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RESEARCH ARTICLE

Assessment of Progression Markers for Cirrhosis in Cystic Fibrosis Patients

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ABSTRACT

Cystic Fibrosis is an autosomal dominant disease that affects the CFTR gene, which is responsible for producing a protein that regulates the transport of chloride ions across the cell membrane. This alteration results in obstruction in the body's ducts and channels, primarily affecting the pulmonary system. Additionally, other systems can be affected. Cystic Fibrosis-related liver disease is a common complication that can lead to steatosis, fibrosis, and multifocal biliary cirrhosis; however, there are no well-established diagnostic criteria yet. The present study was conducted at the Cystic Fibrosis Outpatient Clinic of the Hospital de Clínicas, Federal University of Paraná, examining 98 medical records of patients with cystic fibrosis for at least 10 years. Patients with hepatitis B, hepatitis C, alcohol consumption, or pregnancy were excluded. The objective was to determine the best predictor for the outcome of cirrhosis, and for this purpose, demographic parameters, laboratory tests, and imaging were collected. The statistical analysis was performed using SPSS® software, with quantitative variables described as mean ± standard deviation and categorical variables presented as the number of observations and percentage. ROC curves were adjusted to determine the best predictor, using the Youden index to establish the optimal cutoff point and the corresponding area under the curve to determine the model's efficacy. The study included 80 patients, and 10 of them (12.5%) were diagnosed with Cystic Fibrosis-related cirrhosis. The average age of the cirrhosis group was 21.6 years, with a predominance of males. The most accurate parameter for predisposition to cirrhosis was the APRI score, with a cutoff value of 0.27. Univariate analysis showed that gender, hepatic changes on ultrasound, splenomegaly, increased portal vein diameter, presence of vomiting, levels of alanine transaminase, aspartate transaminase, platelet count, and FIB-4 score were significantly associated with an APRI score value greater than 0.27. This study suggests the hypothesis that the APRI score is the most sensitive and specific tool for defining cirrhosis in patients with cystic fibrosis.

Introduction

Cystic Fibrosis (CF) is the most common autosomal recessive disease in Caucasian populations, affecting the CFTR gene that encodes a protein that regulates the transport of chloride ions across the cell membrane. There are more than two thousand mutations that can trigger CF, with the delta F508 mutation being the most common.

The World Health Organization (WHO) estimates that, in 2018, 70,000 people were affected by cystic fibrosis. At the national level, data from the last record of the Brazilian Group for the Study of Cystic Fibrosis (GBEFC-2019), accounted for 5,773 CF patients in the country, with an incidence of 1: 7,576 live births, according to statistics published by the Brazilian Society of Pulmonology and Phthisiology-2021.

CF mainly affects the pulmonary system, altering the ability to eliminate chloride into the organ's lumen. As a result of this process, the production of dehydrated secretions occurs, which culminates in an obstructive tubulopathy. However, there may also be repercussions on other systems.

From the early reports of the disease, by the end of the 1930s, when high rates of early mortality were observed, there was a significant increase in survival rates for CF patients, given the advances in multidisciplinary diagnosis and treatment. Thus, in addition to pulmonary involvement, manifestations of extrapulmonary locations began to receive attention, among them, hepatic manifestations - the third leading cause of death in the CF population - less frequent, only, than deaths from complications of lung diseases and organ transplants[1,12].

Cystic Fibrosis Associated Liver Disease (CFLD) is a common complication. The CFTR protein, expressed in the apical membrane of cholangiocytes and in the gallbladder epithelium, when altered by the CF mutation, prevents the transmembrane transport of anions, leading to the production of plugs formed by thick biliary secretion, which can lead to steatosis, hepatic fibrosis and multilobar biliary cirrhosis. The prevalence of CFLD remains uncertain in the literature due to the absence of established diagnostic criteria and heterogeneous clinical presentation[2].

Examinations such as ultrasonography (USG), elastography, biopsy and laboratory measurements confirm the involvement of the liver in CF, but do not distinguish the various phenotypic presentations of CFHD nor prevent progression to more severe conditions.

As there is still no gold standard for the accurate diagnosis of CFLD[3], the potential impact that the definition of more accurate criteria for the early detection of biliary cirrhosis would have on the expectation and quality of life of CF patients is verified.

