https://doi.org/10.48047/AFJBS.6.12.2024.5335-5353

African Journal of Biological Sciences
Journal homepage: http://www.afjbs.com
Research Paper Open Access

The Diagnostic Ability of Tissue Inhibitors of Metalloproteinase-1 for staging hepatic fibrosis in chronic hepatitis C patients

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Article History
Volume 6, Issue 12, 2024
Received: 15 June 2024
Accepted: 10 July 2024
Doi:
10.48047/AFJBS.6.12.2024.5335-5353

Abstract

Background: This work aimed to assess the relationships between TIMP-1 and disease severity in patients with chronic hepatitis C (CHC) and to ascertain the usefulness of TIMP-1 in differentiating individuals with different stages of liver fibrosis.

Methods:286 CHCpatients were included in this study. FibroScan was used to diagnose liver fibrosis.

Results:TIMP-1 was directly proportional to liver-fibrosis progression.Individuals with F2-F4 and F4 had significantly higher concentrations of TIMP-1 than patients with F0-F1 and F0-F3, respectively. The mean value of TIMP-1concentration was estimated to be 117.3 and 139.1(ng/mL) in patients with F2-F4and F4 while it was67.4and 78.9 (ng/mL) in case of patients with F0-F1 and F0-F3, respectively.Findings showed patients with F2-F4and F4 displayed 1.74and 1.90-fold increase in TIMP-1over those with F0-F1and F0-F3, respectively. Based on ROC analysis, TIMP-1 enabled the correct identification of F2-F4 yielding 0.83AUCwith 75.0% sensitivity and 66.4% specificity while it produced 0.80 AUC for the correct identification of F4 with 77.0% sensitivity and 71.0% specificity.

Conclusion: Our results indicated that the utilization of TIMP-1 facilitated the categorization of liver fibrosis with a considerable level of precision and efficacy in patients with CHC.
Keywords: Cirrhosis, Extracellular matrix, Fibrosis, Non-invasive, TIMP-1.

Introduction

It's interesting to note that liver fibrosis is treatable, and early intervention can reduce mortality and enhance quality of life. However, unchecked fibrosis can lead to permanent cirrhosis and ultimately liver cancer (Liu et al., 2024). Regrettably, liver fibrosis patients frequently have normal physical tests, no symptoms, and non-specific biochemical abnormalities. As a result, patients with chronic liver illnesses frequently receive a diagnosis far too late, by which time their prognosis for the medium term has already declined. About 78% of instances of hepatocellular carcinoma are at the palliative stage at the time of diagnosis, while 75% of cases of cirrhosis are already decompensated (Canivet and Boursier, 2022). Therefore, it is imperative to focus significant efforts on liver fibrosis early identification, staging, and customized treatment. TIMP-1, sometimes referred to as tissue inhibitor of metalloproteinases-1, is an essential protein that regulates a number of biological processes in the human body. The enzymes known as metalloproteinases (MMPs), which are in charge of rupturing the extracellular matrix (ECM) in tissues, are significantly inhibited by TIMP-1. TIMP-1 supports healthy cell activity and aids in preserving the structural integrity of tissues by blocking these enzymes. Studies indicate that hyperphosphorylation of TIMP-1 may be associated with a number of illnesses, such as cancer, heart problems, and inflammatory diseases (Arpino et al., 2015; Rai and Baird, 2020). TIMP-1 is essential in preserving the fine balance between scar formation and matrix remodeling in the setting of liver fibrosis. Increased TIMP-1 has been linked to the development of liver fibrosis because it prevents MMP activity, which causes collagen and fibrotic tissue to build up. This imbalance reduces the liver's capacity to repair and tilts the scales in favor of fibrosis. In fact, deciphering the complex relationship between TIMP-1 and MMPs is essential to understanding the etiology of liver fibrosis and investigating possible treatment options(Shan et al., 2023; Yao et al., 2022). Our study aims to assess the relationships between TIMP-1 and disease severity in patients with chronic hepatitis C (CHC) and to ascertain the usefulness of circulating TIMP-1 in differentiating individuals with different stages of liver fibrosis, given the crucial role that TIMP-1 plays in liver fibrosis.

Methods

This study comprised 286 Egyptian patients who had received a diagnosis of CHC in a consecutive manner, verified by laboratory testing as well as clinical diagnosis. These patients were prospectively recruited from the Mansoura University hospitals in Mansoura's Tropical Medicine Department. After being thoroughly informed about the diagnostic procedures and the nature of their condition, every patient provided their informed consent. The study protocol adhered to the ethical guidelines set forth in the 1975 Helsinki Declaration.The staging of liver fibrosis was determined by FibroScan (Sandrin et al., 2003) (Echosens, Paris, France).Five different groups were created out of the CHC patients.A total of 36 cases (12.6%) exhibited no fibrosis (F0), 48 cases (16.8%) displayed minimal fibrosis (F1), 67 cases (23.4%) displayed severe fibrosis (F3), and 121 cases (42.3%) demonstrated liver cirrhosis (F4). In all, 188 patients (65.7%) had advanced fibrosis (F3-F4), 202 patients (70.6%) had considerable fibrosis (F2-F4), and 121 patients (42.3%) had cirrhosis (F4).All 286 participants met the following criteria: a negative test for hepatitis B surface antigen using a kit from Biomedica, Sorin, Italy; a positive test for anti-HCV antibodies (ETI-AB-HCVK-3, SorinBiomedica, Diagnostic, Vercelli, Italy),

and a positive result for the presence of HCV-RNA through quantitative polymerase chain reaction assay.All participants also underwent a variety of laboratory evaluations, including a complete blood count performed on blood treated with EDTA-K3, an analysis of the tumor marker alpha fetoprotein (AFP) from CanAg Diagnostics AB, Gothenburg, Sweden, and liver function tests (albumin, total bilirubin, AST, and ALT) using an automated biochemistry analyzer (A15, Biosystem, Spain)

GraphPad Prism package v.5.0 (GraphPad Software, San Diego, CA) and SPSS 20.0 for Microsoft Windows (SPSS Inc.) were two examples of statistical software packages used for the statistical studies. Descriptive statistics were presented as mean ± SD and range or number (percentage) of patients with a particular condition. The student t-test was utilized to assess differences in continuous data, while the Chi-square (X2) test was applied to categorical variables. All tests were two-tailed with statistical significance set at the 0.05 level. The odds ratios were calculated using logistic regression models, and the Pearson correlation coefficient was used to evaluate correlation. Furthermore, the area under the receiver operating characteristic (ROC) curves was used to calculate the diagnostic accuracy of different variables.

Results

According to the study results, there were statistically significant differences in liver fibrosis stages between age groups (P<0.0001). Patients under 50 years old were more likely to have mild to moderate fibrosis, whereas those over 50 years old were more likely to have more advanced stages. In addition, patients with more advanced stages of fibrosis had greater body mass indices than those with milder stages. Furthermore, a significant negative connection (P<0.0001) was found between the platelet count and the rate at which liver fibrosis progresses.

The platelet counts of patients with advanced fibrosis, cirrhosis, and substantial fibrosis were lower than those without these conditions, respectively. Based on P>0.05, there were no significant variations in fasting blood glucoseand INR amongst the various fibrosis groups as shown in Table1. In general, greater ALT levels (> 60 U/L) had been linked to CHC patients with varying stages of fibrosis. As liver fibrosis advanced, there was a non-significant rise (P<0.05) in total bilirubin levels and AST activity. Furthermore, the study's findings showed a negative correlation between albumin levels and the advancement of liver fibrosis. As shown in the Table 1, patients with severe fibrosis stages (F2-F4, F3-F4, and F4) had lower albumin levels than patients with lower or milder fibrosis stages (F0-F1, F0-F2, F0-F3).

