Correlation of Serum TNF-α Levels and Histologic Liver Injury Scores in Pediatric Nonalcoholic Fatty Liver Disease

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Key Words: Tumor necrosis factor α; TNF-α; Nonalcoholic fatty liver disease; NAFLD; Nonalcoholic steatohepatitis; NASH; Insulin resistance; Leptin; Inflammation; Receiver operating characteristic curves

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Abstract

We tested the power of tumor necrosis factor (TNF)-α and/or leptin in predicting the degree of liver involvement in children with nonalcoholic fatty liver disease (NAFLD). We measured serum levels of TNF-α and leptin and computed NAFLD activity score (NAS) (NAS ≥ 5, diagnostic of nonalcoholic steatohepatitis [NASH]) in 72 consecutive biopsy-proven NAFLD cases (training and validation sets, 36 cases each). Univariate analysis evaluated variables significantly associated with a diagnostic NAS. Receiver operating characteristic (ROC) curve analysis assessed the diagnostic value of selected variables in predicting a NAS of 5 or more.

*TNF-α (*P *< .0001), leptin (*P *= .001); triglycerides (*P *= .013), and alkaline phosphatase (*P *= .046) levels were significantly associated with a NAS of 5 or more. TNF-α and leptin levels predicted the risk of NAS of 5 or more. ROC analyses defined cutoff values for TNF-α, leptin, and risk score. They identified 90%, 83%, and 83% of the cases, respectively, with a NAS of 5 or more (true-positive cases) from the validation set.*

TNF-α alone or combined with leptin in a simple risk score can accurately predict a NAS of 5 or more. TNF-α seems to be a specific laboratory marker of NASH.

Obesity, impairment of glucose metabolism, and nonalcoholic fatty liver disease (NAFLD) cluster in westernized countries. These diseases are increasing in incidence in children and are predicted to reach epidemic proportions in the pediatric population in the next decade. In Italy, one fourth of obese children have increased levels of serum aminotransferases, a rough marker of NAFLD[1]; 40% of children with biopsy-proven NAFLD are obese; 51% to 62% are insulin resistant, and 2.3% have overt type 2 diabetes mellitus.[2] NAFLD may develop very early in young (preschool) children, with no difference in sex predominance. Obesity and systemic insulin resistance are strongly associated with increased risk of fibrosis, nonalcoholic steatohepatitis (NASH), and worst NAFLD histologic activity score.[2] Hence, there is a need to develop accurate and reliable noninvasive means to assess the severity of hepatic damage, apart from the presence of comorbid conditions.

An ideal noninvasive diagnostic test for liver histologic features should be simple, readily available, accurate, and not expensive.[3] Together with routine tests, such as liver enzymes, various cytokines might have these features. Strong evidence supports a key role for tumor necrosis factor (TNF)-α and leptin[4] among proinflammatory cytokines in the pathogenesis of the NASH as putative second hits in the 2-hit model proposed by Day and James.[5] Data from animal and clinical studies indicate that TNF-α mediates not only the early stages of fatty liver disease but also the transition to more advanced stages of liver damage,[6-9] whereas leptin exerts direct profibrotic effects.[10,11] Moreover, both adipocytokines take an active part in the pathogenesis of obesity and insulin resistance.[12] Thus, we speculate that both of the aforementioned adipocytokines together with routine laboratory tests may be helpful in selecting patients with NASH.

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Recently, Kleiner et al[13] designed a validated histologic feature scoring system that addresses the full spectrum of NAFLD lesions and proposed an NAFLD activity score (NAS) for use in clinical trials. In the present study, we aimed to test the power of TNF-α and/or leptin alone in predicting the NAS and develop a model consisting of readily available, objective laboratory data to predict the NAS in a case series of biopsy-proven NAFLD, independently of the aforementioned comorbid conditions. To accomplish these goals, cases were consecutively divided into 2 groups of 36 each of training and validation sets. In the former, data were analyzed to construct a model, testing the potential correlation between serum protein levels of TNF-α, leptin, and histologic outcome. In the latter, findings were internally validated.