That way, the present study aims to identify epidemiological differences between participants with biliary cirrhosis secondary to cystic fibrosis and those who have cystic fibrosis without cirrhosis, in order to evaluate the best clinical and laboratory way to predict this hepatic complication in CF patients.

Materials and methods

For inclusion in the study, participants of any age were considered as long as they had a diagnosis of CF equal to or greater than 10 years. Eighteen medical records were excluded from the survey due to the following criteria: diagnosis period of less than 10 years (6), inconclusive or absent genetic testing (11), chronic hepatitis B (1). Exclusion criteria for hepatitis C, pregnancy and alcohol intake in any amount were also considered. The study was approved by the Ethics and Research Committee of HC-UFPR (CAAE 40142620.6.0000.0096).

STUDY DESIGN

Observational and cross-sectional study, of the survey type, in medical records of participants of the Cystic Fibrosis Outpatient Clinic of the Hospital de Clínicas of the Federal University of Paraná. 98 medical records were consulted, in physical and electronic records, from September to October 2021.

CLINICAL, LABORATORY AND RADIOLOGICAL EVALUATION

In data collection, demographic parameters were considered: age, gender and history of meconium ileus. From laboratory data were investigated: type of mutation, aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (AP), direct and indirect bilirubin, serum sodium (Na), potassium (K), creatinine and platelet dosage. Information from imaging exams was included in the study as described in the medical records. The ultrasound terms collected were: changes in the liver, biliary tree and pancreas; increased portal vein diameter; splenomegaly; hepatomegaly; ascites; steatosis and hepatic fibrosis. The value of pulmonary arterial pressure was included in the echocardiography. The presence of gastric and esophageal varices was also recorded when described in an upper digestive endoscopy (UGE). Descriptions of nausea, vomiting, weight loss,

steatorrhea, ascites, collateral circulation and splenomegaly were considered when recorded.

For inclusion in the CF-related liver cirrhosis group, the presence of at least two of the following conditions was considered: (1) hepatomegaly or splenomegaly described in the physical examination; (2) elevation above the upper limit of normal for TGO and/or TGP and/or GGT levels; (3) abnormalities in abdominal ultrasound (biliary tree abnormalities, increased portal vein diameter, splenomegaly, or hepatic fibrosis); (4) presence of esophageal or gastric varices in upper gastrointestinal endoscopy. Participants who did not meet these criteria were included in the control group.

STATISTICAL ANALYSIS

Age, TGP, TGO and platelet values were used to calculate the FIB-4 score (hepatic fibrosis index), while the last two parameters were included for the APRI score (aspartate aminotransferase ratio index on platelets).

For the creation of the database and statistical analysis, the SPSS® software (IBM SPSS Statistics for Windows, version 20.0, NY, USA) was used. The results of quantitative (numerical) variables were described as mean ± standard deviation (SD). Qualitative (categorical) variables were presented by the number of observations (n) and their percentage.

In order to determine the best predictor for the cirrhosis outcome, receiver curves were adjusted among the collected variables. operating characteristic (ROC). The best cut-off point was established according to the Youden index, through the largest area under the corresponding curve (AUC).

Results

From the ROC curve, it was determined that the APRI score was the most accurate parameter for predisposition to cirrhosis, since it had a greater area under the curve (AUC 0.84). The cut-off value for maximum sensitivity and specificity of the APRI score was 0.27 (Fig.1).

Eighty patients with cystic fibrosis were included in the study. Of these, 10 met the criteria defined for CF-associated biliary cirrhosis, which corresponds to 12.5% of the sample. The CFTR delta F508 gene mutation was the most present (55%) in the genetic tests registered in cirrhotic patients. The mean age in this group corresponded to 21.6 years (2.15), with a predominance of males (6:4). Demographic, clinical and laboratory characteristics of patients with CF associated or not with cirrhosis are summarized in Table 1.

Figure 1 - ROC Curves for cirrhosis outcome in cystic fibrosis patients.

