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The Role of Procollagen III N-Terminal Peptide/Platelets ratio inLiver Fibrosis Staging in Chronic Hepatitis C

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Abstract

Background: This work focuses on measuring PIIINP levels in various hepatic fibrosis stages, examining the extent to which it may influence the rate at which liver fibrosis progresses, and estimating its diagnostic performance as a direct and complementary fibrosis marker. **Methods:** This research involved 291 individuals with clinically and laboratory proven chronic hepatitis C. Liver-fibrosis was determined by FibroScan. **Results:** PIIINP level was directly proportional to liver fibrosis progression yielding a Spearman's rank correlation coefficient of 0.51 (*P* <0.0001). The mean value of PIIINP concentration was estimated to be 39.45 and 49.67 (ng/mL) in patients with F2-F4 and F4 while it was 15.60 and 19.77 (ng/mL) in case of patients with F0-F1 and F0-F3, respectively. Findings showed patients with F2-F4 and F4 displayed 2.56-fold and 2.65-fold increase in PIIINP over those with F0-F1 and F0-F3, respectively. To amplify difference in PIIINP among patients with different fibrosis-stages, PIIINP/platelets ratio was devised. Our results showed that PIIINP/platelets ratio enabled correct identification of F2-F4 and F4 showing AUCs 0.86 and 0.85, and displaying 3.72 and 3.56-fold increase over those with F0-F1 and F0-F3, respectively. **Conclusion:** PIIINP/platelets ratio is valuable liver-fibrosis marker that could improve liver fibrosis-staging and monitor disease-progression.

Keywords: Cirrhosis, Extracellular matrix, Fibrosis, marker, PIIINP, Platelets.

Introduction

Despite the fact that liver biopsy is still the gold standard for diagnosing and staging liver fibrosis, its use has considerably decreased due to a number of drawbacks, including the invasiveness of the operation, sampling errors, and inter- and intraobserver variability (Thanapirom et al., 2022). For these reasons, the role of liver biopsy has now been overtaken by the development of non-invasive tests. These methods, which potentially get beyond liver biopsy's drawbacks, are now increasingly frequently employed in standard clinical practice. However, noninvasive diagnostic procedures now used in clinical practice are not sensitive or specific enough to identify occult liver fibrosis in its earliest stages (Zhao et al., 2020). It is noteworthy that an extensive buildup of extracellular-matrix is a hallmark of hepatic fibrosis. The extracellular matrix's normal remodelling process changes during fibrogenesis, leading to an abnormal accumulation of its constituent parts, primarily collagens (Ortiz et al., 2021). Generally, hepatic stellate cells (HSCs) produce procollagen, which is then enzymatically, separated into its C- and N-terminal ends by procollagen C-proteinase and procollagen N-proteinase to become collagen (Jarčuška et al., 2010). Only five of the several collagen subtypes that have been identified have been found in liver tissue: types I, III, IV, V, and VI (Alcolado et al., 1997). In fact, an amino-terminal propeptide (PIIINP) is released into the environment and degraded from type III collagen during its synthesis and deposition. It has been demonstrated that elevated PIIINP in circulation is indicative of continuous fibrotic processes. Stated differently, because type III procollagen peptide is partially isolated from its procollagen precursor, it may serve as a serum indicator of collagen turnover (Safdar et al., 2014). Therefore, our research focuses on measuring PIIINP levels in various hepatic fibrosis stages, examining the extent to which it may influence the rate at which liver fibrosis
progresses, and estimating its diagnostic performance as a direct and complementary fibrosis marker.

Methods

This cross-sectional study involved 291 consecutive Egyptian individuals with chronic hepatitis C. Blood samples were obtained from the department of tropical medicine at the hospitals affiliated with Mansoura University in Egypt. The study protocol complied with the 1975 Helsinki Declaration's ethical principles. The staging of liver fibrosis was determined by FibroScan (Sandrin et al., 2003) (Echosens, Paris, France). Regarding the distribution of fibrosis groups, patients were classified into five groups as the following: a total of 31 (10.65%) individuals showed no fibrosis (F0); 14 males and 17 females with a mean (±SD) age of 45.76 (±11.93) years. A total of 45 (15.46%) individuals showed minimal liver fibrosis (F1); 23 males and 22 females with a mean (±SD) age of 48.85 (±10.31) years. A total of 14 (4.81%) individuals showed moderate liver fibrosis (F2); 7 males and 7 females with a mean (±SD) age of 50.79 (±8.13) years. A total of 73 (25.09%) individuals showed severe liver fibrosis (F3); 49 males and 24 females with a mean (±SD) age of 50.79 (±8.13) years. A total of 128 (43.99%) individuals showed liver cirrhosis (F4); 80 males and 48 females with a mean (±SD) age of 52.77 (±9.23) years.