Materials and Methods

Patients

We enrolled 72 untreated patients (51 males and 21 females), aged 9 to 18 years, and consecutively divided them into the training and validation sets. This study included a subgroup of patients from a previous prospective study.[2] Body mass index (BMI) and BMI *z* score were calculated.[14] Obesity was defined as a BMI more than 2 SD above the 97th percentile adjusted for age and sex. Hypertriglyceridemia and hypercholesterolemia were diagnosed for levels of triglycerides and cholesterol greater than those reported as normal for age, sex, and race.[15] Hypertension was diagnosed according to the guidelines of the task force on blood pressure control in children.[16]

The study protocol conformed to the recommendations of the ethics committee of the Bambino Gesù Children's Hospital, Rome, Italy. The nature and purpose of the study were carefully explained before informed consent was requested from each patient or responsible guardian.

Evaluation of Glucose Metabolism and Insulin Sensitivity

All patients underwent a 2-hour oral glucose tolerance test with the standard 1.75 g of glucose per kg, or a maximum of 75 g. Glucose tolerance was determined according to the American Diabetes Association classification.[17] Insulin resistance was determined by the homeostatic model assessment[18] and insulin sensitivity by the insulin sensitivity index derived from the oral glucose tolerance test.[19]

Biochemical Assays

Serum samples were stored at –80°C for later assays. Serum protein levels of TNF-α were assayed by an immunometric assay (Immulite, Gwynedd, Wales) with an analytic sensitivity of 1.7 pg/mL. Intra-assay and interassay coefficients of variation were 3.6% and 6.5%, respectively. Serum protein levels of leptin were measured by a commercial enzyme-linked immunosorbent assay kit (Lincho Research, St Charles, MO). Intra-assay and interassay coefficients of variation were 4.2% and 4.5%, respectively. The sensitivity of the method is 0.5 ng/mL. Plasma glucose levels were measured in triplicate by the glucose oxidase technique on a Beckman glucose analyzer (Beckman, Fullerton, CA) and plasma insulin levels by a radioimmunoassay (MYRIA Technogenetics, Milan, Italy). Fasting triglycerides and total and high-density lipoprotein cholesterol were measured spectrophotometrically.

Liver Histologic Examination

Biopsies were performed on all children using an automatic core biopsy device (Biopince, Amedic, Sweden). Liver biopsy specimens, routinely processed and analyzed as described elsewhere,[2] were at least 15 mm long and read by the same liver pathologist who was unaware of the patient's clinical and laboratory data. The main histologic features commonly described in NAFLD and NASH, including steatosis, inflammation (portal and lobular), hepatocyte ballooning, and fibrosis, were scored by using the scoring system for NAFLD according to the guidelines of the National Institutes of Health–sponsored Nonalcoholic Steatohepatitis Clinical Research Network.[13] Features of steatosis, lobular inflammation, and hepatocyte ballooning were combined in a score ranging from 0 to 8, the NAFLD activity score (NAS). Cases with NAS of 5 or more are diagnostic of NASH, cases with NAS of 2 or less are diagnostic of simple steatosis, and cases with scores in between are considered indeterminate.[13]

Statistical Analysis

The endpoint of the study was the prevalence of a NAS of 5 or more. Data are given as mean ± SD and/or number (proportion) of affected patients. The Kolmogorov-Smirnov goodness-of-fit test was applied for determining whether sample data likely derive from a normally distributed population. The results for fasting glucose and insulin, insulin sensitivity by the insulin sensitivity index, triglycerides, alanine aminotransferase, aspartate aminotransferase, γ-glutamyltransferase, TNF-α, leptin, and histologic grades diverged significantly from the normal distribution and were log-transformed before analysis. The nonparametric Spearman correlation coefficient was used. The Mann-Whitney *U* test, 1-way analysis of variance, Bonferroni post-hoc test for multiple comparisons, and regression analysis were carried out by standard techniques. Univariate analysis was performed on variables between patients with and without the study end point in the training set. Significant variables from the univariate analysis were included in forward logistic regression models to identify independent factors associated with the end point. Beta coefficients were used to compute risk scores.

