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Role of serum Nogo-B as a biomarker for diagnosis of chronic liver diseases and its severity

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Abstract

Background: Nogo-B is one of the members of the reticulon family. Nogo-B influences the proliferation of the hepatic stellate cells inducing liver fibrotic changes. We aimed at measuring the serum levels of Nogo-B in patients with chronic liver disease (CLD) with different etiologies. Ninety subjects were included, 18 of them were normal healthy individuals and 72 had liver disease (fibrosis/cirrhosis) with different etiologies: post-hepatitis C infection, post-hepatitis B infection, NASH, and autoimmune hepatitis. Serum Nogo-B was assessed using ELISA. Patients were subdivided according to the Child-Pugh score into 3 groups: group 1—Child A (24 patients); group 2—Child B (24 patients); and group 3—Child C (24 patients).

Results: Serum Nogo-B levels were found to be significantly higher in patients (1477.92 ± 1113.50) when compared with healthy control (301.28 ± 180.87) ($p < 0.001$). There was a statistically significant difference in serum Nogo-B level between the three sub-groups of patients ($p < 0.001$). A positive correlation was found between serum Nogo-B and MELD score ($r = 0.46$, p-value < 0.001). However, there was no correlation found between Nogo-B and FIB-4 index or APRI score. There was a significant positive correlation between serum Nogo-B level and coagulation profile and serum bilirubin. An inverse correlation was found between serum Nogo-B with serum albumin. A ROC curve was done to examine the validity of Nogo-B in the diagnosis of liver cirrhosis, and the area under the curve was found to be 0.979, a cutoff value of 600 with a sensitivity of 97.2% and a specificity of 94.4% (p-value < 0.001).

Conclusion: Nogo-B had a high value in the identification of patients with any severity of CLD. There is a highly significant correlation between Nogo-B and the synthetic function of the liver; it could be used as a measure of hepatic functional reserve.

Keywords: Nogo-B, Chronic liver disease, Biomarker

Background

Liver cirrhosis is a major health problem in Egypt and worldwide. Liver diseases are on the top of the etiologies of mortality in Egypt, and it is predicted that there will be an upsurge in liver cirrhosis and hepatocellular carcinoma in the future [1]. The liver has an exceptional capacity to regenerate through various signaling pathways [2]. The regeneration process involves mechanisms which are still obscure and not fully understood.

Nogo-B is one of the members of the reticulon family. The reticulon family of proteins consists of 4 types of proteins which are primarily localized in the endoplasmic reticulum [3]. These proteins are highly expressed in the central nervous system and skeletal muscles [4]. They regulate the ER functions and structure and have an influence on protein trafficking and cell signaling

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regulation [5, 6]. There are four reticulon genes and each gene encodes multiple isoforms. There are 3 gene products for reticulon 4 which are isoform A, B, and C [7]. Reticulon-4B or Nogo-B (Rtn-4B), 55 kDa protein, is found in many tissues, and one of its most important functions is the regulation of vascular remodeling and wound healing [8].

Nogo-B has a role in pathological vascular conditions through its ability to inhibit the migration and proliferation of smooth muscle cells while it enhances the endothelial cell migration [4]. Nogo-B influences proliferation and regeneration of the hepatic stellate cells [9] and induces liver fibrotic changes through stimulation of transforming growth factor β and its TGF β/Smad2 signaling pathway in fibroblasts [10].

Some studies revealed that Nogo-B levels were elevated in liver cirrhosis when compared to healthy controls [11]. Other studies examined the relationship between the levels of Nogo-B and the different clinical characteristics of liver cirrhosis, but the results were still controversial. The expression of Nogo-B in non-parenchymal liver cells was examined, and the levels of tissue Nogo-B were found to be elevated in the patients with liver cirrhosis and also correlated significantly with Child-Pugh scores [12].

In this study, the levels of serum Nogo-B in patients with hepatic dysfunction due to different etiologies were assessed.

Methods

This study was conducted in the Internal Medicine Department, Kasr El-Aini Hospital. A written informed consent was obtained from each participant or a responsible family member after explaining the possible complications of the diagnostic procedures. This study was submitted to and approved by the ethics committee of Kasr Alainy Hospital, Faculty of Medicine, Cairo University, with approval reference number [I-010316].

