Positive Pattern Recognition System Using Alanine Aminotransferase, TypeIV Collagen 7S and E Value (Liver Stiffness) for the Diagnosis of Nonalcoholic Steatohepatitis Based on Natural History

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Abstract

Background: A simple diagnostic system for nonalcoholic fatty liver disease (NAFLD) instead of a biopsy is expected. We investigated the possibility of a positive pattern recognition system for evaluation of nonalcoholic fatty liver (NAFL) and the stages of nonalcoholic steatohepatitis (NASH).

Methods: 68 Japanese patients with biopsy-proven NAFLD were enrolled. Serological biomarkers and markers obtained by medical imaging were investigated to explore candidates for diagnostic system. After selected markers were statistically evaluated, every patient was distributed in pattern combinations.

Results: We selected three markers based on natural history and decided the critical values: alanine aminotransferase/ALT (persistent ≧ 45 IU/L) as hepatitis marker, type Ⅳ collagen 7S (≧ 5.1 ng/ml) as fibrosis one and E value (≧5.5 kPa) as stiffness one. After we statistically evaluated their accuracies, every patient was classified into their combination patterns. Major patterns were limited to four. Comparing relationships between histological classifications and positive patterns , the patients with NAFL were mainly distributed in pattern (ALT, type Ⅳ collagen , E value : -, -, -), those with NASH stage 0-1 in (+, -, +), those with NASH stage 2-3 in (+, +, +), and those with NASH stage 4 in (-, +, +).

Conclusion: The positive patters changed with NAFL and NASH conditions. Our results showed a correlation between the positive patterns using three markers and histological results. Positive pattern recognition system based on natural history is useful in a differential diagnosis of NAFLD and for evaluation of the severity of fibrosis in patients with NASH.

Background

Since the prevalence of diabetes, metabolic syndrome and obesity has increased in the past couple of decades, it is estimated that 20~30% of adult population suffered from a nonalcoholic fatty liver disease (NAFLD) in developed and developing countries[1]. According to the national wide survey, the major complications of diabetes were not only vascular diseases but also liver diseases, especially hepatocellular carcinoma (HCC) and liver cirrhosis (LC). Therefore,

NAFLD has become one of the most common diseases and it is a severe problem in the world[2,3]. NAFLD includes a wide spectrum of liver diseases. The histological changes range from nonalcoholic fatty liver (NAFL) which is generally non-progressive, to nonalcoholic steatohepatitis (NASH) which can progress to chronic hepatitis, liver cirrhosis and sometimes hepatocellular carcinoma[4,5].

Liver biopsy remains a reliable method for the diagnosis of NASH, and it is recommended as a gold standard for staging and grading[6,7]. This procedure, however, is invasive and has complication risks, sampling error and also it is practically difficult to perform for every patient with NAFLD since patient number is too large. Therefore, it is expected to establish a simple, noninvasive system to distinguish NASH from NAFLD and determine NASH stage. Many scoring systems have been reported and a new trial to identify multi-biomolecules for immunological responses and for diagnostic tools by data mining was

also reported[8]. The aim of this study was to investigate the clinical markers and to develop a simple system for a diagnosis and for an evaluation of the severity of liver fibrosis in patient with NASH. To explore and select the candidate of clinical markers, we focused the change of inflammation, fibrosis and diagnostic imaging along with the natural history of NASH, and investigated the possibility of pattern recognition system for NASH diagnosis.

Methods

Ethical committee approval

The protocol of this study was approved by the ethics committee at Tenshi Hospital. Informed consent was obtained from all patients before liver biopsy and blood sampling, and the study was conducted in accordance with the Helsinki Declaration.

Patients

From a total of 269 patients who had liver dysfunctions or had been diagnosed as fatty liver, chronic hepatitis or liver cirrhosis with a medical image diagnostic system at Tenshi Hospital between January 1, 2017 and July 31, 2019, 68 NAFLD patients with well characterized and liver biopsy-confirmed NAFLD were included in this study. The exclusion criteria included patients with a history of hepatic disease, such as chronic hepatitis B or C, autoimmune hepatitis, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), biliary obstruction and Wilson's disease. Men who consumed more than 30g alcohol per day and women who consumed more than 20g alcohol per day were also excluded.

Clinical and laboratory assessment

For body measurement, obesity was defined as body mass index (BMI), and visceral fat (VF) and liver/spleen(L/S) ratio were measured by CT imaging to see a metabolic syndrome and a fatty liver. Blood samples were taken in the morning after an overnight fasting to measure serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), fasting immunoreactive insulin(F-IRI), ferritin, type IV collagen 7S, hyaluronic acid and to count platelets.