The following liver function tests (LFTs) were all measured using an automated biochemistry analyzer (A15, Biosystem, Spain): albumin, total bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The Sysmex Corporation KX-21 automated haematology analyzer, located in Kobe, Japan, was used to perform a complete blood count. Regarding PIIINP, the latter was measured based on ELISA technique (Shanghai Sunred Biological Technology Co. Ltd,
Shanghai, China). All patients underwent HBsAg testing, which was negative (Dia.Pro, Milan, Italy), and anti-HCV antibody testing, which was positive (Biomedica, Sorin, Italy). Using a quantitative polymerase chain reaction assay (COBAS Ampliprep/COBAS TaqMan, Roche Diagnostics, Pleasanton, USA), the presence of HCV-RNA was verified.

Both the SPSS software (15.0; SPSS Inc., Chicago, USA) and the GraphPad Prism package (5.0; GraphPad Software, San Diego, USA) were used to conduct statistical analyses. Mean with standard deviation (SD) were used to express continuous data. The Spearman's rank method was used to assess correlation. One- way analysis of variance (ANOVA) was used to identify significant differences between groups. Statistics were judged significant at P of 0.05. The area under the receiver-operating characteristic (ROC) curves was used to evaluate the diagnostic accuracy. ROC analysis was used to identify the appropriate cut-off values for the most accurate prediction of substantial, advanced fibrosis and cirrhosis. Using a 2 by2 contingency table, common indicators of score performance were determined.

Results

In this work, the majority of the patients (n=173; 59.45%) were men, with an average age of 52.19 (±10.55) years. There was no significant difference between different fibrosis stages with respect to gender (P=0.152). On contrary, there was a statistically significant difference in liver-fibrosis stages among age groups (P <0.0001). As expected, younger patients were diagnosed with mild to moderate fibrosis, but individuals over 50 years old showed signs of more severe stages of the disease. Overall, 215 (73.88%) patients had F2-F4, 201 (69.07%) patients had F3-F4 and 128 (43.99%) patients had F4. Of note, patients who developed significant
fibrosis (F2-F4) and cirrhosis (F4) were accompanied by somewhat higher level of serum AST, ALT and total bilirubin than those with no/mild fibrosis (F0-F1) and non- cirrhosis (F0-F3), respectively, but with no significant difference (P >0.05). On contrary, our results showed that albumin and platelets were adversely correlated withthe progression rate of live fibrosis. Patients with F2-F4 and F4 were accompanied by lower level of albumin and platelets count than those with F0-F1 and F0-F3, respectively, with an extremely significant difference (P <0.0001) as shown in Figure 1.

The next step of this work was dedicated to quantifying PIIINP in different stages of hepatic fibrosis and estimating its diagnostic performances for the diagnosis of different stages of liver fibrosis. PIIINP level was directly proportional to liver fibrosis progression yielding a Spearman's rank correlation coefficient of 0.51 (P <0.0001). Our findings showed that the concentration of PIIINP was found to increase significantly in case of F2-F4 and F4 in contrast to patients who have F0-F1 and F0- F3, respectively, with an extremely significant difference (P <0.0001) as shown in Figure 2 (A, B).

The mean value of PIIINP concentration was estimated to be 39.45 and 49.67 (ng/mL) in patients with F2-F4 and F4 while it was 15.60 and 19.77 (ng/mL) in case of patients with F0-F1 and F0-F3, respectively. Based on the aforementioned results, the findings showed that patients who have F2-F4 and F4 displayed a 2.56-fold and 2.65-fold increase in PIIINP concentration over those who developed F0-F1 and F0-F3, respectively, as depicted in Figure 2 (C, D).