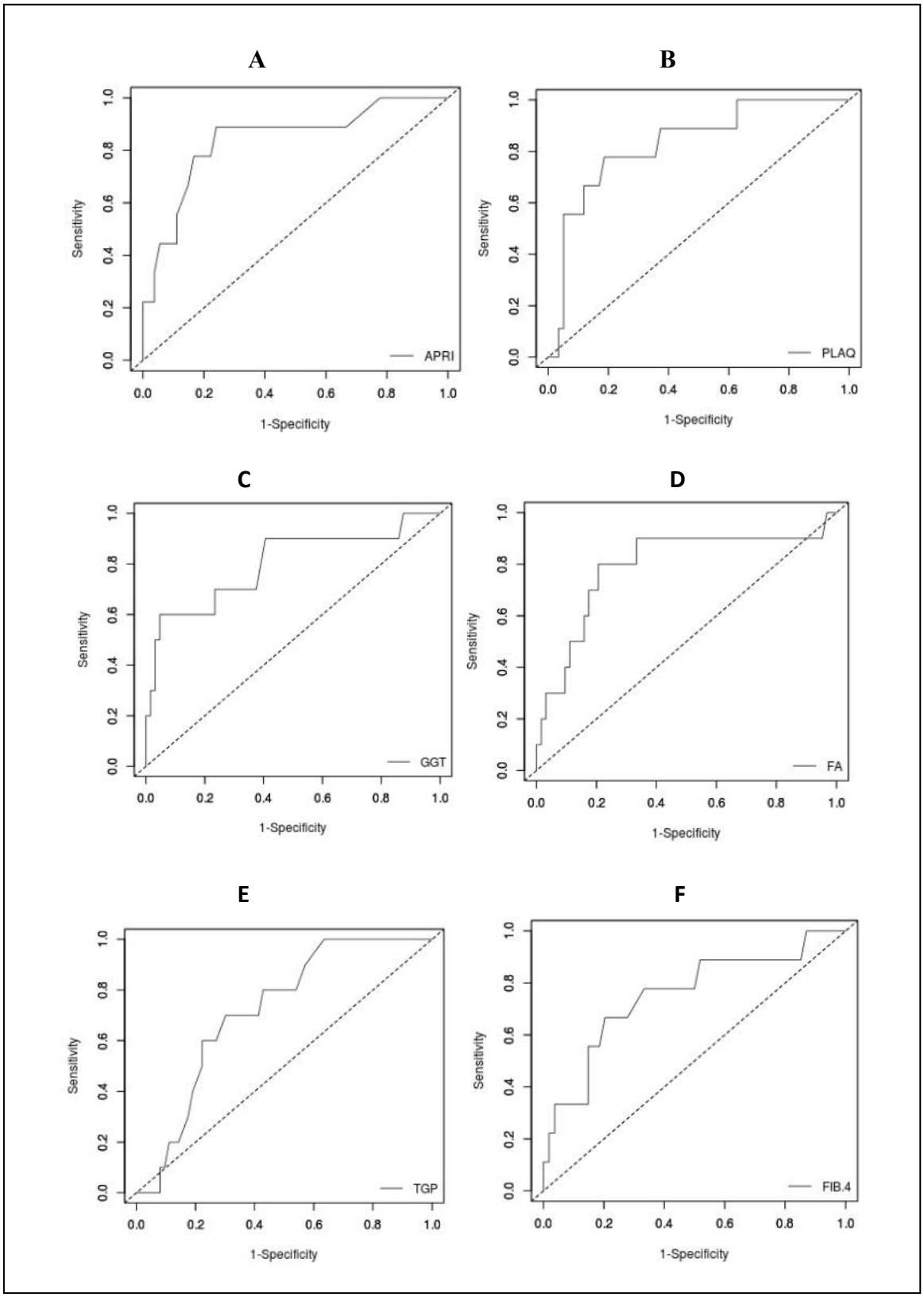

Figure 1: ROC Curves for cirrhosis outcome - (A) APRI, aspartate aminotransferase to platelet ratio index (AUC 0.840, $p < 0.01$); (B) PLT, platelets (AUC 0.830, $p < 0.001$); (C) GGT, gamma-glutamyl transferase (AUC 0.799, $p = 0.001$); (D) ALP, alkaline phosphatase (AUC 0.791, $p = 0.001$); (E) ALT, alanine aminotransferase (AUC 0.718, $p = 0.002$); (F) FIB-4, hepatic fibrosis index (AUC 0.753, $p = 0.010$).