Diagnostic values of laboratory assays and computed risk scores were assessed by the area under the receiver operating

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characteristic (ROC) curve (AUC). ROC analysis is a way of evaluating the accuracy of a diagnostic test by summarizing the potential of the test to discriminate between the absence and presence of a health condition.[20,21]

In the context of the present study, this diagnostic accuracy refers to the ability of TNF-α and leptin levels and the computed risk score to discriminate a NAS of 5 or more from a NAS of 4 or less. With use of these criterion values, NAFLD cases classified correctly as a NAS of 5 or more by TNF-α, leptin, and/or the computed risk score values represent the true-positive cases, whereas cases with a NAS of 5 or more but classified as a NAS of 4 or less represent false-negative cases. Cases classified correctly as a NAS of 4 or less represent true-negative cases, and cases with a NAS of 4 or less but classified as a NAS of 5 or more represent false-positive cases.

The sensitivity of the test is the probability that TNF-α, leptin, and computed risk score values will classify a case as a NAS of 5 or more when the case is truly a NAS of 5 or more; the specificity is the probability that TNF-α, leptin, and the computed risk score values will classify a case as a NAS of 4 or less when the case is truly a NAS of 4 or less (true-negative). In ROC analysis, the true-positive rate (sensitivity) is plotted against the false-positive rate (1 – specificity) across a range of values from the diagnostic test. This provides an estimate of the cutoff that corresponds to the best tradeoff between sensitivity and 1 – specificity (ie, minimal false-negative and false-positive cases), suggesting the best accuracy of TNF-α, leptin, and/or the computed risk score values to discriminate between cases with a NAS of 5 or more and those with a NAS of 4 or less. The decision threshold for the best tradeoff is the criterion value with the highest accuracy that maximizes the sum of the sensitivity and specificity.[22] One index reflecting the overall accuracy of the diagnostic test derived from an ROC analysis is the AUC.[21] This is a useful quantitative and descriptive expression of how close the AUC is to the ideal area of 1.

The level of significance was set at α = .05. SPSS 11.5 for Windows (SPSS, Chicago, IL) was used for statistical analyses.

Results

Characteristics of Studied Patients and Liver Histologic Features

The prevalence of impaired fasting glucose was 11% (8/72); impaired glucose tolerance, 10% (7/72); and type 2 diabetes mellitus, 3% (2/72). Obesity was present in 29 (40%), hypercholesterolemia in 18 (25%), and hypertriglyceridemia in 48 (67%) of subjects. Hypertension was diagnosed in 24 subjects (33%).

Steatosis was found in all biopsy specimens. It was mostly macrovesicular but associated with microvesicular lesions. It was diffuse or scattered lobular and, in 10 cases, zonally distributed. In 59 biopsy specimens (82%), inflammation was present. The inflammatory infiltrate was mainly composed of lymphocytes and neutrophils, and, when granulomas were present, mononuclear histiocytic cells and eosinophils were associated. Hepatocyte ballooning was found in 34 biopsy specimens (47%). Periodic acid–Schiff–diastase–positive cells containing phagocytosed cellular debris were present in the portal tract and/or sinusoids. Glycogenated nuclei of variable dimensions were found in 38 cases (53%); this nuclear change was noted mostly in zone 1. No Mallory hyaline was noted in any case, and mild iron deposition was present in 3 cases. A diagnosis of NASH was formulated in 22 cases (31%; NAS ≥ 5) and simple steatosis (NAS ≤ 2) in 26 (36%), and 24 cases (33%) were considered indeterminate. Increased fibrosis was observed in 49 cases (68%). It was mostly of mild (stage 1) severity with only 4 children showing septal fibrosis (stage 3). Among the 43 cases of stage 1 fibrosis, 3 were 1a, 7 were 1b, and 33 were 1c. No case showed cirrhotic stage disease by liver biopsy. **Table 1** shows levels of TNF-α according to liver histologic features.