Herein, in order to amplify the difference in PIIINP values among patients with different fibrosis stages and increase its attitude, PIIINP/ platelets ratio was devised that showed better findings compared to that obtained by PIIINP *per se*. The diagnostic value of PIIINP/ platelets ratio was assessed in the present study based on
ROC analysis. Our results showed that the developed ratio enabled the correct identification of F2-F4 and F4 showing AUCs of 0.86 and 0.85, respectively, as displayed in Figure 3A, B. As anticipated, the level of PIIINP/ platelets ratio was found to increase significantly with the progression of liver disease. Interestingly, patients with F2-F4 and F4 displayed a 3.72 and 3.56-fold increase in PIIINP/ platelets ratio over those who developed F0-F1 and F0-F3, respectively, as shown in Figure 3C, D. Of note, the best cutoff value for optimal prediction of significant fibrosis and cirrhosis were determined based on the ROC curve. Take both sensitivity and specificity into account, the cutoff point was selected according to maximum number of sensitivity and specificity. Then, the distribution of PIIINP/Platelets ratio values at the specified cutoff point for significant fibrosis and cirrhosis versus those without was depicted as scatter/ dot diagram as provided in Figure 3E, F.

Discussion

Actually, a number of regular blood tests have been studied as potential indirect indicators of fibrosis in individuals with CHC. All of the laboratory tests are readily available, but none of them directly represent the pathophysiology of hepatic fibrogenesis, which is mediated by hepatic stellate cells (Almpanis et al., 2016; Fontana et al., 2008). Patients with cirrhosis and severe liver disease frequently have slight variations in their test results, which typically lie within normal ranges (Ahmed et al., 2018). Therefore, diagnosing or ruling out those who could have chronic liver disorders shouldn't be limited to standard laboratory tests. Therefore, the purpose of this work was to estimate PIIINP's diagnostic performances for the detection of various stages of liver fibrosis and to quantify PIIINP in different stages of hepatic fibrosis. The study's findings indicated that the concentration of this substance was raised and that it increased considerably ($P < 0.0001$) as liver fibrosis progressed. Of
note, patients with F2-F4, F3-F4 and F4 displayed a 2.56, 2.44, 2.65-fold increase in PIIINP concentration over those who developed F0-F1, F0-F2 and F0-F3, respectively. Hepatic stellate cells are the main matrix-producing cells that drive liver fibrosis and play a major part in the disease (Lee et al., 2021). Activated HSCs concentrate in the liver as a result of overproduction of ECM components such as collagen, fibronectin, elastin, laminin, and proteoglycan (Blokland et al., 2020). When type III collagen is synthesized, the N-terminal propeptide of PIIINP splits, resulting in fibrogenesis and the blood's release of ECM fragments (Gudowska et al., 2017). As a result, propeptide of PIIINP concentration can be used as a direct measure of collagen synthesis and deposition in the extracellular area (Cross et al., 2010). Remarkably, PIIINP is among the non-invasive markers of liver-fibrosis in CHC that is most well researched (Wang et al., 2020). Several studies showed a correlation of PIIINP levels with liver-inflammation and fibrosis-stage

in CHC patients (Wong et al., 2014; Zeng et al., 2018). Furthermore, PIIINP level is higher in individuals with chronic hepatitis C in higher stages of fibrosis, and it is a useful marker to distinguish between advanced and moderate fibrosis, according to Nielsen et al. (Nielsen et al., 2015). ECM remodeling as an attempt to repair damaged tissue in response to liver injury is the only plausible explanation for elevated PIIINP levels in advanced fibrosis. The synthesis of collagen and other ECM components is increased in cases ofsignificant liver injury (Gudowska et al., 2017). PIIINP concentrations in the basal membrane are higher in hepatic fibrosis, which is brought on by ongoing liver damage. In acute hepatitis, PIIINP will be associated with aminotransferase levels, which indicate the degree of fibrosis (Castera, 2012; Gressner et al., 2007).