Table 1 - Demographic, clinical, and laboratory characteristics according to the presence or absence of cirrhosis in cystic fibrosis.

	DHCF	CF	*P value
Demography			
Age	21.60 (+-2,15)	24.11(+-1.11	0,413
Time of diagnosis (years)	18.10 (+-0.58)	18.96(+- 0.69)	0.645
Sex (female)	4 (40%)	32 (45.7%)	1
Meconium Ileus	2 (20%)	6 (8.5%)	0.261
Ecocardiography			
High pulmonary artery pressure	1 (12,5%)	7 (17,5%)	1
Ultrassonography			
Hepatic alteration	9 (90%)	20 (28,5%)	< 0,001
Billiary tree alteration	3 (30%)	5 (7,4%)	0,063
Splenomegaly	5(50%)	3(4,4%)	0.004
Hepatomegaly	4(40%)	3(4,4%)	0.004
Ascitis	1(10%)	0	0.128
Steatosis	3 (30%)	12 (17.6%)	0.395
Elastography alteration	3 (75%)	0	0.024
Fibrosis	3 (33.3%)	1 (1.5%)	0.005
Pancreatic alteration	2 (20%)	9 (13.4%)	0.629
Endoscopic			
Esophageal varices	4(57.1%)	0	1
Clinical			
Nausea	4 (40%)	4 (5.7%)	0.007
Vomiting	3 (30%)	1 (1.4%)	0.005
Weight loss	4 (40%)	7 (10%)	0.027
Steatorrhea	0	3 (4.2%)	1
Ascitis	1 (10%)	0	0.125
Colateral circulation	1 (10%)	0	0.125
Splenomegaly	2 (20%)	2 (2.8%)	0.074
Laboratory			
AST (U/L)	30.40 (+- 2.68)	25.13 (+- 2.27)	0.370
ALT (U/L)	33.10 (+- 5.31)	24.11 (+- 2.04)	0.109
GGT (U/L)	124.60 (+- 46.96)	24.67 (+- 3.91)	< 0.001
AP (U/L)	285.50 (+- 63.75)	143.81 (+-11.49)	< 0.001
CB (mg/dL)	0.45 (+- 0.09)	0.61 (+- 0.31)	0.821
UB (mg/dL	0.46 (+- 0.14)	0.36 (+- 0.03)	0.362
Na (mmol/L)	133.33 (+- 1.49)	138.96 (0.72)	0.119
K (mmol/L)	4.68 (+- 0.28)	4.53 (+- 0.07)	0.481
Creatinin (mg/dL)	0.80 (0.05)	0.75 (+- 0.75)	0.435
Platelets (/mm³)	184222.22 (+- 34996.73)	320992.37 (+- 14629.15)	0.001
Score			
FIB-4	1.95 (+-1.02)	0.55 (+- 0.13)	0.010

Caption: * Fisher's exact test or Student's t-test; $p < 0.05$. Quantitative variables were expressed as mean ± standard deviation. Qualitative variables were expressed as frequency and percentage. APRI, aspartate aminotransferase to platelet ratio index; AP, pulmonary artery; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; CB, conjugated bilirubin; UB, unconjugated bilirubin; Na, sodium; K, potassium; FIB-4, hepatic fibrosis index.

Univariate analysis based on APRI greater than 0.27 revealed statistical significance for gender ($p = 0.019$), liver alteration on USG ($p = 0.031$), increased portal vein diameter on USG ($p <0.001$), splenomegaly on USG ($p = 0.018$), description of vomiting ($p = 0.040$), splenomegaly on physical examination ($p = 0.040$), SPT ($p = 0.011$), GST ($p = 0.006$), platelet count ($p < 0.001$) and FIB- 4 ($p <0.001$), as per Table 2.

Table 2 - Demographic, clinical, and laboratory characteristics according to APRI values > 0.27 and APRI values < 0.27.