The subsequent step of this investigation was dedicated to evaluating TIMP-1 levels across various stages of hepatic fibrosis and assessing its diagnostic accuracy for different liver fibrosis stages.The results showed that individuals with F2-F4 had significantly higher concentrations of TIMP-1 than patients with F0-F1 (Figure 1A). For F2-F4 patients, the average TIMP-1 level was determined to be 117.3 (ng/mL), but for F0-F1 patients, it was 67.4 (ng/mL). As seen in Figure 1B, these data showed that F2-F4 patients had 1.74-fold higher TIMP-1 levels than F0-F1 patients.Additionally, compared to F0-F2 patients, there was a discernible increase in TIMP-1 levels in F3-F4 patients (Figure 2A). Figure 2B illustrates a 1.79-fold increase in TIMP-1 levels for F3-F4 patients compared to F0-F2 patients, with the mean TIMP-1 concentrations for these patients being 119.8 (ng/mL) and 69.6 (ng/mL), respectively.Furthermore, compared to non-cirrhotic individuals, the results showed a considerable rise in TIMP-1 levels in cirrhotic individuals (Figure 3A). For cirrhotic individuals, the mean TIMP-1 concentration was 139.1 (ng/mL), while for non-cirrhotic persons, it was 78.9 (ng/mL). As shown in Figure 3B, it was found that cirrhotic patients had 1.90 times higher TIMP-1 levels than non-cirrhotic (F0-F3) people.

The ROC curve was used in the current study to assess TIMP-1's diagnostic effectiveness. As shown in Figure 4A, the results showed that TIMP-1 could correctly identify F2-F4 patients with an AUC of 0.83. TIMP-1 levels above 82.35 indicated considerable fibrosis, whereas levels equal to or below 82.35 showed non-significant fibrosis. A cutoff value of 82.35 was found for the best diagnosis of significant fibrosis. TIMP-1 demonstrated a sensitivity of 75.0% and specificity of 66.4% in identifying F2-F4 individuals at this threshold. Figure 4B shows a scatter/dot plot that illustrates the distribution of TIMP-1 values at this cutoff point for patients with F2-F4 against those without.Moreover, Figure 5 showed the correlation between a patient's TIMP-1 levels and the probability that they had F2-F4. A TIMP-1 value of 82.35 or higher indicated a higher likelihood that the patient had F2-F4, whereas a value of 82.35 or lower indicated a lower likelihood.Figure 6A illustrates the AUC of 0.84 that was obtained for identifying F3-F4. Similar to this, a cutoff value of 85.1 was found using the ROC curve to get the best prediction for F3–F4. TIMP-1 showed 74.0% sensitivity and 71.0% specificity at this level for detecting F3-F4. A scatter/dot plot in Figure 6B showed the distribution of TIMP-1 values at this cutoff point for patients with F3-F4 versus those with F0-F2. Patient F3-F4 likelihood increases with TIMP-1 >85.1. On the other hand, the likelihood of the patient having F3-F4 decreases with TIMP-1≤85.1 (Figure 7).

TIMP-1 application on cirrhotic individuals produced an AUC of 0.80, as shown in Figure 8A. 88.4 was found to be the ideal cutoff value using the ROC curve to predict cirrhosis (F4). TIMP-1 demonstrated 77.0% sensitivity and a 71.0% specificity for cirrhosis detection at this threshold (F4). Figure 8B shows a scatter/dot diagram that shows the distribution of TIMP-1 at the liver cirrhosis cutoff point in relation to liver cirrhosis patients lacking F0-F3. F4 classification was more likely to be assigned to patients whose TIMP-1 levels were greater than 88.4. on the other hand, individuals who had TIMP-1 levels higher than 88.4 were more likely to be categorized as F4. On the other hand, those with TIMP-1 ≤ 88.4 were less likely to be classified as F4 (Figure 9).

Discussion

A chronic liver illness called hepatic fibrosis is caused by an excessive buildup of fibrous connective tissue in the extracellular matrix (ECM) surrounding injured tissues. Hepatic fibrosis, the main pathogenic factor for liver failure and a crucial feature in individuals with end-stage liver disease, including HCC, is the main source of chronic liver damage (Antar et al., 2023). It is thought that the overproduction of ECM in hepatic stellate cells causes a six-fold increase in ECM fragments deposited in Disse's space, which in turn causes liver damage and the development of liver fibrosis (Gudowska et al., 2017).In fact, among CHC patients, the chance of disease progression is substantially correlated with the severity of hepatic fibrosis and inflammation at the time of diagnosis. Furthermore, decisions about antiviral therapy and disease surveillance are influenced by the histologic stage in CHC patients(Ginès et al., 2022). Of note, Fibrosis can now be assessed using a variety of non-invasive blood and image-based assays that combine physiological, physical, or biological approaches, such as tissue stiffness imaging or serum biomarkers.(Bera et al., 2024; Patel and Sebastiani, 2020). However, these non-invasive tests face limitations such as variability, inadequate accuracy, and error risk (Patel and Sebastiani, 2020). There are very little longitudinal data that use these tests for screening. Non-invasive test screening could be less expensive, but it needs to be validated(Serra-Burriel et al., 2019). Therefore, there is a rising medical need for the development of non-invasive tests that can reliably predict the initial stage of the disease as well as the evolution of fibrosis over time. Therefore, the purpose of this study was to evaluate how well serum TIMP- estimates hepatic

fibrosis staging in CHC patients.Aminotransferase activity is a critical disease measure and hepatic damage indicator in CHC patients. AST is mostly present in the mitochondria and is also utilized as a marker of liver damage in CHC patients. ALT is found in the cytoplasm of hepatocytes, where it transfers amino groups(Kobayashi et al., 2011; Maulidia et al., 2020). In this investigation, we found a correlation between higher ALT levels (> 60 U/L) and various fibrosis phases in our CHC patients. Although the difference was not statistically significant, patients with substantial fibrosis, advanced fibrosis, and cirrhosis, respectively, had somewhat lower serum ALT activity than those without these conditions. As liver fibrosis advanced, there was a non-significant rise ($P<0.05$) in AST activity.

Many growth factors that are essential for organ development, tissue regeneration, and repair processes are present in platelets(Kurokawa et al., 2016). Research has shown that in chronic liver disease, platelets release growth factors that promote tissue healing in addition to causing liver damage through T cell-mediated immunological responses(Meyer et al., 2015; Nowatari et al., 2014). Clinical data indicates that platelet count may be used to assess liver steatosis and fibrosis severity in patients with nonalcoholic fatty liver disease (NAFLD) as well as to distinguish between severe fibrosis in hepatitis C patients (Khokhar, 2003; Yoneda et al., 2011). Indeed, people with CHC infection typically have a lower platelet count. Numerous factors, such as decreased thrombopoietin production, which may hinder platelet formation in the bone marrow, are frequently implicated in this thrombocytopenia. Furthermore, sequestration of platelets might result from splenomegaly, a typical hallmark of CHC, which lowers the number of circulating platelets. Furthermore, an HCV infection can directly impair bone marrow function, which lowers the synthesis of platelets(Huang et al., 2022). According to our research,the pace at which liver fibrosis progresses and platelet count were significantly negatively associated ($P<0.0001$). The platelet counts of patients with F2-F4, F3-F4, and F4 were shown to be lower than those with F0-F1, F0-F2, F0-F3, respectively. Albumin, an exclusive product of the liver, is the predominant protein in circulation, with 60% of the total albumin pool located in the interstitial compartment. This protein is vital for maintaining vascular oncotic pressure. A decline in oncotic pressure, attributed to reduced albumin levels, leads to fluid extravasation into the peritoneal cavity, resulting in ascites. Additionally, albumin plays a crucial role in transporting various molecules like bilirubin, free fatty acids, drugs, and hormones. As the only protein produced solely by the liver, albumin makes up the majority of the proteins in circulation; the interstitial compartment contains 60% of the total albumin pool. The preservation of vascular oncotic pressure depends on this protein. As a result of decreased albumin levels, there is a drop in oncotic pressure, which causes fluid to leak into the peritoneal cavity and cause ascites. Furthermore, albumin is essential for the transportation of many other compounds, including hormones, medicines, free fatty acids, and bilirubin (Trebicka, 2022). Reduced serum albumin levels are a sign of hepatic impairment. Serum albumin levels are not immediately impacted by acute liver failure, but albumin production is reduced by chronic liver failure, which is frequently caused by cirrhosis (Nagao and Sata, 2010). Reduced albumin levels are a frequent side effect of CHC, especially in cases of severe liver disease(Heybe and Mehta, 2024). Our results showed that albumin level was inversely proportional to liver fibrosis progression. Patients with F2-F4, F3-F4, and F4 were accompanied by lower albumin level than those with F0-F1, F0-F2, F0-F3, respectively.

It is interesting to note that liver fibrosis, which is largely defined by an imbalance in the synthesis and breakdown of extracellular matrix, is commonly acknowledged as a common consequence of many chronic liver lesions. Interstitial collagen and other matrix components

accumulate as a result of this imbalance. Scar tissue and liver fibrosis are the results of the ongoing buildup of collagen I and III in hepatic fibrosis (Zhang et al., 2016).