Values of serum protein levels of TNF-α and leptin significantly correlated (r_o = 0.251; *P* = .03).

Training and Validation Sets and Predictors of a NAS of 5 or More

Anthropometric and biochemical characteristics of patients in both sets are reported in **Table 2**. No significant differences were found except in systolic blood pressure (*P* =

Table 1
Serum TNF-α Level by Histologic Grading in the Studied Population (N = 72)*

Score	No. of Cases	TNF-α (pg/mL)
Steatosis†		
1	27	5.10 ± 0.97
2	26	6.78 ± 2.41
3	19	8.43 ± 3.27
Inflammation‡		
0	13	5.98 ± 1.91
1	53	6.40 ± 2.67
2	6	9.55 ± 1.58
Ballooning§		
0	37	5.18 ± 1.18
1	17	6.49 ± 2.05
2	18	9.55 ± 1.58
Fibrosis‖		
0	31	5.45 ± 1.49
1	41	7.45 ± 2.96
NASH score¶		
1-2	26	5.16 ± 1
3-4	24	5.38 ± 1.24
5-7	22	9.59 ± 2.58

NASH, nonalcoholic steatohepatitis; TNF, tumor necrosis factor.
* Data are represented as mean ± SD. *P* values are reported for the Bonferroni post-hoc test for multiple comparisons.
† 1 vs 2, *P* = .05; 2 vs 3 and 1 vs 3, *P* < .0001.
‡ 0 vs 2 and 1 vs 2, *P* = .01.
§ 0 vs 2 and 1 vs 2, *P* = .0001.
‖ *P* = .0001.
¶ 1-2 vs 5-7 and 3-4 vs 5-7, *P* = .0001.

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.005). A NAS of 5 or more was found in 12 patients from the training set and 10 from the validation set.

Variables associated with the presence of a NAS of 5 or more were first assessed by univariate analysis **Table 3** in the training set. It showed statistically significant differences (NAS ≤ 4 vs NAS ≥ 5) in circulating levels of alkaline phosphatase, triglycerides, TNF-α, and leptin. Regression analyses were run to assess the best predictors of NAS among the aforementioned variables. Accordingly, TNF-α and leptin simply and accurately predicted the risk of having a high NAS. The following risk score was obtained:

Risk Score = 0.440 + (1.454 × ln leptin) + (4.617 × ln TNF-α)

ROC curves for fasting leptin and TNF-α levels and for the computed risk score in the training and validation sets are shown in **Figure 1**, with the sensitivity of the test (true-positive

Table 2
Comparison of Cases in the Training and Validation Sets*

Variable	Training Set (n = 36)	Validation Set (n = 36)
Age (y)	12.11 ± 3.31	11.57 ± 3.40
BMI (kg/m^2)	26.56 ± 4.57	26.16 ± 3.84
BMI *z* score	1.82 ± 0.74	1.84 ± 0.77
Aspartate aminotransferase (U/L)	51.58 ± 34.12	45.17 ± 14.62
Alanine aminotransferase (U/L)	84.97 ± 36.12	60.94 ± 24.68
γ-Glutamyl transferase (U/L)	26.78 ± 19.78	20.83 ± 19.66
Alkaline phosphatase (U/L)	666 ± 170	643 ± 219
Albumin, g/dL (g/L)	4.52 ± 0.38 (45.2 ± 3.8)	4.61 ± 0.39 (46.1 ± 3.9)
Bilirubin, mg/dL (μmol/L)	0.67 ± 0.21 (11 ± 4)	0.65 ± 0.18 (11 ± 3)
Platelet count, × 10^3/μL (× 10^9/L)	292 ± 6 (292 ± 6)	286 ± 6 (286 ± 6)
WBC count, /μL (× 10^9/L)	7,000 ± 1,300 (7.0 ± 1.3)	7,300 ± 2,100 (7.3 ± 2.1)
Cholesterol, mg/dL (mmol/L)	161.03 ± 32.21 (4.16 ± 0.83)	149.33 ± 33.49 (3.86 ± 0.87)
Triglycerides, mg/dL (mmol/L)	98.47 ± 59.61 (1.11 ± 0.67)	87.11 ± 52.40 (0.98 ± 0.59)
TNF-α (pg/mL)	6.66 ± 2.46	6.51 ± 2.80
Serum leptin (ng/mL)	16.35 ± 7.06	16.80 ± 8.43
Fasting glucose, mg/dL (mmol/L)	81.67 ± 13.31 (4.5 ± 0.7)	82.25 ± 8.54 (4.6 ± 0.4)
Fasting insulin, μU/L (pmol/L)	11.18 ± 6.36 (80 ± 46)	11.32 ± 5.20 (81 ± 37)
HOMA-IR	2.70 ± 1.42	2.55 ± 1.18
ISI-comp	4.52 ± 2.02	4.36 ± 1.88
Blood pressure (mm Hg)		
Systolic	119.29 ± 16.45	108.80 ± 10.07†
Diastolic	66.35 ± 9.33	68.40 ± 7.57