	APRI > 0.27	APRI < 0.27	P value
Demography			
Age	25.68 (+- 2.92)	23.21 (+- 0.96)	0,413
Time of diagnosis (years)	19.63 (+-1.71)	18.61 (+- 0.60)	0.645
Sex (female)	4 (21%)	32 (52.4%)	1
Meconium Ileus	1 (5.2%)	7 (11.4%)	0.261
Ecocardiography			
High pulmonary artery pressure	3 (33.3%)	4 (11.1%)	0.094
Ultrassonography			
Hepatic alteration	11 (57.9%)	18 (29.5%)	0.031
Billiary tree alteration	2 (10.5%)	6 (10.3%)	1
Splenomegaly	5 (26.3%)	3 (5%)	< 0.001
Hepatomegaly	3 (15.7%)	4 (6.7%)	0.352
Ascitis	1 (5.2%)	0	0.244
Steatosis	3 (14.7%)	12 (20.3%)	1
Elastography alteration	2 (40%)	1 (16.6%)	0.545
Fibrosis	2 (11.1%)	2 (3.5%)	0.247
Pancreatic alteration	3 (15.7%)	8 (13.7%)	1
Endoscopic			
Esophageal varices	3 (60%)	1 (33.3%)	1
Clinical			
Nausea	4 (21%)	4 (6.5%)	0.086
Vomiting	3 (15.7%)	1 (1.6%)	0.040
Weight loss	4 (21%)	7 (11.4%)	0.281
Steatorrhea	1 (5.2%)	2 (3.2%)	0.562
Ascitis	1 (5.2%)	0	0.237
Colateral circulation	1 (5.2%)	0	0.237
Splenomegaly	3 (15.7%)	1 (1.6%)	0.040
Laboratory			
ALT (U/L)	34.37 (+- 4.96)	22.85 (+- 1.94)	0.011
AST (U/L)	34.16 (+- 3.07)	22.24 (+- 2.23)	0.006
GGT (U/L)	61.63 (+- 23.01)	30.07 (+- 7.14)	0.085
AP (U/L)	193.74 (+- 28.74)	152.48 (+- 16.17)	0.204
CB (mg/dL)	0.37 (+- 0.05)	0.67 (+- 0.36)	0.618
UB (mg/dL	0.49 (+- 0.09)	0.34 (+- 0.03)	0.081
Na (mmol/L)	138.58 (+- 1.46)	138.33 (+- 0.59)	0.859
K (mmol/L)	4.71 (+- 0.16)	4.46 (+- 0.07)	0.133
Creatinin (mg/dL)	0.81 (+- 0.04)	0.74 (+- 0.02)	0.149
Platelets (/mm^3)	199926.32 (+- 25496.05)	342815.31 (+- 14057)	< 0.001
Score			
FIB-4	1.80 (+- 0.58)	0.30 (+- 0.01)	< 0.001

Caption: * Fisher's exact test or Student's t-test; $p < 0.05$. Quantitative variables were expressed as mean ± standard deviation. Qualitative variables were expressed as frequency and percentage. APRI, aspartate aminotransferase to platelet ratio index; AP, pulmonary artery; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; CB, conjugated bilirubin; UB, unconjugated bilirubin; Na, sodium; K, potassium; FIB-4, hepatic fibrosis index.

Discussion

The potential impact of defining more accurate criteria for early detection of biliary cirrhosis on the life expectancy and quality of life of CF patients is evident, given the lack of a gold standard for the precise diagnosis of CFLD[2]. The implications of extrapulmonary involvement have gained more attention with the increased life expectancy of CF patients, which has risen from 16 to 50 years in the past five decades due to advances in multidisciplinary diagnostic and therapeutic approaches [3].

Liver biopsy, considered the gold standard for fibrosis staging and cirrhosis diagnosis, is a method

with limited applicability. Flass and Narcewicz (2013) point out risks and costs associated with the procedure, as well as limitations related to sampling and varying interpretations, highlighting the need for the development of reliable and validated tests for investigating CF liver disease[10].