Regrettably, PIIINP's somewhat modest sensitivity and specificity (78 and 81%) have restricted its clinical application. Therefore, these cannot be trusted to determine fibrosis grading (Castera, 2012; Jarčuška et al., 2010). The PIIINP/platelets ratio was developed in this instance to enhance the variation in PIIINP values among patients with varying degrees of fibrosis and to improve its attitude. The results were superior to those obtained by using PIIINP alone. The results demonstrated that patients with advanced fibrosis (F3-F4), cirrhosis (F4), and significant fibrosis (F2- F4) had PIIINP/platelets ratios that were 2.75, 3.44, and 3.75 times higher than those with no/mild fibrosis (F0-F1), non-advanced fibrosis (F0-F2), and non-cirrhosis (F0- F3), respectively. Our results showed that PIIINP/ platelets ratio enabled the correct identification of F2-F4, F3-F4 and F4 showing AUCs of 0.86, 0.84 and 0.85, respectively.

In fact, APRI and FibroTest are the most extensively utilized and verified tests, mostly for viral hepatitis C. For F2-F4 and F4, respectively, a recent comprehensive review of 172 papers on hepatitis C found that the median AUCs for FibroTest and APRI were 0.79 and 0.86 and 0.77 and 0.84, respectively (Chou and Wasson, 2013). The mean normalized AUC for the diagnosis of F2-F4 was 0.84 in a meta-analysis that examined data from 6378 people who had the FibroTest and biopsies (3501 with HCV infection and 1457 with HBV), with no discernible differences between patients with HCV (0.85) and HBV (0.80) (Poynard et al., 2007). An further meta-analysis examined data from 33 studies involving 6259 HCV patients; the mean AUCs of APRI in identifying F2-F4 and F4 were 0.77 and 0.83, respectively (Lin et al., 2011). In general, cirrhosis is more accurate than biomarkersat identifying intermediate levels of fibrosis.

Conclusion

The PIIINP/platelets ratio is a useful noninvasive, simple, accurate, and affordable measure of liver fibrosis that can help with hepatic fibrosis staging and track the effectiveness of treatment in individuals with HCV infection.

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Author’s contributions

All authors have made a substantial, direct, and intellectual contribution to the work. Mohamed El-Far, Abdelfattah M. Attallah, Mohamed S. Albannan and Khaled Farid were involved in the supervision of the study. All authors contributed to the final manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

The 1975 Helsinki Declaration's ethical principles were followed by the study protocol. This study was authorized by the Mansoura University Hospitals' ethical andscientific committees (Code Number: R.20.12.1124).

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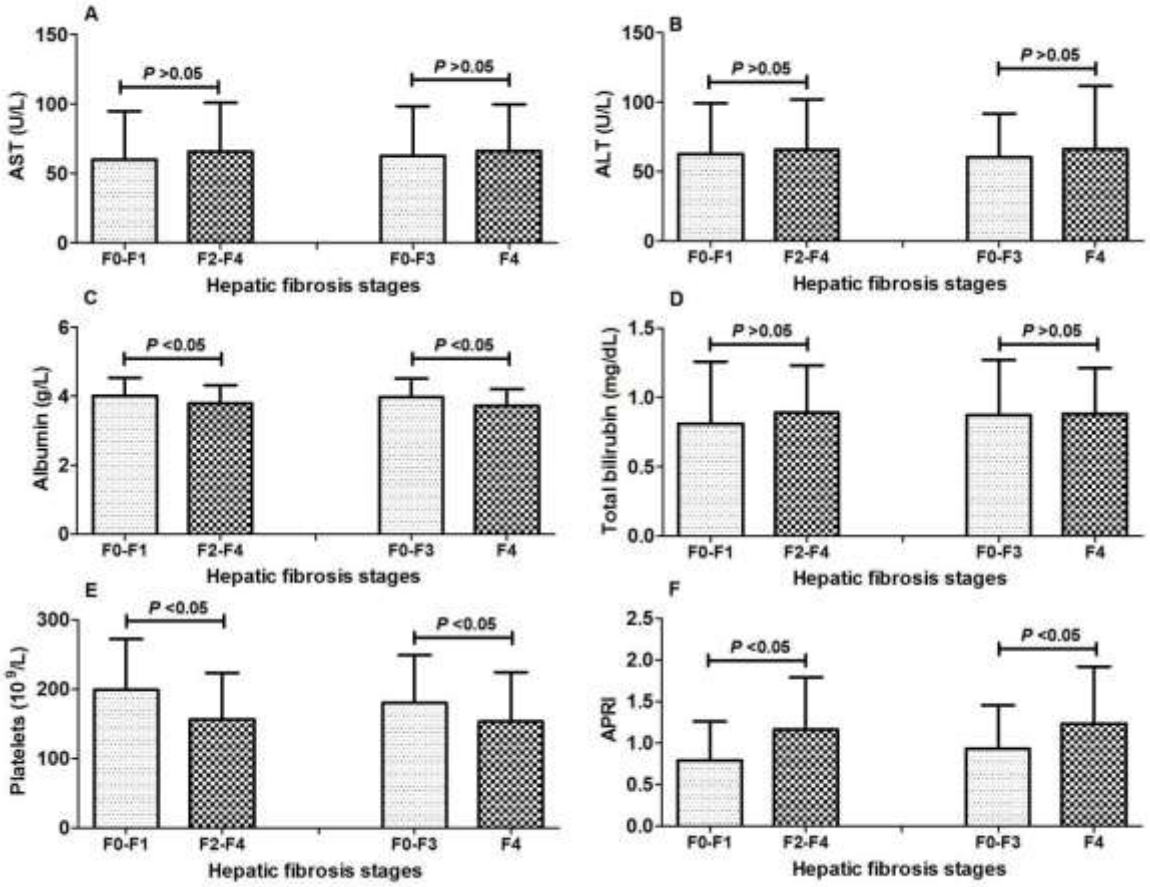