BMI, body mass index; HOMA-IR, insulin resistance determined by the homeostatic model assessment; ISI-comp, insulin sensitivity determined by the insulin sensitivity index.
* Data are presented as mean ± SD. Biochemical analytes were assayed in fasting condition.
† P = .005.

Table 3
Univariate Analysis of Variables Associated With the Study End Point (NAS ≥ 5) in the Training Set (n = 36)

Variable	NAS ≤ 4	NAS ≥ 5	*P*
Age (y)	12.26 ± 3.63	11.78 ± 2.54	.698
BMI (kg/m^2)	26.01 ± 4.59	28.29 ± 4.39	.260
BMI *z* score	1.72 ± 0.83	2.05 ± 0.47	.228
Aspartate aminotransferase (U/L)	43.44 ± 18.79	70.09 ± 51.8	.120
Alanine aminotransferase (U/L)	66.76 ± 40.30	76.36 ± 40.06	.431
γ-Glutamyl transferase (U/L)	26.80 ± 19.24	26.73 ± 21.90	.118
Alkaline phosphatase (U/L)	623.37 ± 164	767.63 ± 146	**.046**
Albumin, g/dL (g/L)	4.50 ± 0.38 (45.0 ± 3.8)	4.56 ± 0.38 (45.6 ± 3.8)	.681
Bilirubin, mg/dL (μmol/L)	0.68 ± 0.18 (12 ± 3)	0.63 ± 0.27 (11 ± 5)	.50
Platelet count, × 10^3/μL (× 10^9/L)	300 ± 68 (300 ± 68)	272 ± 45 (272 ± 45)	.240
WBC count, /μL (× 10^9/L)	7,100 ± 1,400 (7.1 ± 1.4)	6,900 ± 1,200 (6.9 ± 1.2)	.772
Cholesterol, mg/dL (mmol/L)	155.12 ± 29.79 (4.01 ± 0.77)	174.45 ± 34.87 (4.51 ± 0.90)	.098
Triglycerides, mg/dL (mmol/L)	82.32 ± 32.14 (0.93 ± 0.36)	135.18 ± 88.47 (1.53 ± 1.00)	**.013**
TNF-α (pg/mL)	5.48 ± 1.18	9.34 ± 2.52	**.0001**
Serum leptin (ng/mL)	13.93 ± 6.61	21.87 ± 4.65	**.001**
Fasting glucose, mg/dL (mmol/L)	84.17 ± 15.18 (4.7 ± 0.8)	75.90 ± 3.69 (4.2 ± 0.2)	.102
Fasting insulin, μU/L (pmol/L)	12.13 ± 7.08 (87 ± 51)	11.32 ± 4.55 (82 ± 33)	.729
Blood pressure (mm Hg)			
Systolic	117.87 ± 17.04	123.38 ± 14.92	.424
Diastolic	66.09 ± 9.30	67.13 ± 10.01	.791

NAS, nonalcoholic fatty liver disease activity score.
* Values are reported as mean ± SD. Significant *P* values are in bold type.