This observational case-control study included 90 subjects, 18 subjects were normal healthy individuals and 72 patients with liver disease (fibrosis/cirrhosis) due to the different etiologies such as post-hepatitis C infection, post-hepatitis B infection, NASH, and autoimmune hepatitis.

Patients were subjected to full clinical history and examination. They were classified into 4 groups: control group—composed of 18 normal healthy persons; group I—24 patients with chronic liver disease, Child A; group II—24 patients with chronic liver disease, Child B; and group III—24 patients with chronic liver disease, Child C.

Venous blood samples were taken for the analysis of the serum level of Nogo-B (enzyme-linked immunosorbent assay, ELISA, Human Nogo-B ELISA Kit, supplied by Chongqing Biospes Co., Ltd. (7F, Bldg B, High-tech Venture Park, Jiulongpo District, Chongqing, China), according to the manufacturer's instructions), anti-bilharzial antibodies in the serum (Schistosoma mansoni IgG Human ELISA Kit, GenWay Biotech, Inc. 6777 Nancy Ridge Drive, San Diego, CA 9212, intended for the qualitative determination of IgG class antibodies against Schistosoma mansoni in human serum), HCV antibodies (ELISA, Murex anti-HCV (version 4.0) is an enzyme immunoassay for the detection of antibodies to hepatitis C virus (HCV) in human serum, Murex Biotech S.A. (Pty) Ltd., Kyalami Boulevard, Kyalami Business Park, Kyalami, Republic of South Africa), and serum ferritin (Human Ferritin Enzyme Immunoassay Test Kit, supplied by BIOCHECK, INC.(323 Vintage Park Dr, Foster City, CA 94404, USA, according to the manufacturer's instructions).

Liver function tests and liver enzymes were also done: serum albumin, prothrombin time, concentration, INR, serum bilirubin, ALT, and AST, and renal function tests: serum urea, creatinine, and complete blood count.

Abdominal ultrasound and upper gastrointestinal endoscopy were done to all patients. Patients with other etiologies of liver affection rather than hepatitis C (HBV, AIH, NASH) were included in the study according to their past documented investigations.

The exclusion criteria included the following: patients have diseases which might affect plasma Nogo-B levels as cardiovascular disease (including hypertension), central nervous system disorders, chronic obstructive pulmonary diseases (COPD), and pulmonary artery hypertension.

Samples were withdrawn, left to clot at room temperature for 10 min, and then centrifuged at the speed of 2000–3000 rpm for 5 min, and the supernatant was collected and stored at − 20 °C till the time of analysis.

MELD score was calculated as 3.78[Ln serum bilirubin (mg/dL)] + 11.2[Ln INR] + 9.57[Ln serum creatinine (mg/dL)] + 6.43. MELD-Na = MELD + 1.59 [135 − Na]; APRI score: [(AST/upper limit of normal)/platelet count (109/L)] × 100; and FIB -4 index: (age [years] × AST [IU/L])/(platelet count [109/L] × (ALT [IU/L]) 1/2).

Statistical methods

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data, and frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests [13]. For comparing categorical data, the chi-square (χ^2) test was performed. The exact test was used instead

when the expected frequency is less than 5. Correlations between quantitative variables were done using the Spearman correlation coefficient. ROC curve was constructed with the area under curve analysis performed to detect the best cutoff value of Nogo-B, MELD, APRI, and FIB-4 for the detection of patients [14].

Results

Ninety participants were recruited in this study. They were divided into patients' group (72 patients with liver diseases of different etiologies) and control group (18 normal healthy individuals, age- and sex-matched). The mean age of the patients was 53.72 ± 12.46 years, 47 males (65.3%) and 25 females (34.7%), while the mean age of the control subjects was 36.00 ± 9.94 years and divided into 9 males (50%) and 9 females (50%).