Patients were assigned a diagnosis of lifestyle related diseases. Dyslipidemia

and hypertension were diagnosed according to each diagnostic criterion. Diabetic patients were assigned a diagnosis of diabetes mellitus (DM) if they had documented use of oral DM medication or got diagnosed to DM criteria,

Transient elastography

Liver stiffness[9] was measured in the right lobe of the liver, using a Fibroscan (Echo Sens, Paris, France) through the intercostal spaces of patients in the dorsal decubitus position. The vibration induces an elastic shear wave and the propagation and velocity of the wave are measured by simultaneous

ultrasonography and expressed in kilopascals (kPa). The median value was determined as a representative of the liver elastic modulus. The details have been described in previous report[9]. The median values obtained by vibration controlled transient elastography (VCTE) were called as E (elasticity) value. The Controlled Attenuation Parameter (CAP) specifically targets liver steatosis using a process based on Fibroscan. Principle of CAP measurement has been described elsewhere[10]. CAP was computed only when the associated liver stiffness measurement was valid. Both stiffness and CAP were obtained simultaneously. The final CAP value was the median of individual values and was expressed in dB/m.

Histological assessment

Consecutive patients with NAFLD (n=68) undergoing liver biopsies at Tenshi Hospital, Sapporo, Japan were recruited. Histological scoring was performed by an expert pathologist blinded to all clinical information, according to the NASH Clinical Research Network Scoring System[6]. The level of disease severity was assessed using the NAS (NAFLD activity score) as the unweighted sum of scores of steatosis, hepatocyte ballooning and lobular inflammation. According to the liver histology, three groups were identified:

Subgroup 1 (NAFL) included patients with simple steatosis or non-alcoholic fatty liver (NAFL =NAFLD but no NASH) (n=19)

Subgroup 2 (NASH): NASH without advanced fibrosis (fibrosis score 0-1) (n=33)

Subgroup 3 (NASH with fibrosis): NASH with advanced fibrosis (fibrosis score 2-3), but no cirrhosis (n=14).

Subgroup 4 (NASH with liver cirrhosis): NASH with cirrhosis (fibrosis score 4) (n=2)

After histopathological evaluation, the presence of NAFL was confirmed in 19 patients (7 males and 12 females: mean age of 64.1 years), that of NASH stage 0-1 was in 33 patients (13 males and 20 females: mean age of 55.9 years), that of NASH stage 2-3 was in 14 patients (5 males and 9 females: mean age of 60.9 years) and that of NASH stage 4 was in 2 patients (1 male and 1 female: mean age of 68.5 years), which were enrolled in the present study (Fig1).

Statistical analysis

The quantitative measurements are presented as the means and standard deviation (SD) or results are presented as numbers with percentages.

Statistical differences between NAFL and NASH were determined using the t test for quantitative data or chi-square test for categorical date (numbers with percentages). Multivariate analysis was performed by logistic regression analysis to identify variables independently associated with the presence of NASH and those associated with the natural history of NASH. We calculated the sensitivity, specificity to assess the accuracy of the clinical scoring system in determining NASH and NAFL. Using these results, we

constructed receiver operating characteristic (ROC) curves by plotting the sensitivity against 1-specificity at each value. The diagnostic performance of the prediction models was assessed by analysis of ROC curves. The area under the ROC curve (AUROC) was used as the statistical measure of accuracy[11] with values close to 1.0 indicating high diagnostic accuracy. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated to evaluate the accuracy of selected scores. P value were calculated by t test or chi-squared test, and differences were considered statistically significant at $p< 0.0001$.

Results

Patient characteristics

Table 1 summarizes the clinical characteristics of NAFL and NASH patients, showing body measurements, diagnosis of lifestyle related disease, biochemical data, diagnostic imaging marker, E, CAP measured with Fibroscan, liver/spleen (L/S) ratio measured with CT scan.

Statistical analysis showed that presence of type 2 DM, F-IRI and E value were significant variables. Type 2 DM could not be a marker, and F-IRI would be useful for NASH diagnosis. However, we did not select either type 2 DM or F-IRI in this study, since these markers would not change liver histology.

Selection of the predictors for NASH

We focused that inflammation and fibrosis markers should be selected as the predictors for NASH, since histological imaging was certainly affected by them according to natural history of NASH.