Figure 1: Clinical features of chronic hepatitis C patients in different fibrosis stages included in the present study.

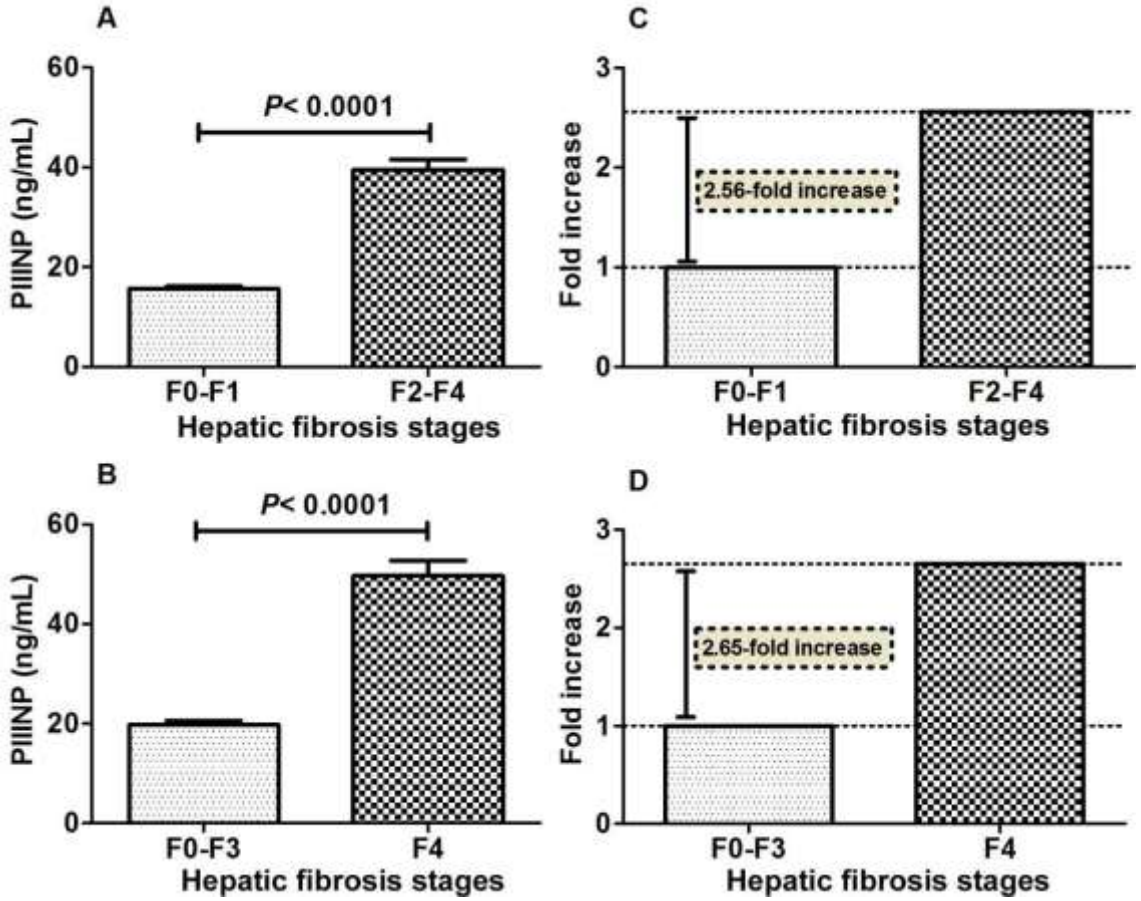

Figure 2: Quantification of PIIINP in addition to its observed fold changes in sera of chronic hepatitis C patients **(A, B)** the levels of PIIINP among different fibrosis stages; **(C, D)** the distribution of observed fold changes for PIIINP among different liver fibrosis stages. *P* >0.05 is considered non-significant, *P* <0.05 