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result) plotted against the false-positive results (1 – specificity) across a range of values from the diagnostic test. Overall accuracy of the diagnostic test deriving from the ROC analysis was estimated by the AUC. According to the ROC analysis in the training set, cutoff values were chosen to discriminate patients with a NAS of 5 or more, and accuracy of these values in predicting the disease are given in **Table 4**. By applying the cutoff values to the validation set, sensitivity, specificity, and positive and negative predictive values overlapped values reported in the training set. A decision tree using these cutoff values is depicted in **Figure 2**. A value of TNF-α of 7.9 pg/mL or more has a sensitivity of 82% and a specificity of 96% (Table 4), which means that this value is able to identify 82% of subjects with a NAS of 5 or more (true-positive cases) and 96% with a NAS of 4 or less (true-negative cases) among the selected population in the training set. By applying this cutoff in the validation

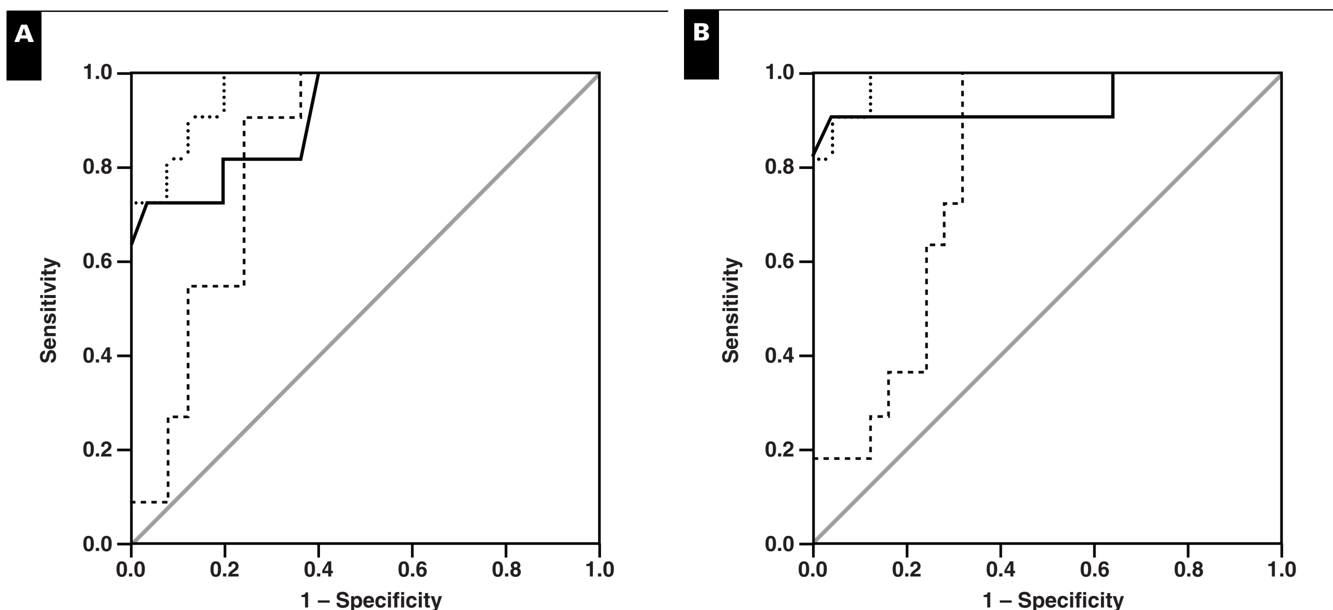

Figure 1 Receiver operating characteristic curves of variables in the prediction of the nonalcoholic fatty liver disease activity score (NAS) given as area under the curve (AUC) and 95% confidence interval (CI). The further the curves lie above the reference line (gray line), the more accurate the test. Tumor necrosis factor (TNF)-α is represented by a solid line, leptin by a dashed line, and risk score by a dotted line. An AUC of 1.0 is characteristic of an ideal test, whereas an AUC of ≤ 0.5 indicates a test of no diagnostic value. In the training set (**A**) AUC values are 0.911 (95% CI, 0.809-1.00) for TNF-α, 0.833 (95% CI, 0.702-0.964) for leptin, and 0.964 (95% CI, 0.911-1.00) for the computed risk score. In the validation set (**B**), AUC values are 0.940 (95% CI, 0.829-1.00) for TNF-α, 0.796 (95% CI, 0.653-0.940) for leptin, and 0.985 (95% CI, 0.956-1.00) for the computed risk score.

Table 4
Accuracy of TNF-α, Leptin, and the Computed Risk Score Cutoff Values in Predicting the NAS in the Training Set (n = 36)*

Variable	No. of TP Cases	No. of FP Cases	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
TNF-α						
≤5.9 pg/mL	2	16	18	36	11	5
≥7.9 pg/mL	9	1	82	96	90	96
Leptin						
≤14.9 ng/mL	1	16	9	36	5	47
≥20.4 ng/mL	6	6	54	76	50	79
Risk score						
≤12.9	1	20	9	2	4	33
≥13.5	9	2	81	92	82	92

FP, false-positive; NAS, nonalcoholic fatty liver disease activity score; NPV, negative predictive value; PPV, positive predictive value; TNF, tumor necrosis factor; TP, true-positive.