In the production and degradation of collagen, MMPs and their associated inhibitors - specifically, TIMPs - play a critical role. TIMPs are known to perform a variety of tasks, such as controlling protease activity, migration, and cell development. Different TIMPs family members' expression patterns have been connected to a number of chronic illnesses. Several investigations have shown that TIMP-1 expression gradually rises as liver fibrosis progresses, reaching a peak at the cirrhosis stage (Busk et al., 2014; Wu et al., 2017). In fact, TIMP-1's mechanisms for accelerating the development of liver fibrosis entail a complicated web of interactions that include suppressing MMPs, encouraging cell survival, and regulating inflammatory responses, all of which eventually contribute to the build-up of extracellular matrix and fibrosis.The ECM is broken down and remodeled by MMPs, which TIMP-1 binds to and suppresses. Fibrosis results from TIMP-1's inhibition of MMPs, which encourages the buildup of collagen and other ECM components. Additionally, it has been documented that TIMP-1 can prevent hepatocytes and HSCs - two important components of hepatic fibrogenesis - from going through apoptosis, or programmed cell death. TIMP-1 keeps these cells from dying so they can continue to make collagen and other fibrotic components. Furthermore, TIMP-1 has the ability to promote HSC activation, which is the process by which collagen and other ECM components are produced. Fibrosis is reinforced by a positive feedback loop that is created when activated HSCs produce TIMP-1. TIMP-1 can also support a pro-fibrotic environment by influencing the ratio of pro- to anti-inflammatory cytokines. This could be a factor in the ongoing liver damage and fibrosis(Nalluri et al., 2015; Thiele et al., 2017; Wang et al., 2011). Furthermore, it has been documented that TIMP-1 gene expression in liver tissue increases with the severity of liver fibrosis(Nie et al., 2002). Because TIMP-1 can decrease MMP1, MMP-8, and MMP-13 activities in liver tissues, which encourage ECM buildup and collagen deposition and exacerbate liver fibrosis, these genes have been identified as biomarkers of liver fibrosis(Nie et al., 2004). Our results showed that the concentration of TIMP-1 was found to increase significantly with liver fibrosis progression. Moreover, the findings showed that TIMP-1 enabled the correct identification of F2-F4, F3-F4 and F4 patients showing an AUC of 0.83, 0.84 and 0.80, respectively.

Conclusion

Our results indicated that the utilization of TIMP-1 facilitated the categorization of liver fibrosis with a considerable level of precision and efficacy in patients with CHC.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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 andKhaledFaridwere 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 and scientific committees (Code Number: R.20.12.1123).

References

Antar, S. A., Ashour, N. A., Marawan, M. E., and Al-Karmalawy, A. A. (2023). Fibrosis: types, effects, markers, mechanisms for disease progression, and its relation with oxidative stress, immunity, and inflammation. *International Journal of Molecular Sciences* 24 (4), 4004.

Arpino, V., Brock, M., and Gill, S. E. (2015). The role of TIMPs in regulation of extracellular matrix proteolysis. *Matrix Biology* 44-46 247-254.

Bera, C., Hamdan-Perez, N., and Patel, K. (2024). Non-Invasive Assessment of Liver Fibrosis in Hepatitis B Patients. *Journal of Clinical Medicine* 13 (4), 1046.

Busk, T. M., Bendtsen, F., Nielsen, H. J., Jensen, V., Brünner, N., and Møller, S. (2014). TIMP-1 in patients with cirrhosis: relation to liver dysfunction, portal hypertension, and hemodynamic changes. *Scandinavian Journal of Gastroenterology* 49 (9), 1103-1110.

Canivet, C. M., and Boursier, J. (2022). Screening for liver fibrosis in the general population: where do we stand in 2022? *Diagnostics* 13 (1), 91.

Ginès, P., Castera, L., Lammert, F., Graupera, I., Serra-Burriel, M., Allen, A. M., Wong, V. W. S., Hartmann, P., Thiele, M., Caballeria, L., de Knegt, R. J., Grgurevic, I., Augustin, S., Tsochatzis, E. A., Schattenberg, J. M., Guha, I. N., Martini, A., Morillas, R. M., Garcia-Retortillo, M., de Koning, H. J., Fabrellas, N., Pich, J., Ma, A. T., Diaz, M. A., Roulot, D., Newsome, P. N., Manns, M., Kamath, P. S., Krag, A., and for the LiverScreen Consortium, I. (2022). Population screening for liver fibrosis: Toward early diagnosis and intervention for chronic liver diseases. *Hepatology* 75 (1).

Gudowska, M., Gruszewska, E., Panasiuk, A., Cylwik, B., Swiderska, M., Flisiak, R., Szmitkowski, M., and Chrostek, L. (2017). High serum N-terminal propeptide of procollagen type III concentration is associated with liver diseases. *Przeglad gastroenterologiczny* 12 (3), 203-207.

Heybe, M. A., and Mehta, K. J. (2024). Role of albumin infusion in cirrhosis-associated complications. *Clinical and Experimental Medicine* 24 (1), 58.

Huang, C.-E., Chang, J.-J., Wu, Y.-Y., Huang, S.-H., Chen, W.-M., Hsu, C.-C., Lu, C.-H., Hung, C.-H., Shi, C.-S., Lee, K.-D., Chen, C.-C., and Chen, M.-C. (2022). Different impacts of common risk factors associated with thrombocytopenia in patients with hepatitis B virus and hepatitis C virus infection. *Biomedical Journal* 45 (5), 788-797.

Khokhar, N. (2003). Serum aminotransferase levels and platelet count as predictive factor of fibrosis and cirrhosis in patients with chronic hepatitis C infection. *JPMA The Journal of the Pakistan Medical Association* 53 (3), 101-104.

Kobayashi, Y., Kawaguchi, Y., Mizuta, T., Kuwashiro, T., Oeda, S., Oza, N., Takahashi, H., Iwane, S., Eguchi, Y., Anzai, K., Ozaki, I., and Fujimoto, K. (2011). Metabolic factors are associated with serum alanine aminotransferase levels in patients with chronic hepatitis C. *Journal of gastroenterology* 46 (4), 529-535.

Kurokawa, T., Zheng, Y. W., and Ohkohchi, N. (2016). Novel functions of platelets in the liver. *Journal of gastroenterology and hepatology* 31 (4), 745-751.

Liu, Q.-Q., Chen, J., Ma, T., Huang, W., and Lu, C.-H. (2024). DCDC2 inhibits hepatic stellate cell activation and ameliorates CCl4-induced liver fibrosis by suppressing Wnt/β-catenin signaling. *Scientific Reports* 14 (1), 9425.