The main hypothesis of this article is that the APRI score categorizes as the best predictor of hepatic cirrhosis in patients with cystic fibrosis. It has proven to be a good predictor of severe fibrosis and its complications in patients with CFLD (AUC 0.81; cutoff > 0.264), compared to histopathological data [4]. The APRI score was the most accurate parameter for predisposition to cirrhosis in the current case-control study, as it exhibited a larger area under the curve (AUC 0.84). The cutoff value for maximum sensitivity and specificity of the score was 0.27, and these data are consistent with several other studies, such as that of Kitson et al., which compared the APRI score with similar clinical criteria and obtained similar results (AUC 0.77), proposing APRI as a precise score to identify CFLD[5]. The significant sensitivity (88.9%) and specificity (75.9%) data from our study suggest that APRI is a good predictor for the outcome of biliary cirrhosis in patients with cystic fibrosis.

In the population with significant outcomes for cirrhosis regarding the consequences of hepatic fibrosis, there were also significant alterations in the ultrasonographic examination of hepatic changes and portal vein diameter, predominantly reported in the group with APRI above the cutoff value (57.9%, p = 0.031). However, this parameter has limited correlation power as no further specifications regarding the type of changes visualized in the image were found. This finding corroborates with investigations described in the literature that used the same parameter to diagnose portal hypertension in CF patients, in which detailed descriptions, such as parenchymal thickening, nodularity at the edge, or periportal hyperechogenicity, would provide greater assistance in detecting cirrhosis at early stages. Another ultrasound parameter with significance (p <0.001) was the increased diameter of the portal vein, present only in the population with a significant outcome for cirrhosis (36.8%). This result aligns with findings described in the literature [5,6].

Previous studies have cited the higher accuracy of the APRI score for defining portal hypertension [4,5]. Splenomegaly in the APRI > 0.27 group, observed in both imaging analysis (p = 0.018) and physical examination descriptions (p = 0.040), with percentages of 26.3% and 15.7%, respectively, can also serve as a secondary finding indicating evolving cirrhotic disease.

Male sex is a factor associated with a higher risk of hepatic involvement in CF patients in previous studies, as described in national literature [7,8]. This association was confirmed in the analysis of the APRI > 0.27 group, which had a predominantly male composition (79%, p = 0.019). Vomiting, TGO, TGP, and platelet count are nonspecific findings that could be attributed to both cirrhosis and other clinical variables of cystic fibrosis, showing statistical differences in comparative analysis with the control group.

In addition to APRI, another evaluated score was the FIB-4, calculated based on the patients' age, TGO and TGP values, and platelet count. Except for age, the other three parameters showed statistical significance, and as a result, FIB-4 also did (p < 0.001). In a pediatric cohort, FIB-4 was found to be significantly superior to APRI in predicting portal hypertension, although it had a lower AUC (0.70) compared to APRI (AUC 0.81) for predicting cirrhosis, which was not statistically significant [5]. The finding of FIB-4 as an important predictor of portal hypertension can be justified by considering the time factor in the development of this complication.

When conducting retrospective epidemiological research, starting from data collection and final analysis, in a pathology such as CF liver disease (DHFC) without standardized diagnostic criteria or associated marker research linked to clinical data, the analysis becomes more vulnerable to measurement errors and frequency variation. The observed percentage in this study of cirrhosis (12.5%) and esophageal varices (5%) in CF patients is in line with current literature (5-10%, as per Amaral et al., 2007). However, the cross-sectional observation and the fact that no patient underwent liver biopsy may have underestimated this percentage[11].

Despite the limitations, the obtained results were significant, as they demonstrate reliable sensitivity and specificity for the correlation between clinical, biochemical, and ultrasound criteria when the outcome is cirrhosis. However, there is still no confirmation of effective methods for early detection of individuals at risk of developing cirrhosis, an area where future studies should focus.

Conclusion

This study has some noteworthy limitations, such as a small sample size and limitations in the complete evaluation of medical records with ultrasound descriptions. Additionally, the study's observational nature prevents drawing conclusions about the causality of the comparisons. Furthermore, the absence of histopathological evaluation of liver

cirrhosis, with the diagnosis relying solely on clinical variables, is another limitation.

In summary, this study suggests the hypothesis that the APRI score is the most sensitive and specific clinical and laboratory score for defining cirrhosis in patients with cystic fibrosis, a restricted population with limited tools for such assessment. It is recommended that future studies focus on the prospective diagnostic evaluation of APRI as a predictor of early liver damage.

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