Patients were further classified according to the Child-Pugh score into 3 groups: group 1—Child A (24 patients); group 2—Child B (24 patients); and group 3—Child C (24 patients). As for the liver disease etiologies, 54 patients had HCV infection (75%), 6 patients had HBV infection (8.3%), 3 patients had autoimmune hepatitis (4.1%), and 9 patients had non-alcoholic steatohepatitis (12.5%). Clinical and laboratory data of the patients and the healthy control groups are listed in Table 1.

Serum Nogo-B levels were found to be significantly higher in patients (1477.92 ± 1113.50) when compared with healthy control (301.28 ± 180.87) ($p < 0.001$) as shown in Fig. 1.

There was a statistically significant difference in the serum Nogo-B level between the three sub-groups of patients ($p < 0.001$). A high significant difference was found between the three sub-groups of patients as regards FIB-4 score ($p < 0.001$), APRI score ($p < 0.05$), and MELD score ($p < 0.001$) (Table 2).

Serum levels of Nogo-B were significantly higher in patients with Child C (2006.67 ± 1286.32) when compared with other sub-groups with Child A (787.71 ± 245.88) or Child B (1639.37 ± 1138.58) (p-value < 0.001) (Fig. 2).

It was found that there was a positive correlation between serum Nogo-B and MELD score ($r = 0.46$, p-value < 0.001). However, there was no correlation found between Nogo-B and FIB-4 index or APRI score. There was a significant positive correlation between serum Nogo-B level and coagulation profile and serum bilirubin. An inverse correlation was found between serum Nogo-B and serum albumin (Table 3).

Table 1 Comparison between the three groups of patients and the control group as regards laboratory data using the Kruskal-Wallis test

Variables		Group I ($n = 24$)	Group II ($n = 24$)	Group III ($n = 24$)	Control ($n = 18$)	P^1	P^2
Hematemesis and melena		21	15	6			
Bleeding tendency		2	4	7			
Hepatic encephalopathy		0	2	8			
Jaundice		0	5	17			
Ultrasound findings	Hepatomegaly	20	21	6			
	Liver cirrhosis	19	24	24			
	Splenomegaly	20	23	23			
	Ascites	3	21	24			
Varices (upper GI endoscopy)		18	24	24			
HB		8.36 ± 2.26	8.09 ± 1.48	9.04 ± 2.17	12.49 ± 1.98	***< 0.001***	0.338
PLT		122.75 ± 89.33	113.00 ± 70.54	103.75 ± 62.60	302.00 ± 63.24	***< 0.001***	0.850
Urea		45.46 ± 28.14	57.67 ± 30.67	56.54 ± 24.23	30.44 ± 5.76	***< 0.001***	0.159
Creatinine		0.91 ± 0.32	1.05 ± 0.25	1.26 ± 0.41	1.02 ± 0.17	0.800	***0.004***
Na		138.04 ± 3.09	136.46 ± 3.66	134.96 ± 5.86	139.83 ± 3.54	***0.004***	***0.045***
ALT		30.25 ± 35.15	32.54 ± 17.18	60.87 ± 58.49	23.06 ± 7.30	***0.048***	***< 0.001***
AST		41.12 ± 31.01	51.88 ± 28.04	100.63 ± 93.31	24.11 ± 7.31	***< 0.001***	***< 0.001***
Alb		3.28 ± 0.46	2.75 ± 0.30	2.21 ± 0.43	4.93 ± 0.57	***< 0.001***	***< 0.001***
Bil		.89 ± 0.43	1.43 ± 0.66	3.37 ± 2.21	0.96 ± 0.17	***0.026***	***< 0.001***
PC		69.92 ± 10.34	59.12 ± 11.98	47.67 ± 14.47	91.28 ± 7.34	***< 0.001***	***< 0.001***
Ferritin		61.52 ± 129.04	45.06 ± 60.87	44.48 ± 54.71	25.80 ± 19.45	0.525	0.882
Positive anti-bilharzial ab		12	17	11			

HB hemoglobin, *TLC* total leukocytic count, *PLT* platelets, *Na* sodium, *K* potassium, *ALT* alanine transaminase, *AST* aspartate transaminase, *Alb* albumin, *Bil* bilirubin, *PC* prothrombin concentration, *PT* prothrombin time, P^1 between all patients and controls, P^2 between the different groups of patients