Maulidia, V. N. a. R., Wardhani, P., and Setyoboedi, B. (2020). AST, ALT and Albumin Level in Chronic Hepatitis B Patients with and without Complications of Cirrhosis and Hepatocellular Carcinoma. *INDONESIAN JOURNAL OF CLINICAL PATHOLOGY AND MEDICAL LABORATORY* 26 (3), 344-349.
Meyer, J., Lejmi, E., Fontana, P., Morel, P., Gonelle-Gispert, C., and Bühler, L. (2015). A focus on the role of platelets in liver regeneration: Do platelet-endothelial cell interactions initiate the regenerative process? *Journal of hepatology* 63 (5), 1263-1271.
Nagao, Y., and Sata, M. (2010). Serum albumin and mortality risk in a hyperendemic area of HCV infection in Japan. *Virology journal* 7 375.
Nalluri, S., Ghoshal-Gupta, S., Kutiyanawalla, A., Gayatri, S., Lee, B. R., Jiwani, S., Rojiani, A. M., and Rojiani, M. V. (2015). TIMP-1 Inhibits Apoptosis in Lung Adenocarcinoma Cells via Interaction with Bcl-2. *PloS one* 10 (9), e0137673.
Nie, Q.-H., Cheng, Y.-Q., Xie, Y.-M., Zhou, Y.-X., Bai, X.-G., and Cao, Y.-Z. (2002). Methodologic research on TIMP-1, TIMP-2 detection as a new diagnostic index for hepatic fibrosis and its significance. *World journal of gastroenterology* 8 (2), 282.
Nie, Q.-H., Duan, G.-R., Luo, X.-D., Xie, Y.-M., Luo, H., Zhou, Y.-X., and Pan, B.-R. (2004). Expression of TIMP-1 and TIMP-2 in rats with hepatic fibrosis. *World journal of gastroenterology* 10 (1), 86.
Nowatari, T., Murata, S., Fukunaga, K., and Ohkohchi, N. (2014). Role of platelets in chronic liver disease and acute liver injury. *Hepatology research : the official journal of the Japan Society of Hepatology* 44 (2), 165-172.
Patel, K., and Sebastiani, G. (2020). Limitations of non-invasive tests for assessment of liver fibrosis. *JHEP reports : innovation in hepatology* 2 (2), 100067.
Rai, G. P., and Baird, S. K. (2020). Tissue inhibitor of matrix metalloproteinase-3 has both anti-metastatic and anti-tumourigenic properties. 37 (1), 69-76.
Sandrin, L., Fourquet, B., Hasquenoph, J. M., Yon, S., Fournier, C., Mal, F., Christidis, C., Ziol, M., Poulet, B., Kazemi, F., Beaugrand, M., and Palau, R. (2003). Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound in medicine & biology* 29 (12), 1705-1713.
Serra-Burriel, M., Graupera, I., Torán, P., Thiele, M., Roulot, D., Wong, V. W.-S., Guha, I. N., Fabrellas, N., Arslanow, A., and Expósito, C. (2019). Transient elastography for screening of liver fibrosis: cost-effectiveness analysis from six prospective cohorts in Europe and Asia. *Journal of hepatology* 71 (6), 1141-1151.
Shan, L., Wang, F., Zhai, D., Meng, X., Liu, J., and Lv, X. (2023). Matrix metalloproteinases induce extracellular matrix degradation through various pathways to alleviate hepatic fibrosis. *Biomedicine & Pharmacotherapy* 161 114472.
Thiele, N. D., Wirth, J. W., Steins, D., Koop, A. C., Ittrich, H., Lohse, A. W., and Kluwe, J. (2017). TIMP-1 is upregulated, but not essential in hepatic fibrogenesis and carcinogenesis in mice. *Sci Rep* 7 (1), 714.
Trebicka, J. (2022). Role of albumin in the treatment of decompensated liver cirrhosis. *Current Opinion in Gastroenterology* 38 (3).
Wang, H., Lafdil, F., Wang, L., Yin, S., Feng, D., and Gao, B. (2011). Tissue inhibitor of metalloproteinase 1 (TIMP-1) deficiency exacerbates carbon tetrachloride-induced liver injury and fibrosis in mice: involvement of hepatocyte STAT3 in TIMP-1 production. *Cell & Bioscience* 1 (1), 14.

Wu, L., Zhang, Q., Mo, W., Feng, J., Li, S., Li, J., Liu, T., Xu, S., Wang, W., and Lu, X. (2017). Quercetin prevents hepatic fibrosis by inhibiting hepatic stellate cell activation and reducing autophagy via the TGF-β1/Smads and PI3K/Akt pathways. *Scientific reports* 7 (1), 9289.

Yao, H., Yang, X., Yan, M., Fang, X., Wang, Y., Qi, H., and Sun, L. (2022). Correlation of Serum M-CSF, CER, and TIMP-1 Levels with Liver Fibrosis in Viral Hepatitis. *Computational and Mathematical Methods in Medicine* 2022 (1), 6736225.

Yoneda, M., Fujii, H., Sumida, Y., Hyogo, H., Itoh, Y., Ono, M., Eguchi, Y., Suzuki, Y., Aoki, N., Kanemasa, K., Imajo, K., Chayama, K., Saibara, T., Kawada, N., Fujimoto, K., Kohgo, Y., Yoshikawa, T., and Okanoue, T. (2011). Platelet count for predicting fibrosis in nonalcoholic fatty liver disease. *Journal of gastroenterology* 46 (11), 1300-1306.

Zhang, C. Y., Yuan, W. G., He, P., Lei, J. H., and Wang, C. X. (2016). Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets. *World journal of gastroenterology* 22 (48), 10512-10522.

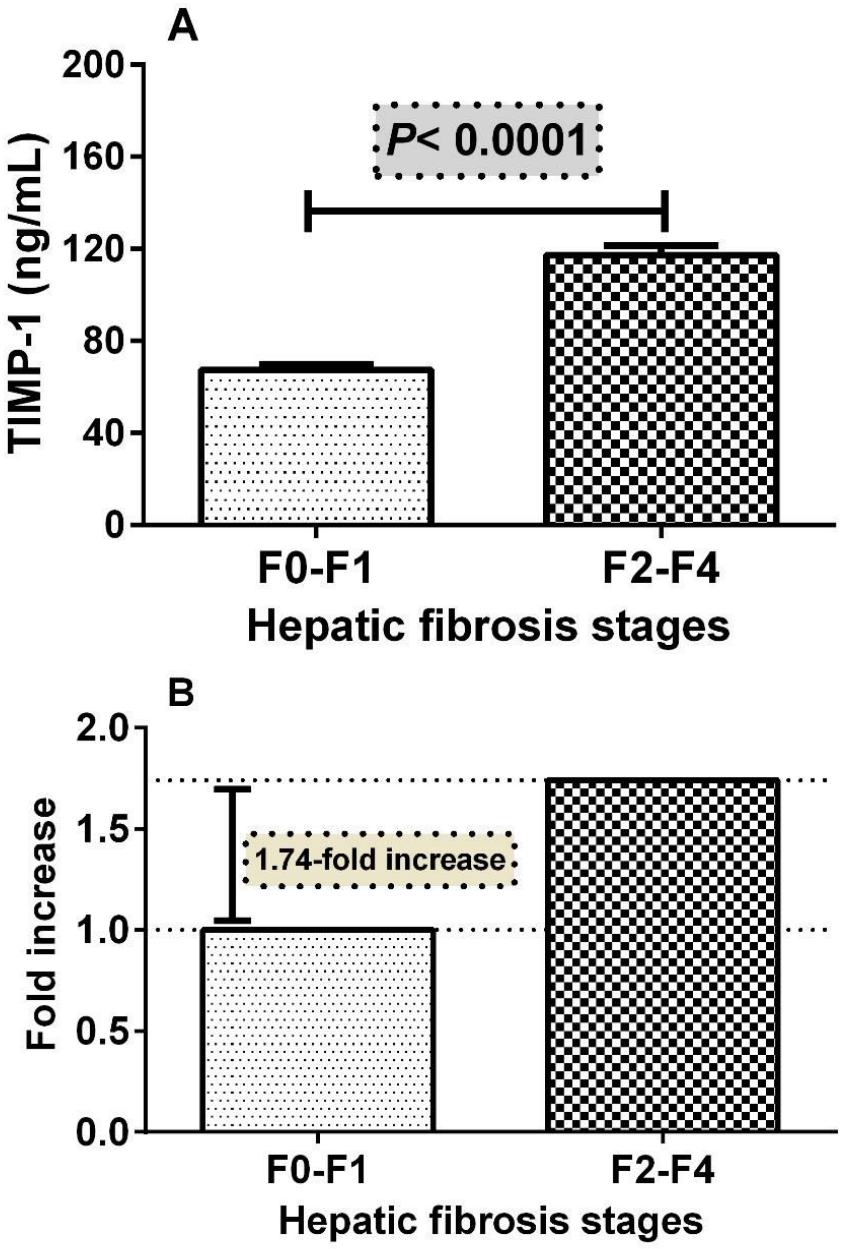

Figure 1. Quantification of TIMP-1 in addition to its observed fold changes in serum of chronic hepatitis C patients (A) The levels of TIMP-1 and (B) The distribution of observed fold changes for TIMP-1 for differentiating patients with significant fibrosis (F2-F4) from those with no/mild fibrosis (F0-F1). $P>0.05$ is considered non-significant, $P<0.05$ is considered significant.