ALT is a marker of hepatitis level. ALT was not good enough for differential diagnosis of NASH from NAFL (p value 0.0186, Table1). However, we have experienced many NASH patients who showed persistent abnormality of ALT (more than 45 IU/L) for 3 months and more, while NAFL patients showed only a temporary higher value (Fig2). To evaluate the accuracy of persistent ALT (≧45 IU/L) in discriminating between NAFL and NASH, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPT) were calculated as 79.6, 84.2, 92.9 and 61.5%, respectively. The ratio of persistent ALT abnormality was 3/19 (15.8%) for NAFL, and 39/49 (79.6%) for NASH. Statistical analysis showed it significant: NAFL vs all NASH, chi-square test <0.0001, shown in Table 2.

Type IV collagen 7S is known as a marker of liver fibrosis. But the results of this study about type IV collagen 7S did not show the significant t test (p value 0.0013) between NAFL vs NASH (Table 1). It is shown that type IV collagen 7S values of $NASH_{S2\text{-}3}$ (5.9±1.1 ng/ml) and NASH s**4**(12.2±2.5 ng/ml) were higher than those of NAFL (3.7±0.4ng/ml) and $NASH_{S0\text{-}1}$(4.4±1.4ng/ml) in Fig 3-a.

Receiver-operating characteristic (ROC) curves of the type IV collagen 7S were plotted in Fig 3-b. The first ROC curve (1) depicts the ability to discriminate between NAFL patients and NASH patients. At a cutoff value 3.9 ng/ml, the sensitivity, specificity, PPV and NPV were 72.3, 84.2, 91.9 and 55.2%, respectively.

Area under the curve (AUROC) was 0.823. Strictly speaking, type Ⅳ collagen 7S could not be good enough to differentiate all NASH from NAFL. According to the second ROC curve (2) for differentiation between NAFL / $NASH_{S0-1}$patients and $NASH_{S2-3}$ / NASH s4 patients, the sensitivity, specificity, PPV and NPV were 92.9, 86.5, 65.0 and 97.8% at a cutoff value 5.1ng/ml and the AUROC was 0.896 (Fig 3-b). T test revealed a significant difference (p value <0.0001) between NAFL / $NASH_{S0-1}$vs $NASH_{S2-3}$ / NASH s4 (Table 2).

E value (kPa) was selected as the third marker. E value expresses the level of liver stiffness measurement with Fibroscan. Fig 4-a shows that the distribution of E values changed higher from NAFL, $NASH_{S0-1}$, $NASH_{S2-3}$ to NASHs4 together with the fibrosis and/or chronic inflammation. The median liver stiffness values were NAFL 4.9±1.1, $NASH_{S0-1}$ 8.8±3.0, $NASH_{S2-3}$ 13.3±6.2 and NASHs4 48.5±14.2 kPa. The AUROC of the E value were plotted in Fig.4-b. This curve depicts the ability to distinguish between NAFL patients and all NASH patients. The sensitivity, specificity, PPV and NPV were 93.6, 83.3, 91.7 and 82.4% respectively at a cutoff value 5.5 kPa. The AUROC was 0.926 (Fig.4-b). Furthermore, t test revealed a significant difference (p value <0.0001) between NAFL and NASH (Table 2).

From the results shown above, we selected three markers: (1) persistent abnormality of **A**LT as a hepatitis one, (2) type Ⅳ **c**ollagen 7S as a liver fibrosis one and (3) **E** value as a liver stiffness measurement including fibrosis and chronic inflammatory activity. These markers complementarily can detect the characters of NASH. We called the ACE system using three markers as a pattern recognition method.

Pathological conditions of NASH change from fatty liver to chronic hepatitis, liver cirrhosis and hepatoma along a natural history. The positive patterns using three markers must change along the different state, since the medical conditions of NAFL and every stage of NASH reflect the difference of hepatitis, fibrosis and stiffness.

Positive pattern combinations using three markers (ACE system)

The total number of combination would be eight: all positive 1, two positive one negative 3, one positive two negative 3, and all negative 1, shown in Fig. 5-a (□□□). Fourteen patients of NAFL showed all negative □, 19 patients of $NASH_{S0-1}$ showed positive for ALT and E value □ and 11 patients of $NASH_{S2-3}$ showed all positive □. Almost none of the patients showed type Ⅳ collagen 7S positive and E value negative □ and □. Since the sensitivity of E value was higher than that of type IV collagen 7S, both □ and □ groups could be deleted from Fig. 5-b.