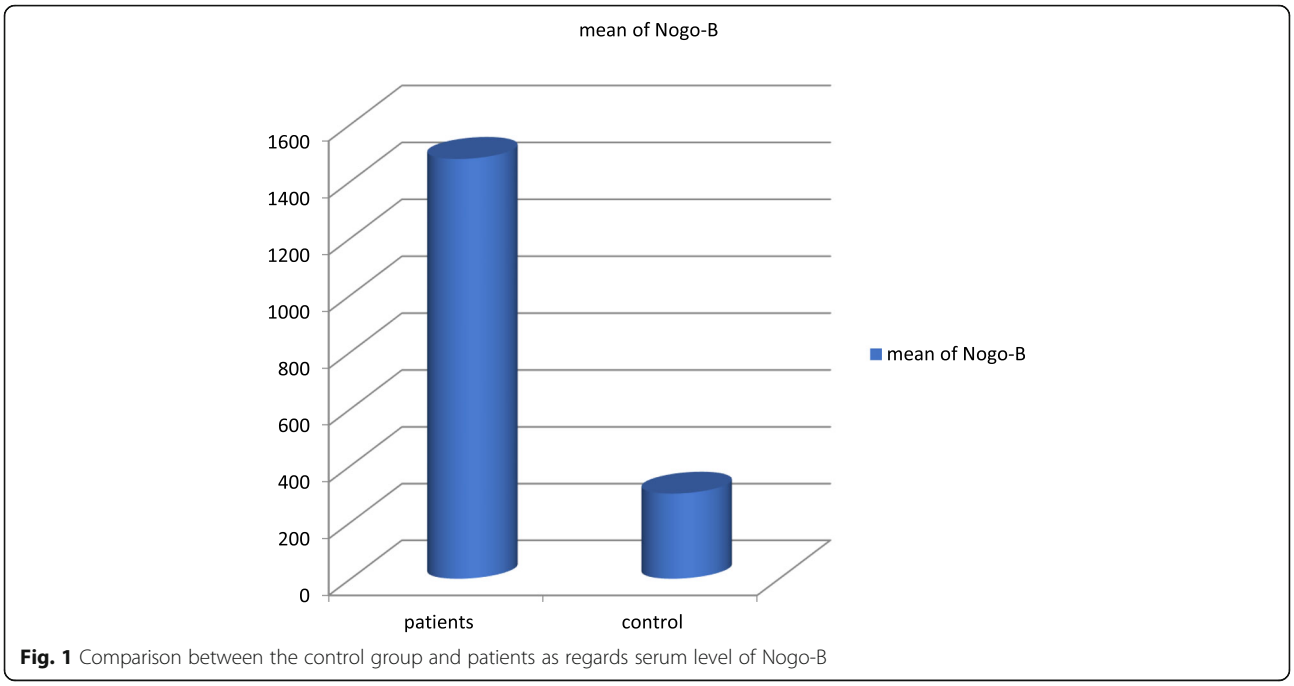

Fig. 1 Comparison between the control group and patients as regards serum level of Nogo-B

A ROC curve was done to examine the validity of Nogo-B in the diagnosis of liver cirrhosis, and the area under the curve was found to be 0.979, a cutoff value of 600 with a sensitivity of 97.2% and a specificity of 94.4% (p-value < 0.001; Table 4 and Fig. 3). It has also a sensitivity of 100% in the detection of Child A and B patients (AUC 0.954 and 0.984 at a cutoff value of 540 and 600, respectively); it decreased to 95.8% in Child C patients (AUC 1.000 at a cutoff value of 927.5) with a specificity of 88.9%, 94.4%, and 100%, respectively. Nogo-B has a very high significance in the differentiation between Child A and B groups and Child A and C groups (p-value < 0.001).

Discussion

Liver cirrhosis is a major cause of mortality around the world; the development of cirrhosis has been considered to be an irreversible event [15].

The prognosis of patients with liver cirrhosis often depends on their hepatic functional reserve. Clinical classification systems, such as MELD and Child-Pugh scoring systems, are widely used. Radiographic examination of the remnant liver volumes is also helpful [16].