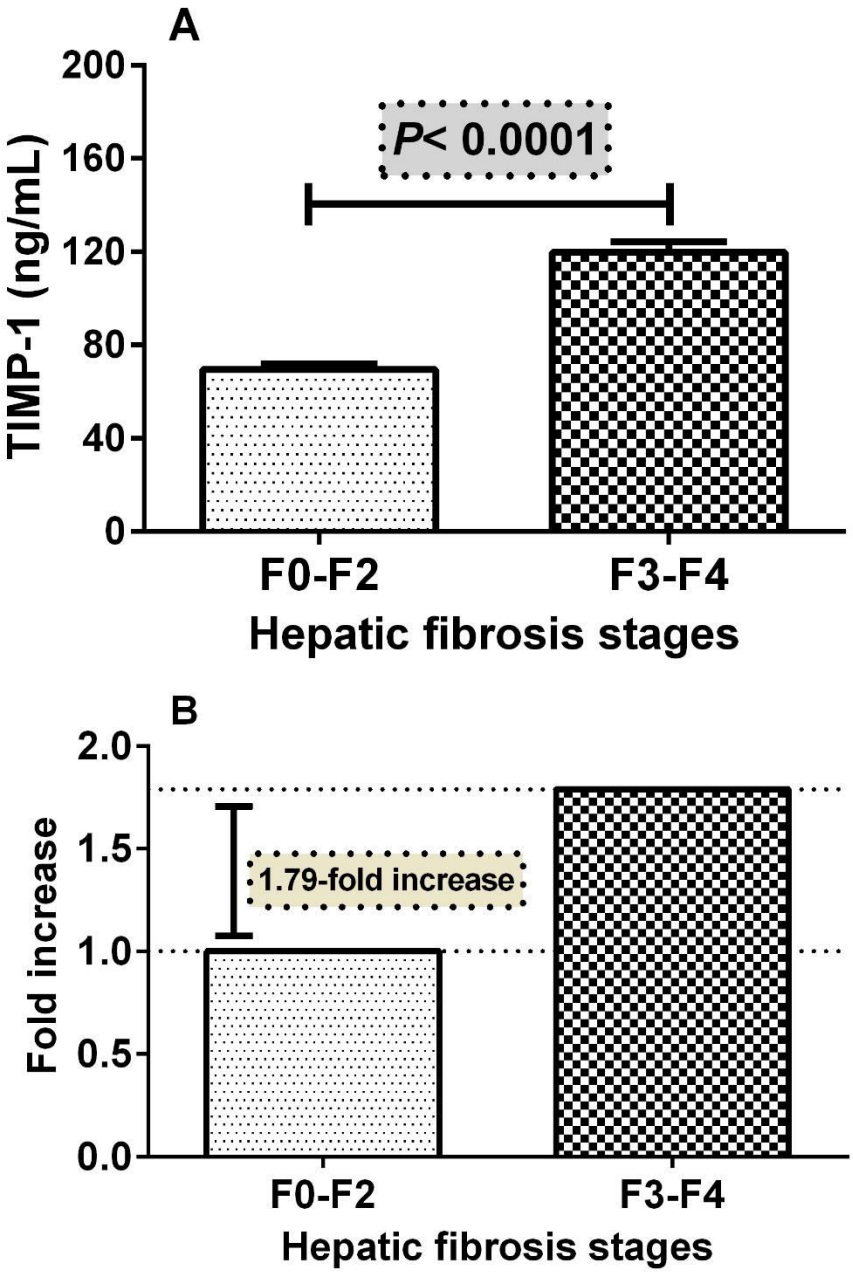

Figure 2. Quantification of TIMP-1 in addition to its observed fold changes in serum of chronic hepatitis C patients (A) The levels of TIMP-1 and (B) The distribution of observed fold changes for TIMP-1 for differentiating patients with advanced fibrosis (F3-F4) from those with non-advanced fibrosis (F0-F2). $P>0.05$ is considered non-significant, $P<0.05$ is considered significant and $P<0.0001$ is considered extremely significant.

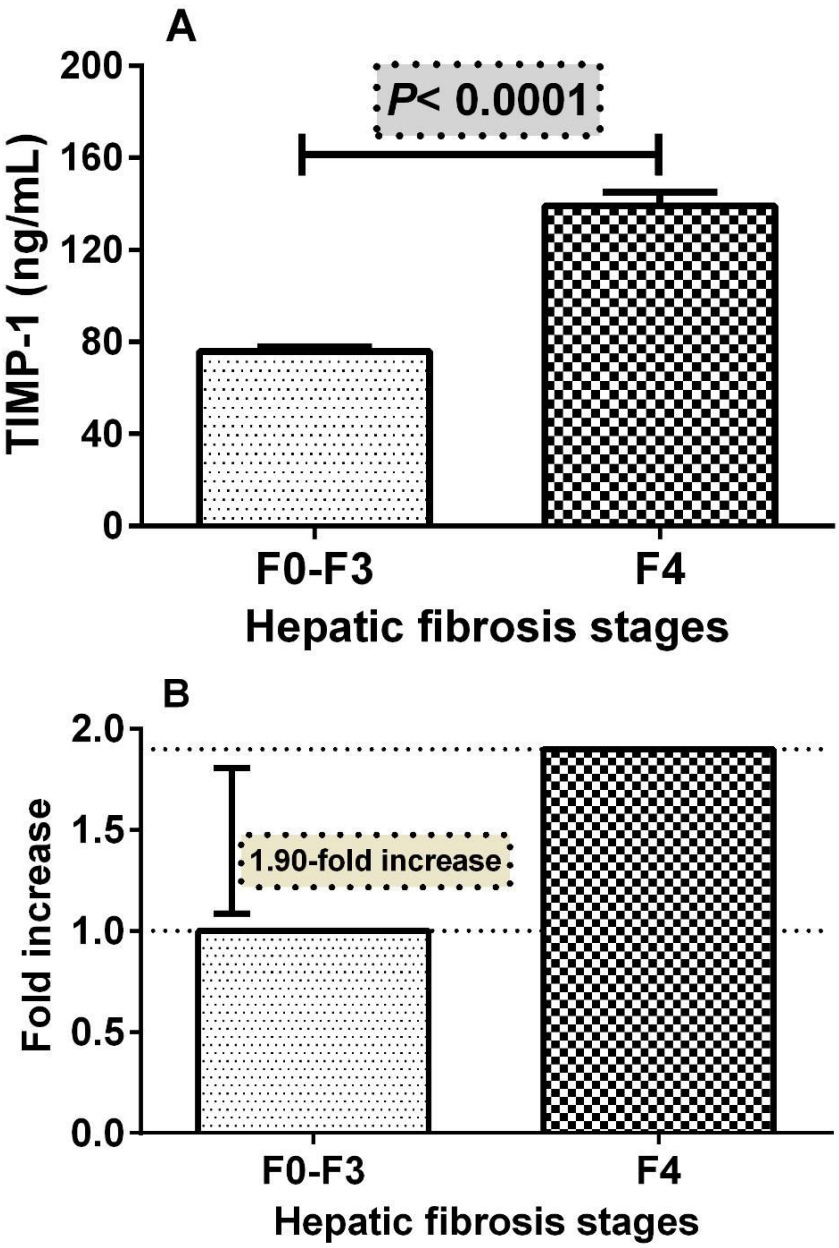

Figure 3. Quantification of TIMP-1 in addition to its observed fold changes in serum of chronic hepatitis C patients **(A)** The levels of TIMP-1 and **(B)** The distribution of observed fold changes for TIMP-1 for differentiating patients with cirrhosis (F4) from those without cirrhosis (F0-F3). $P>0.05$ is considered non-significant, $P<0.05$ is considered significant and $P<0.0001$ is considered extremely significant.