* The highest values are the criterion values with the highest accuracy to maximize the sum of specificity and sensitivity. The lowest value represents an empirical minimum value across the entire range of scores to test accuracy. The highest value provides an estimate of the cutoff that corresponds to the best tradeoff between sensitivity and 1 – specificity, which suggests the best accuracy of leptin, TNF-α, and computed risk score to discriminate between the absence and presence of a NAS of ≥ 5. TP cases are children with nonalcoholic fatty liver disease whose disease was classified correctly as NAS ≥ 5 by TNF-α, leptin, and/or the computed risk score. FP cases are children with NAS ≤ 4 whose disease was incorrectly classified as NAS ≥ 5. The sensitivity is computed as the ratio between TP cases and the number of subjects with a NAS ≥ 5. The specificity is calculated as the ratio between true-negative cases and the number of patients with a NAS ≤ 4; PPV represents the probability that disease is present when the test result is positive; NPV represents the probability that the disease is not present when the test result is negative.

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set (Figure 2), this value of TNF-α identifies 90% of true-positive and 97% of true-negative cases.

Discussion

We found a strong association between serum protein levels of TNF-α and histologic features of NAFLD as defined according to the Kleiner scoring system.[13] Levels of the circulating cytokines significantly increased as the NAS worsened (Table 1). Among the laboratory tests routinely performed in our unit, levels of leptin, alkaline phosphatase, and triglycerides were significantly associated with the primary end point of the study. Nevertheless, in an attempt to develop a simple mathematical model to predict a NAS of 5 or more in this cohort of children with NAFLD, only TNF-α and leptin entered the mathematical model that more accurately predicted the degree of liver involvement.

There is a strong rationale for measuring the levels of TNF-α in NAFLD. The cytokine has a key role in mediating liver injury, given its ability to induce inflammation and apoptosis in hepatocytes under conditions of oxidative stress.[23,24] In susceptible people, oxidative stress, lipid peroxidation, and TNF-α overexpression will induce cell death, inflammation, and fibrosis.[23,24] Evidence comes from studies performed in humans,[9,10,25-27] but contrasting findings have been reported in adults[28,29] and in children with NAFLD.[30] In a pediatric cohort of 50 children for whom liver histologic data were not available, Mandato et al[30] failed to find a significant increase in TNF-α levels in children with hypertransaminasemia and ultrasonographic liver brightness compared with children with levels of liver enzymes within the normal range.[30] This finding may be due to the lack of a significant association between enzyme levels and liver histologic features in NAFLD and NASH.[31] Thus, by measuring liver enzyme levels instead of considering liver histologic features, the authors might have missed the association between levels of the inflammatory adipocytokines and diagnosis of uncomplicated liver steatosis or NASH.