In this study, we evaluated the relationship between serum Nogo-B and chronic liver disease in Egyptian patients. This study revealed that the serum Nogo-B level was very high in patients with liver diseases compared to the control group. The serum level of Nogo-B was significantly higher in patients with Child B and Child C compared with those patients with Child A.

Our findings signified the high sensitivity of serum Nogo-B as a biomarker that can be used for the evaluation of patients with chronic liver disease. Using the ROC curve for predicting significant fibrosis and cirrhosis, the sensitivity of Nogo-B was high reaching up to 97.2% and with a specificity of 94.4%.

Table 2 Comparison between the three groups of patients as regards serum Nogo-B, MELD, APRI, and FIB-4 scores

Variables	Group I	Group II	Group III	p-value
Nogo-B, mean+SD	787.71 ± 245.88	1639.37 ± 1138.58	2006.67 ± 1286.32	***< 0.001***
APRI, mean+SD	1.46 ± 1.48	1.56 ± 1.08	4.16 ± 8.19	***0.005***
FIB-4, mean+SD	4.86 ± 3.78	5.63 ± 3.19	9.48 ± 10.50	***0.035***
MELD, mean+SD	10.29 ± 2.44	13.83 ± 3.28	20.29 ± 5.04	***< 0.001***

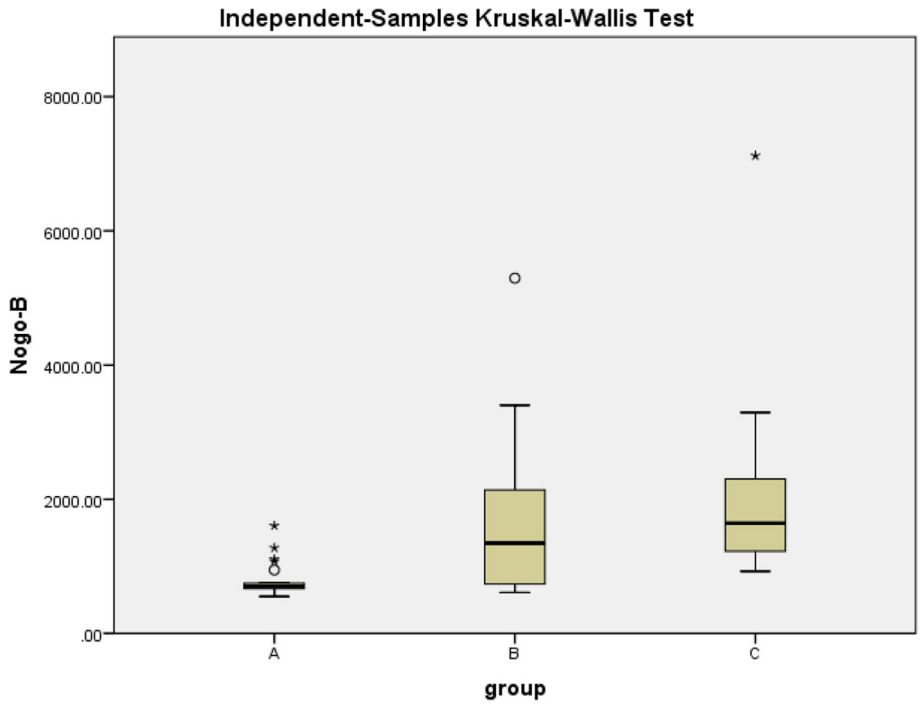

Fig. 2 Comparison between the three groups of patients as regards serum level of Nogo-B levels

Nogo-B was evaluated in many studies in plasma and liver tissues of patients with liver cirrhosis, and these studies showed that plasma Nogo-B levels were significantly higher in patients with liver cirrhosis than in healthy controls and were the highest in Child-Pugh class C patients. Plasma Nogo-B levels were positively correlated with Child-Pugh classes. There was no relationship between plasma Nogo-B levels and the etiology of liver diseases [12].

In 2017, Jin-Kyu et al. proved that Nogo-B is permissive of M1 (classically activated macrophages) polarization of Kupffer cells and accentuating liver injury in acute liver disease (ALD). They found a significant positive correlation between Nogo-B-positive Kupffer cells and disease severity in ALD patients [17].

