p*
Endoglin	0.3 (0-I)	1.9 (1-3)	<0.001

Data are expressed as median (range).

and mild NASH, the iNOS staining score being significantly higher in severe than in mild NASH (2.6 (1-3) *versus* 0.9 (0-l)) p<0.001. Positive iNOS reactivity was found only in the cytoplasm of hepatocytes. No iNOS expression was observed in liver samples with isolated steatosis or which were histologically normal. On the other hand, in severe NASH the NTY staining was mainly detected in cellular clusters, formed by hepatocytes and SLC, adopting a focal pattern distributed throughout the hepatic lobules. In contrast, in mild NASH a low level of NTY immunoreactivity was found in scattered hepatocytes. Comparing NTY scoring in both types of NASH (see Table 5), NTY staining was significantly stronger in severe than in mild NASH (1.8 (1-3) *versus* 0.4 (0-l)) p<0.001. Finally, in all cases of histologically normal liver or with steatosis alone, no NTY expression was observed.

Discussion

The majority of obese patients studied *(69.5%)* had histological findings of NASH and, noticeably, 88% of them had an abnormal stage of fibrosis. Moreover, we have demonstrated that the presence of hepatic fibrosis associated positively with older ages, with greater degrees of steatosis, and with increased grades of inflammation at liver biopsy. Although these risk factors have been identified in a subgroup of NASH patients with marked obesity, which is a potential weakness of this study, the recent report by Angulo et al. (30) shows that older age and obesity, whatever their degree, are independent predictor factors of liver fibrosis in NASH, strongly suggesting that the results of our study could be extrapolated to the moderately or mildly obese NASH patients most commonly seen in clinical practice.

The main strength of the present study is that liver biopsy findings are available in obese individuals not selected upon histological or biological criteria (e.g. persistently abnormal liver function tests) allowing conclusions to be drawn about the prevalence of NASH in the asymptomatic obese population. We show in this paper that, despite different inclusion criteria used, the prevalence of this entity as well as the frequency of fibrotic liver disease in obese persons appear to be higher than those reported in two studies published 10 years ago (8,31). The clinical relevance of the data reported herein is even higher because of the increasing prevalence of obesity detected worldwide. In the UK, the prevalence of obesity increased from 8% to *15%* between 1980 and 1995 (32), and in the USA from 12.3% to 20% among men and *16.5%* to 24.9% among women between l97~80 and 1988-94 (33).

Using the average estimate that 18% of the general population in Western countries is obese and that 69.5% of markedly obese individuals had histologic findings of NASH with variable stages of fibrosis we might, therefore, consider NASH as the most prevalent form of liver disease worldwide, and with a significant potential to progress to fibrotic liver disease. This latter assumption has been further reinforced by recent results supporting progression of NASH as the most likely cause of cryptogenic cirrhosis (12).

Non-insulin-dependent diabetes mellitus (NIDDM) and hyperlipidemia are commonly associated with NASH (5~6). In our series, only six and nine had NIDDM and hyperlipidemia, respectively. While a trend to score high in fibrosis was observed in this group of patients, no significant association between the presence of either NIDDM or hyperlipidemia and the stage of NASH was found. Another feature increasingly reported in patients with NASH is

the presence of abnormal serum tests of iron metabolism with evidence of increased hepatic iron concentration (34). Conversely, only 15% of our patients had elevated serum levels of ferritin but none of them had histological evidence of hepatic iron overload.

An important percentage (54%) of our patients with severe NASH had normal liver function tests. This finding is worth emphasizing because the current impression of most clinicians is that significant progressive liver disease is unlikely in the absence of alterations in liver function tests. However, the small sample size of this study does not justify performing liver biopsy on all obese patients with persistently normal liver function tests, but the identification of patients at high risk of having NASH, that is older and markedly obese persons with associated conditions, such as diabetes mellitus, would be useful for this subgroup of patients who could benefit from liver biopsy, allowing a more accurate therapeutic strategy to be designed aimed at preventing the development of liver fibrosis.

Oxidative stress (OS) appears to play a key role in the pathogenesis of NASH (35-39). In that setting, nitric oxide may potentiate cytotoxicity through its reaction with superoxide anion, yielding peroxynitrite (40,41), a strong oxidant agent that promotes tyrosine nitration forming NTY (42). The finding that an abnormal intrahepatic accumulation of NTY is associated with the histological severity of NASH strongly suggests that nitric oxide-related oxidative injury may play a significant role in the pathogenesis of this liver disease. An additional factor contributing to hepatocellular damage could be OS-triggered lipid peroxidation (LP). End-products of LP, such as 4-hydroxy-noneal and malondialdehyde, are capable of activating
NF-KB (43), a nuclear factor regulating the transcription of several genes involved in the inflammatory response, including JCAM-1 and iNOS (44,45). The up-regulated intrahepatic expression of both genes in NASH suggests the existence of an increased NF-KB activity in the liver tissue of these patients.

Recently, some experimental data have emerged favoring a role for endotoxin-mediated cytokine release in the development of liver damage in NASH. Yang et al. (46) have shown that obese mice with severe steatosis have much more sensitivity to bacterial endotoxin than lean ones. They also showed that liver injury appeared to be mediated by both TNF-a and IFN-y. Supporting these data is the fact that increased levels of circulating TNF-a have been found in obese mice (47). Since it has been demonstrated that proinflammatory cytokines are directly implicated in the up-regulation of ICAM- I and iNOS (18,48), the enhanced intrahepatic expression of these molecules in NASH reinforces the hypothesis that locally-released proinflammatory cytokines could play a significant role in the pathogenesis of this liver disease. A potential source of cytokines, such as TNF-a and INF-y, is macrophages and activated T lymphocytes infiltrating the liver tissue of NASH patients. Furthermore, to our knowledge this is the first study showing an enhanced intrahepatic endoglin expression in NASH, mainly located on sinusoidal endothelial cells and probably on stellate cells. It is noteworthy that the intrahepatic expression level of endoglin correlated significantly with the presence of fibrosis. Given that sinusoidal endothelial cells and stellate cells are the major cellular sources of extracellular matrix proteins in liver injury (49), it is conceivable that the upregulated expression on these cells of a molecule, such as endoglin, that functionally behaves as a receptor for TOF-a *(50)* would increase the cellular responsiveness to TGF-a, the most potent fibrogenic factor (51), triggering fibrogenesis with the consequent potential risk of progression to cirrhosis.

In conclusion, in obese patients with NASH, age as well as degree of steatosis and grade of inflammation at liver biopsy are risk factors of fibrosis. Knowledge of these data could be useful to evaluate the prognosis in patients with NASH and to design more specific therapeutic strategies aimed at overcoming those pathogenic factors, such as OS and cytokine-mediated injury, contributing to progression of liver disease.

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