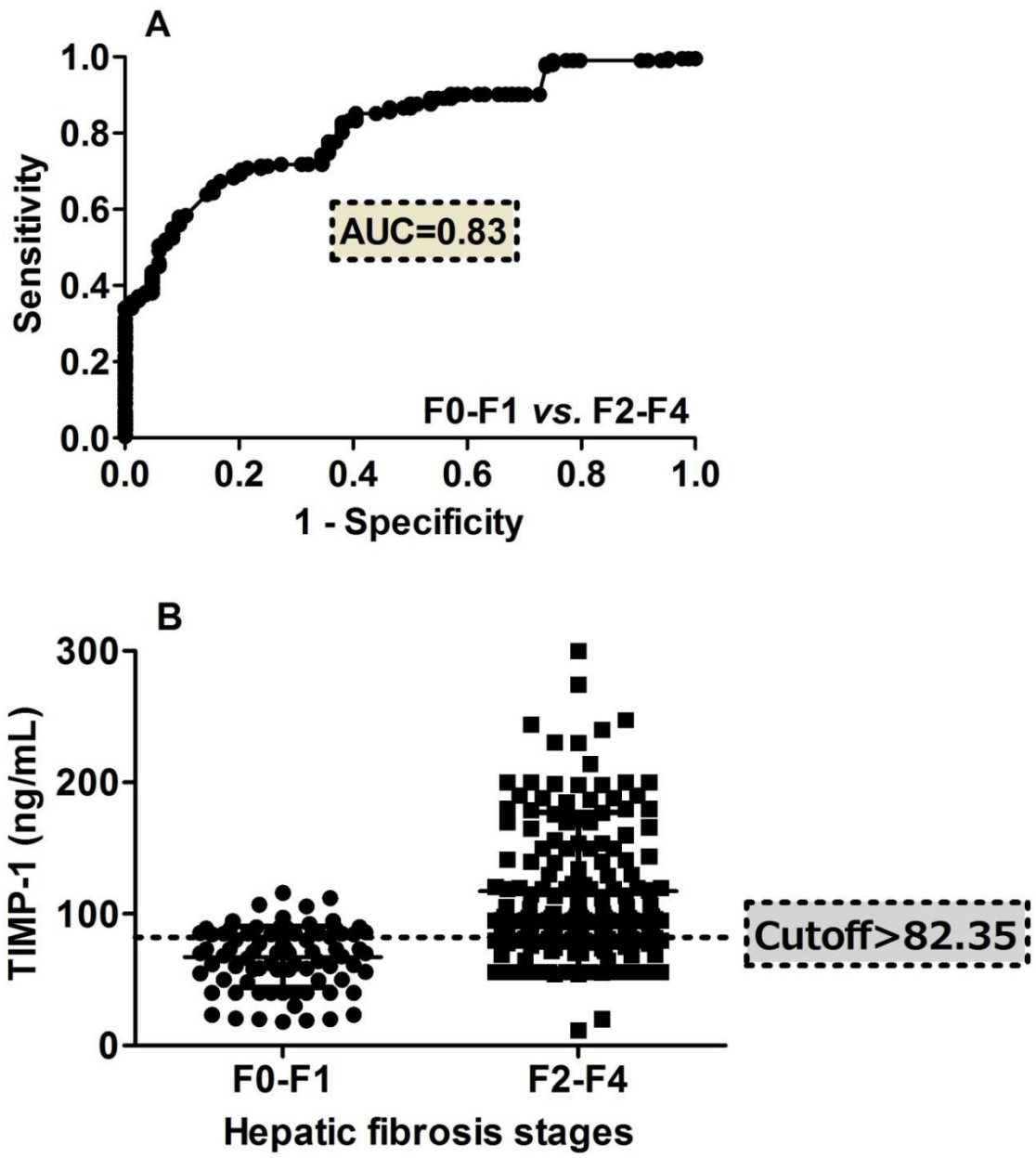

Figure 4. Diagnostic accuracy and distribution of TIMP-1 for separating patients who developed significant fibrosis (F2-F4) from those without significant fibrosis (F0-F1).

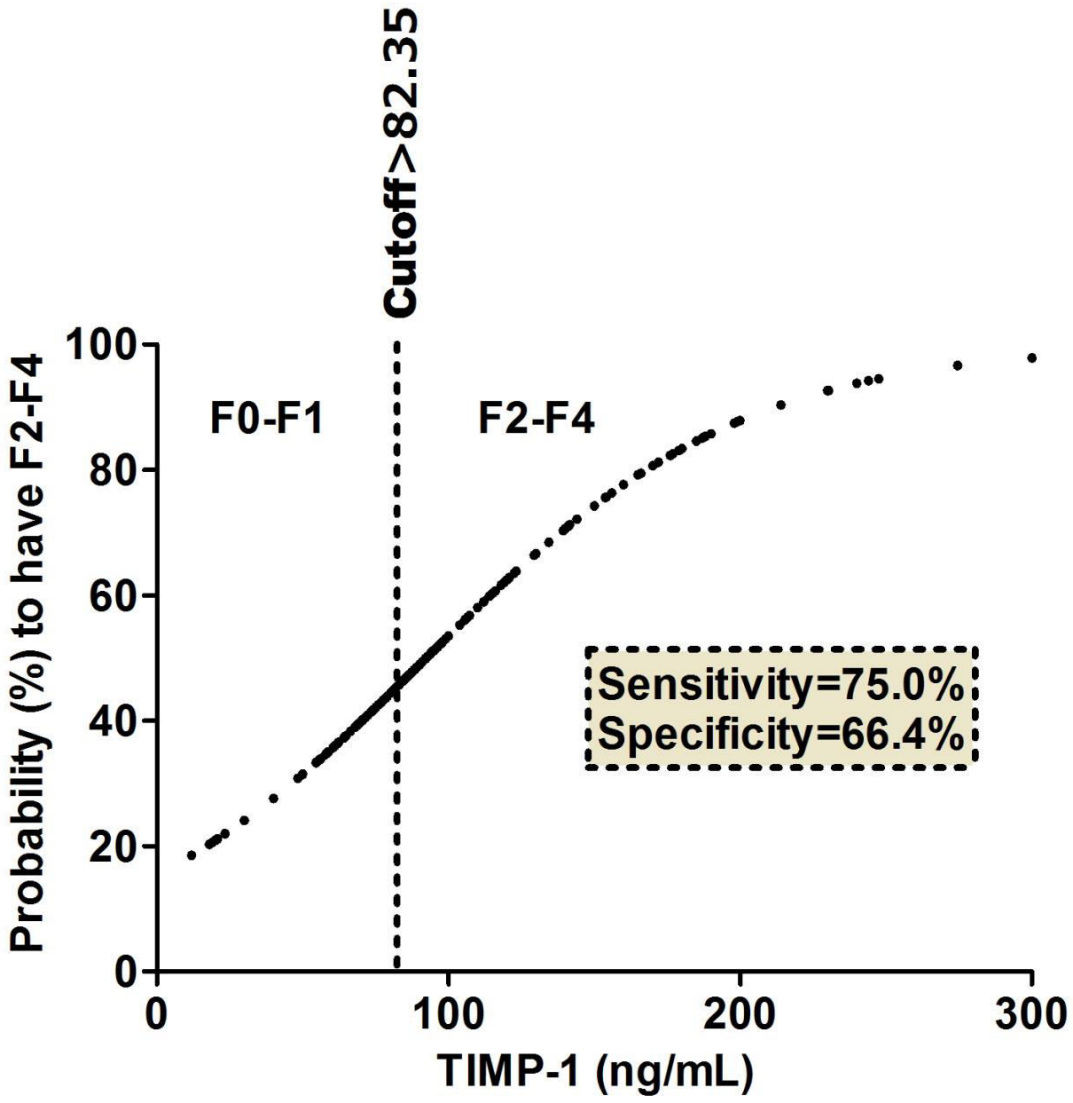

Figure 5. The probability of patients to have significant fibrosis (F2-F4) in relation to TIMP-1. TIMP-1 greater than 82.35 indicates F2-F4 while PIIINP/Platelets ratio equal to or less than 82.35 indicates F0-F1.