Table 3 Correlation between serum NOGO-B, MELD, APRI, and FIB-4 scores and other laboratory parameters in the three groups of patients

	Correlation coefficient (*r*)	*p*-value
APRI (%)	0.167	*0.161*
FIB-4	0.190	*0.110*
MELD	0.460	***< 0.001***
ALT	0.195	*0.102*
AST	0.146	*0.221*
Alb	− 0.424	***< 0.001***
Bil	0.372	***0.001***
PC	− 0.490	***< 0.001***
Ferritin	*0.026*	0.826

The expression of Nogo-B in hepatocytes was firstly studied in a prior study in 2011 in rats and humans by Dahai et al. who stated that Nogo-B was highly expressed in non-parenchymal cells and minimally in hepatocytes in human and rat liver; Nogo-B protein levels were found to be significantly elevated in fibrotic/cirrhotic liver. They found also that Nogo-B gene deletion reduced hepatic fibrosis and may block the development of portal hypertension [9].

We found a strong positive correlation between Nogo-B and MELD in the patient groups (p-value < 0.001) denoting the ability of Nogo-B to predict mortality in a cirrhotic patient with the same sensitivity of MELD score.

There was also a highly significant correlation between Nogo-B and synthetic function of the liver; serum albumin, coagulation profile with p-value <0.001, significantly correlated with serum total bilirubin with p-value < 0.05. This suggested that plasma Nogo-B might be a good surrogate marker for the evaluation of liver functional reserve in patients with liver fibrosis/cirrhosis.

No correlation was found regarding some other parameters like platelet count, ALT, AST, and ferritin. As Nogo-B is not produced by hepatocytes, this miscorrelation between plasma Nogo-B and ALT and AST was

Table 4 Validity of serum Nogo-B, APRI, and FIB-4 in detecting hepatic patients

Variables	AUC	*p*-value	Cutoff value	Sensitivity (%)	Specificity (%)
Nogo-B	0.979	***< 0.001***	600	97.2	94.4
APRI (%)	0.954	***< 0.001***	0.5350	87.5	100
FIB-4	0.983	***< 0.001***	1.587	93.1	100

expected, suggesting that serum Nogo-B level does not reflect the inflammatory status of the liver. Nogo-B and platelet miscorrelation negates the ability of Nogo-B to detect portal hypertension. These findings were also proved by Maoyao et al. [12].

Maoyao et al. found no correlation between Nogo-B and the presence of varices in contrast to our study as most of our patients had varices. This could be related to the high prevalence of bilharziasis in Egypt which led to the early development of vascular decompensation and portal hypertension without evident cellular decompensation or in Child A group. Repeated attacks of hematemesis with blood transfusion and the use of iron supplements may explain the miscorrelation between Nogo-B and hemoglobin and ferritin [12].

Thus, the serum Nogo-B may be considered a good simple noninvasive test for evaluating the degree of liver fibrosis and hepatic functional reserve after exclusion of other conditions affecting its level. Serum Nogo-B level was positively correlated with the synthetic function of the liver (albumin, coagulation profile, and serum bilirubin) in contrast to the FIB-4 index and APRI score. APRI score and FIB-4 index can reflect the degree of inflammation of hepatocytes taking into consideration decreased or normal AST and ALT levels in advanced cirrhosis.

Conclusion

Nogo-B has a high value in the identification of patients with any degree of liver fibrosis with a high positive correlation with different child classes. Nogo-B had the same significance of MELD score in the prediction of mortality in cirrhotic patients. There is a highly significant correlation between Nogo-B and the synthetic function of the liver; it could be used as a measure of hepatic functional reserve. Serum Nogo-B level does not reflect inflammation of the liver or portal hypertension.