is considered significant, and *P* <0.0001 is considered extremely significant.

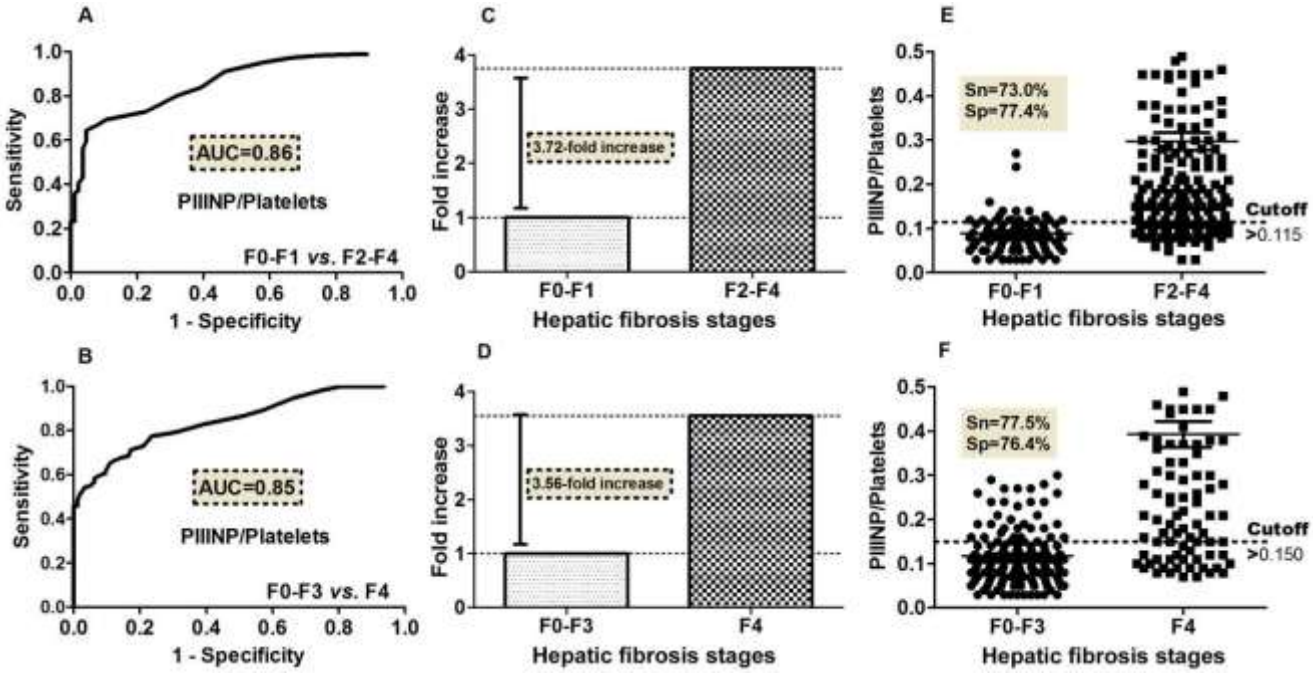

Figure 3: The diagnostic performance and distribution of PIIINP/Platelets ratio for separating **(A, C, E)** Patients who developed significant fibrosis (F2-F4) from those without (F0-F1); **(C, D, F)** Patients who developed cirrhosis (F4) from those without (F0-F3). The cutoff points were chosen based on ROC analysis. Abbreviations: Sn: Sensitivity, SP: Specificity.