As for a role for leptin in NASH, the hormone has been shown to have a prominent role in hepatic fibrosis in animal models of disease,[32] mainly acting through the activation of hepatic stellate cells[33] and the enhanced release of osteopontin, a proinflammatory cytokine.[34] Leptin knockout mice are protected from hepatic inflammation and fibrosis in the methionine-choline–deficient diet–induced model of NASH.[34] In humans with NAFLD, in agreement with the present findings, levels of leptin are increased.[35]

TNF-α and leptin entered a mathematical model able to predict NASH in our patients, whereas alkaline phosphatase and triglycerides were removed. By testing the score in the validation set, we observed that levels of TNF-α alone are adequate in clinical study to discriminate patients with a NAS of 5 or more (Figure 2). So far, clinical features such as a high BMI or older age could be considered markers of a high NAS.[2] This is the first attempt to identify biochemical markers of liver involvement in pediatric NAFLD independent of comorbid conditions. Of note, a very recent study by Poniachik et al[36] found that increased production of interleukin 1α and TNF-α by in vitro stimulated whole blood cell cultures occurs in obese adults with NAFLD. The response is more marked in steatohepatitis than in uncomplicated steatosis, and it significantly correlates with the histologic score.

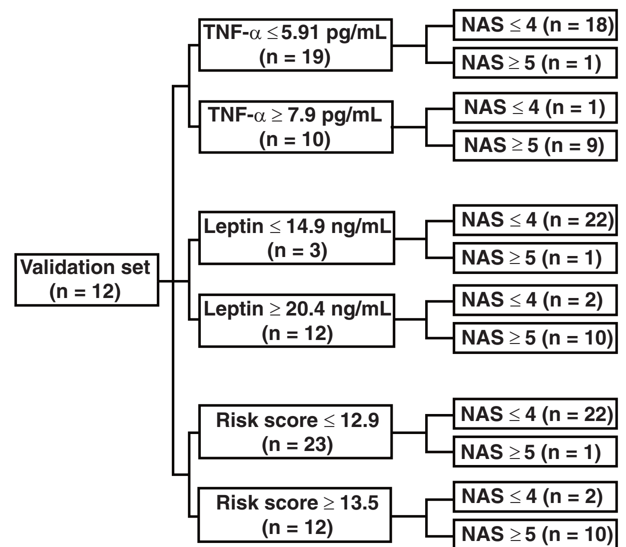

Figure 2 Decision tree using the cutoff values that resulted from the receiver operating characteristic analysis for tumor necrosis factor (TNF)-α, leptin, and the computed risk score. NAS, nonalcoholic fatty liver disease activity score.

The strength of our study derives from the observation that in the literature, few studies concern liver biopsy–proven NAFLD in children.[2,37] Thus, the small number of patients in this study must be balanced against the difficulty of obtaining a cohort of young subjects with liver biopsy–proven NAFLD. Despite the interesting association between TNF-α levels and NAS, TNF-α measurement is strongly recommended in clinical practice. The control population herein is inadequate to establish the true false-positive nature of this analysis because levels of TNF-α may increase in several pathologic conditions such as inflammatory diseases and cancer. Thus, our results are likely to be unreliable in the general pediatric population, and they need to be validated in an external series. TNF-α measurement cannot replace liver biopsy, which remains the "gold-standard" technique in the diagnosis of NAFLD and NASH.[31] Moreover, measurement of a laboratory marker cannot be used as a predictor of disease as long as normal reference values vary between different assays. TNF-α alone is better than leptin or other biochemical markers for accurately predicting a NAS of 5 or more in up to 90% of children with NAFLD.

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