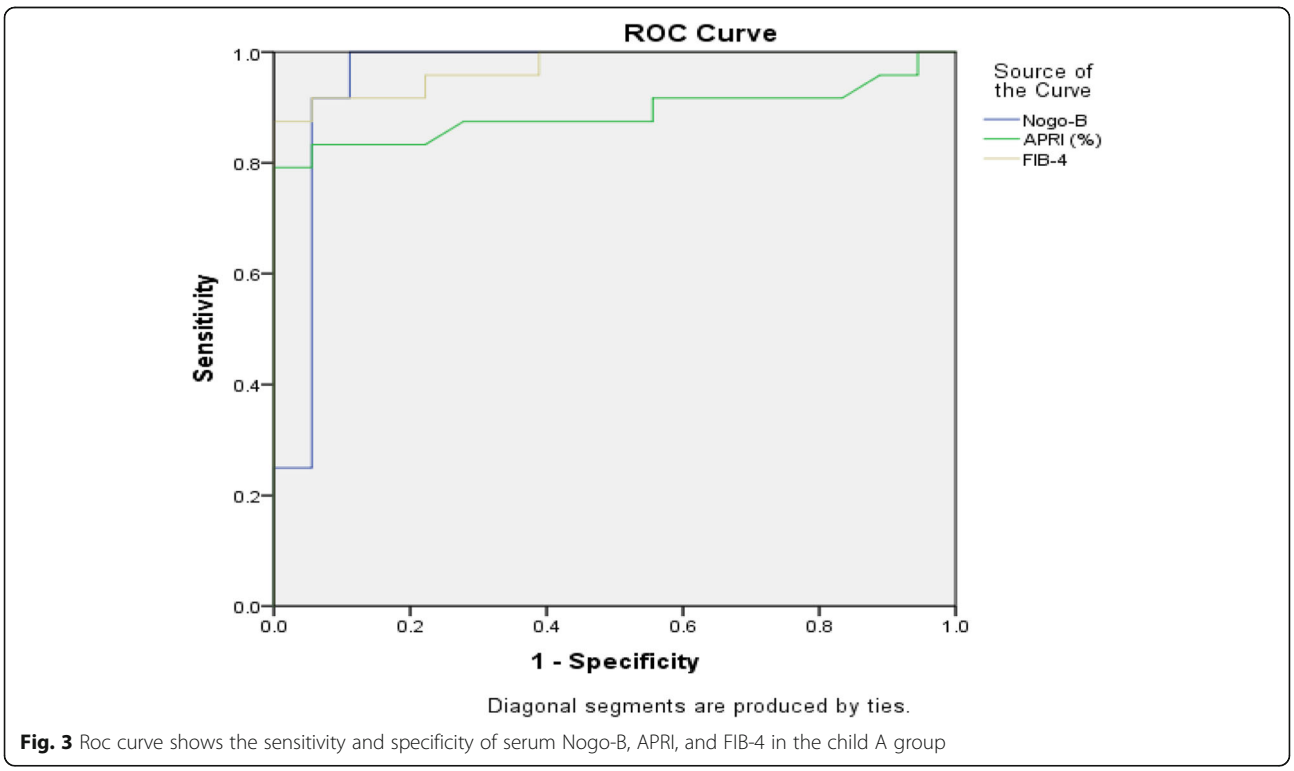

Fig. 3 Roc curve shows the sensitivity and specificity of serum Nogo-B, APRI, and FIB-4 in the child A group

Abbreviations
CLD: Chronic liver disease; ELISA: Enzyme-linked immunosorbent assay; COPD: Chronic obstructive pulmonary diseases; AIH: Autoimmune hepatitis; NASH: Non-alcoholic steatohepatitis; INR: International normalization ratio; ALT: Alanine transaminase; AST: Aspartate transaminase; MELD: Model for end-stage liver disease; APRI: AST to platelet ratio index; FIB-4score: Fibrosis 4 score; ALD: Acute liver disease; ROC: Receiver operator curve; AUC: Area under curve

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Authors' contributions
AF: study selection, data extraction, bias assessment, revision, and supervision. WA: literature search and study selection. AE: data collection, sampling, and writing. HMH: laboratory work, literature search, study selection, and critical appraisal. ARL: literature search, writing, and critical appraisal. KMA: writing, literature search, and data review. All authors read and approved the final manuscript for publication.

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Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
This study was submitted to and approved by the ethics committee of Kasr Alainy Hospital, Faculty of Medicine, Cairo University, with approval reference number [I-010316]. A written informed consent was obtained from each participant or a responsible family member after explaining the possible complications of the diagnostic procedures.