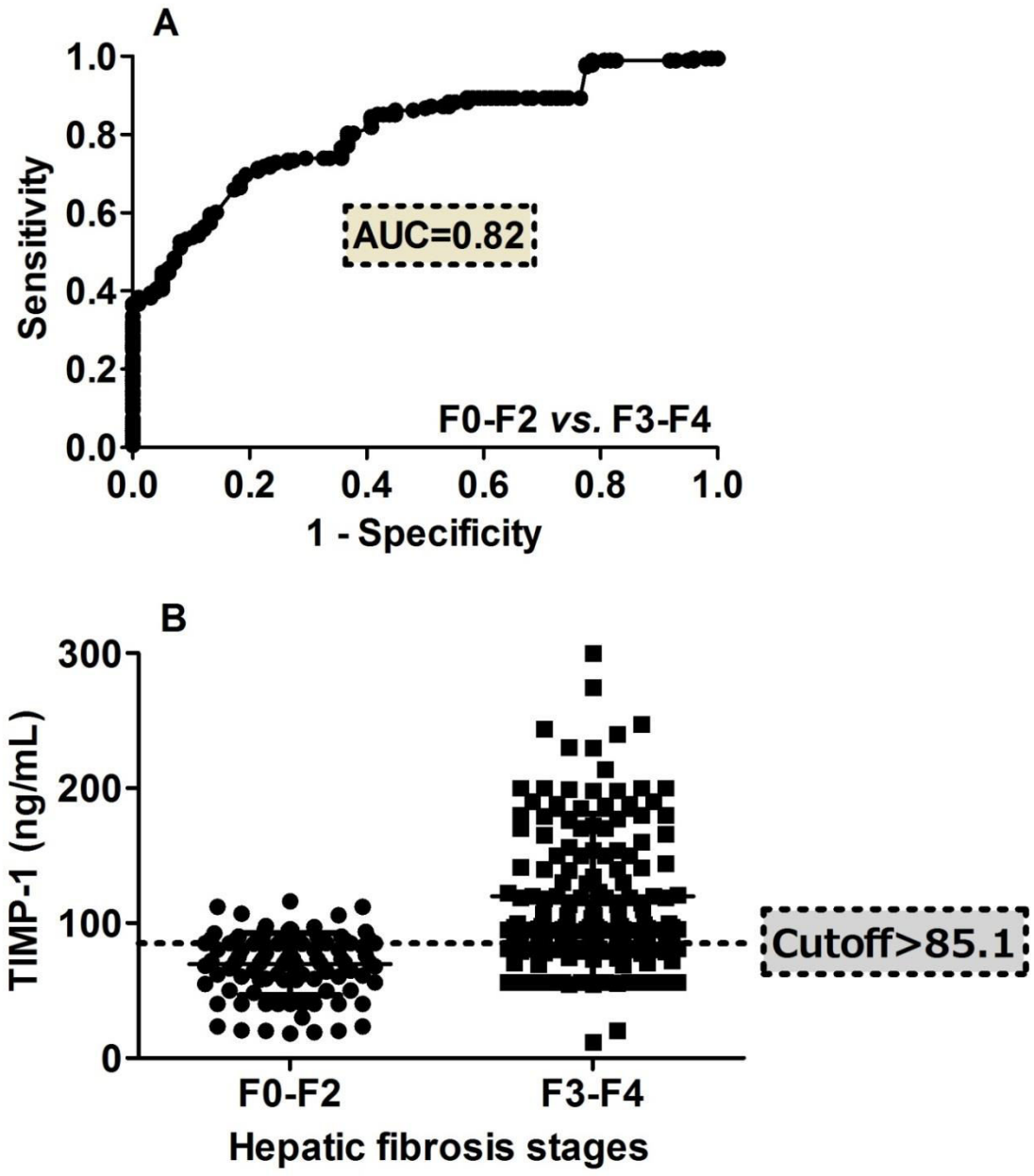

Figure 6. Diagnostic accuracy and distribution of TIMP-1 for separating patients who developed advanced fibrosis (F3-F4) from those without advanced fibrosis (F0-F2).

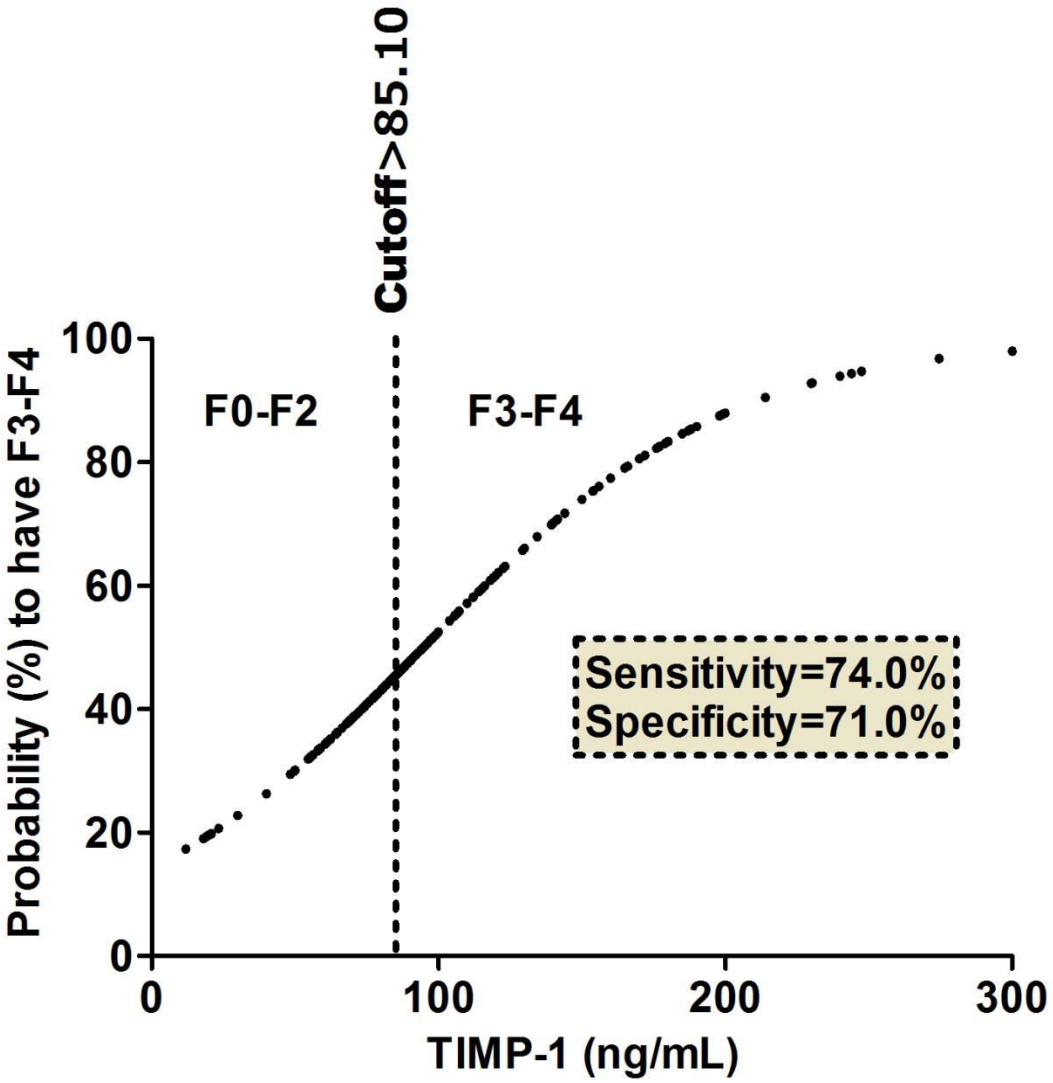

Figure 7. The probability of patients to have advanced fibrosis (F3-F4) in relation to TIMP-1. TIMP-1 greater than 85.10 indicates F2-F4 while PIIINP/Platelets ratio equal to or less than 85.10 indicates F0-F1.