Consent for publication
All patients were anonymous, and a written informed consent was obtained for publication.

Competing interests
The authors declare that they have no competing interests.

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References
1. Deuffic-Burban S, Mohamed MK, Larouze B, Carrat F, Valleron A-J (2006) Expected increase in hepatitis C-related mortality in Egypt due to pre-2000 infections. J Hepatol. 44(3):455–461. https://doi.org/10.1016/j.jhep.2005.08.008
2. Michalopoulos GK (2010) Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. Am J Pathol. 176(1):2–13. https://doi.org/10.2353/ajpath.2010.090675
3. Teng FYH, Tang BL (2008) Cell autonomous function of Nogo and reticulons: the emerging story at the endoplasmic reticulum. *J Cell Physiol*. 216(2):303–308. https://doi.org/10.1002/jcp.21434
4. Oertle T, Schwab ME (2003) Nogo and its paRTNers. Trends Cell Biol. 13(4):187–194. https://doi.org/10.1016/s0962-8924(03)00035-7
5. Yang YS, Strittmatter SM (2007) The reticulons: a family of proteins with diverse functions. Genome Biol. 8(12):234. https://doi.org/10.1186/gb-2007-8-12-234
6. Wakana Y, Koyama S, Nakajima K et al (2005) Reticulon 3 is involved in membrane trafficking between the endoplasmic reticulum and Golgi. Biochem Biophys Res Commun. 334(4):1198–1205. https://doi.org/10.1016/j.bbrc.2005.07.012
7. Huber AB, Weinmann O, Brösamle C, Oertle T, Schwab ME (2002) Patterns of Nogo mRNA and protein expression in the developing and adult rat and after CNS lesions. J Neurosci 22(9):3553–3567
8. Yu J, Fernández-Hernando C, Suarez Y et al (2009) Reticulon 4B (Nogo-B) is necessary for macrophage infiltration and tissue repair. Proc Natl Acad Sci U S A. 106(41):17511–17516. https://doi.org/10.1073/pnas.0907359106
9. Zhang D, Utsumi T, Huang H-C et al (2011) Reticulon 4B (Nogo-B) is a novel regulator of hepatic fibrosis. Hepatology. 53(4):1306–1315. https://doi.org/10.1002/hep.24200
10. Gao L, Utsumi T, Tashiro K et al (2013) Reticulon 4B (Nogo-B) facilitates hepatocyte proliferation and liver regeneration in mice. Hepatology. 57(5):1992–2003. https://doi.org/10.1002/hep.26235
11. Men R, Wen M, Dan X et al (2015) Nogo-B: a potential indicator for hepatic cirrhosis and regulator in hepatic stellate cell activation. Hepatol Res. 45(1):113–122. https://doi.org/10.1111/hepr.12324
12. Wen M, Men R, Yang Z et al (2015) The value of circulating Nogo-B for evaluating hepatic functional reserve in patients with cirrhosis. Dis Markers. 2015:419124. https://doi.org/10.1155/2015/419124
13. Chan YH (2003) Biostatistics 103: qualitative data-tests of independence. Singapore Med J. 44(10):498–503
14. Chan YH (2003) Biostatistics 102: quantitative data–parametric & non-parametric tests. Blood Press 140(24.08):0–79
15. Chan ESL, Montesinos MC, Fernandez P et al (2006) Adenosine A(2A) receptors play a role in the pathogenesis of hepatic cirrhosis. Br J Pharmacol. 148(8):1144–1155. https://doi.org/10.1038/sj.bjp.0706812
16. Ge P-L, Du S-D, Mao Y-L (2014) Advances in preoperative assessment of liver function. Hepatobiliary Pancreat Dis Int. 13(4):361–370. https://doi.org/10.1016/s1499-3872(14)60267-8
17. Park J-K, Shao M, Kim MY et al (2017) An endoplasmic reticulum protein, Nogo-B, facilitates alcoholic liver disease through regulation of Kupffer cell polarization. Hepatology. 65(5):1720–1734. https://doi.org/10.1002/hep.29051

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