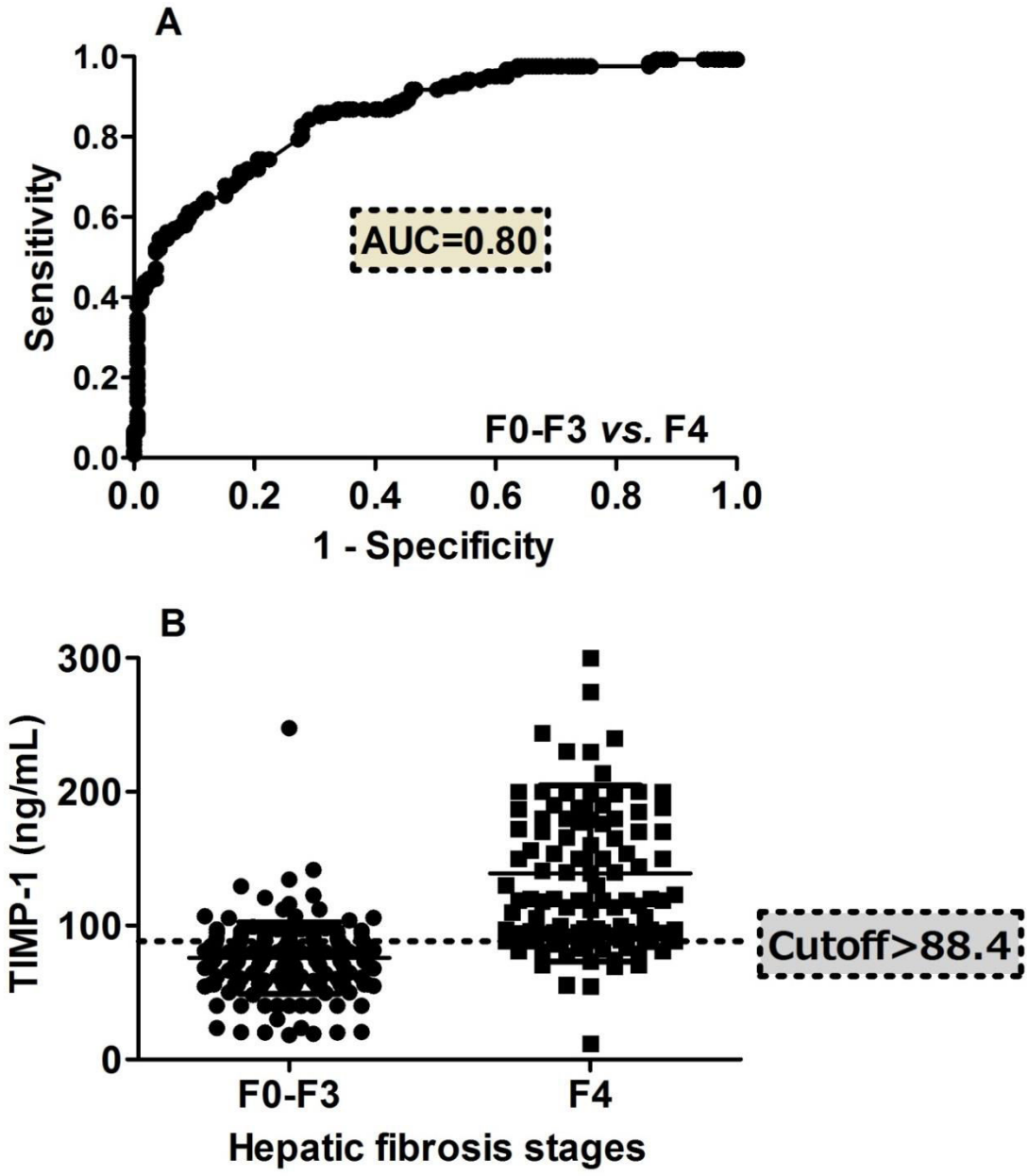

Figure 8.Diagnostic accuracy and distribution of TIMP-1 for separating patients who developed cirrhosis (F4) from those without cirrhosis (F4).

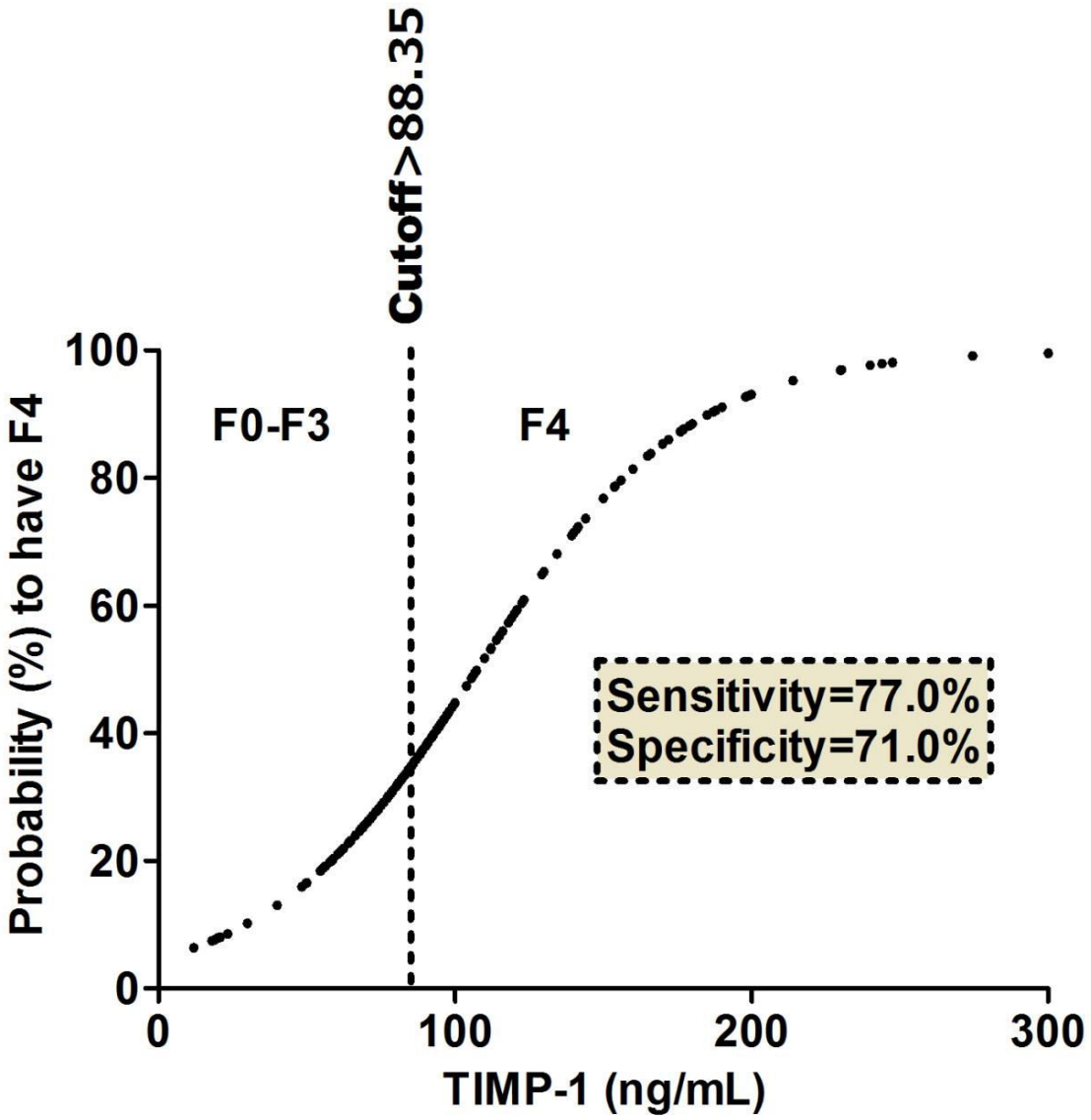

Figure 9. The probability of patients to have cirrhosis (F4) in relation to TIMP-1. TIMP-1 greater than 88.35 indicates F2-F4 while PIIINP/Platelets ratio equal to or less than 88.35 indicates F0-F1.

Table 1. Patients' characteristics

Variables [a]	Different hepatic fibrosis stages								
	F0-F1	**F2-F4**	***P***	**F0-F2**	**F3-F4**	***P***	**F0-F3**	**F4**	***P*** [*]
Age (years)	47.5±11.0	54.5±9.1	<0.0001	48.0±10.6	54.8±9.1	<0.0001	50.9±10.6	54.8±9.1	0.001
BMI (kg/m^2)	24.6±7.4	32.2±11.8	0.004	26.3±8.8	32.2±11.9	0.018	30.1±13.2	29.3±5.0	0.748
FBG (mg/dL)	105±32	111±35	0.213	104±30	111±39	0.083	109±35	109±39	0.853
INR	1.0±0.1	1.1±0.1	0.063	1.0±0.1	1.1±0.1	0.008	1.0±0.1	1.1±0.1	0.557
Platelets (×10^9/L)	199±73	156±67	<0.0001	196±8	155±66	<0.0001	180±70	153±71	0.002
AST (U/L)	60.0±34.6	65.6±35.2	0.213	59.8±34.1	66.1±35.2	0.143	62.5±36.1	66.1±33.8	0.392
AST/ALT	1.1±0.6	1.0±0.1	0.550	1.1±0.5	1.1±0.1	0.908	1.08±0.5	1.12±0.61	0.453
Albumin (g/dL)	4.0±0.5	3.8±0.5	0.002	4.0±0.5	3.7±0.5	0.0001	3.9±0.5	3.7±0.4	0.0001
Total bilirubin	0.8±0.4	0.9±0.3	0.097	0.8±0.4	0.9±0.3	0.085	0.8±0.4	0.9±0.3	0.725

Values were expressed as mean ± SD. [a]Reference values: Body mass index (BMI) 18.5 – 24.9; Fasting blood glucose (FBG) 70-110 mg/dL; International normalized ratio (INR) 1; Platelet count 150-400 ×109/L.Aspartate aminotransferase (AST) (male up to 37 U/l, female up to 31 U/l); albumin 38-54 g/dL; total bilirubin up to 1 mg/dL.

[*]$P>0.05$ is considered non-significant, $P<0.05$ is considered significant..