Fig.5-b summarizes the relationship between the positive patterns using three markers and pathological condition (NAFL, NASHs0-1, NASHs2-3 and NASH s4). Considering the natural history of NAFL and NASH, major conditions are following: NAFL pattern □ all negative, NASH hepatitis pattern □ (ALT(+),□ collagen(-), E(+)), NASH fibrosis pattern □ (ALT(+), □ collagen(+), E (+) all positive) and NASH liver cirrhosis pattern □ (ALT(-), □ collagen(+), E (+)). Pattern □ (ALT(+), □ collagen(-), E(-)) suggested early hepatitis pattern, which showed the stage when NAFL and NASH could not be distinguished. Pattern □ (ALT(-), □ collagen(-), E(+)) was shown by some of $NASH_{S0-1}$ patients. It is supposed that NASH patients had hepatitis before, but

had not during liver function tests. These patients who were pathologically diagnosed as $NASH_{S0-1}$, showed that only E value was positive. This condition suggests that E value measurement is a sensitive for liver stiffness remained in NASH patients even if the inflammation happened in the past. Described above, pattern (NASH post-hepatitis pattern) would be situated between and .

Frequency distributions of NAFL and NASH cases are shown in Fig.6. NAFL, NASH s0-1, NASH s2-3, NASH s4 cases had peaks at combination, , , and , respectively. It should be noted that these NAFLD cases lined up in order and properly distributed in the combination patterns.

Comparing the ACE score to previously reported scoring systems

FIB4 index[12,13], NAFLD fibrosis score (NFS)[14,15] and BARD score[16,17]were previously established and were utilized for predicting advanced fibrosis (stage3 and 4) versus others. On the other hand, NAFIC score[18] used to be utilized to differentiate between NAFL and NASH patients. Formulas of these scoring systems were described in Table 3.

In order to compare the difference between our system and others for differentiating NAFL and NASH, each positive pattern combination was weighted 0 ~ 3points as ACE score.

ACE score was determined; combination NAFL main was given 0 point, and 0.5point, (early NASH) 1point, (middle NASH) 2points, and (late NASH) 3points respectively.

The AUROCs of these scoring systems were summarized in Table3 and Fig 7. The AUROC was greatest for ACE score (0.953), followed by NAFIC score (0.898), FIB4 index (0.633), NFS (0.592) and BARD score (0.542) (Table 3). According to these results, NAFIC score was useful for differentiating NASH from NAFL, but ACE score was superior to other scores to distinguish NAFLD patients.

Discussion

We could establish the positive pattern recognition system using three markers to differentiate NASH from NAFLD. Furthermore, the results of positive patterns correlated with histological staging of NASH and NAFL.

Many scoring systems[19-22]for diagnosis of NAFLD instead of liver biopsies have been reported. As most of them were based on the formulas using several

biomarkers, the results were expressed as numeric values. However, the concept of our study is different, because the results were expressed as positive pattern combinations decided by existence of chronic hepatitis, progression of liver fibrosis and degree of liver stiffness. Though the histological evaluation is about steatosis, hepatocyte ballooning and inflammation as a grading and fibrosis as a staging,[7] major tissue characteristic of image must be basically influenced by inflammation and fibrosis. Consequently, if

both chronic hepatitis and progressive fibrosis were properly estimated, it may be possible to decide the stage of NASH along with natural history.

The main purposes of recent studies have tended to detect advanced fibrosis NASH. Because the progression of fibrosis is the major risk factor of NASH to determine its prognosis[23,24]. However a satisfactory treatment for advanced fibrosis NASH and NASH□LC has not been established yet. Therefore, it is the most important that we can make an accurate diagnosis of early NASH and then therapeutic interventions (medication, body weight control, control of insulin resistance) should be begun in an early stage to prevent serious complications.

On the other hand, NASH is not a single disease state, since NASH would change from fatty liver to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. According to the clinical observations of NASH patients in general, the hepatitis has been persistent in early and middle period, if there is no treatment (□, □ in Fig.5-a,b), while NAFL patients do not have persistent hepatitis, if any, only temporary hepatitis. Therefore, persistent ALT abnormality could be a marker to differentiate NASH from NAFL. Another point is that ALT values show abnormally up in NASH/ hepatitis~ early fibrosis stage and then it becomes down within normal limit when there is no hepatocyte to break in the decompensated liver cirrhosis stage, since ALT is a deviation enzyme.

These are the reasons that it has been told that ALT is not suitable as a marker.

As ALT would change along with natural history and be useful for positive pattern recognition, we selected the persistent ALT abnormality as a marker. The optimal cut off value was persistent 45 IU/ml of ALT for more than 3 months and the results for sensitivity, specificity, PPV and NPV were 83.0, 84.2, 92.9 and 66.7%, respectively. The ratio of persistent abnormality of ALT was 15.8% for NAFL, and 83.0% for $NASH_{S0-1}$ + $NASH_{S2-3}$ and 0% NASHs4. Chi-square test showed statistically significant difference ($P<0.0001$) between NAFL and NASH (Table 2). Utilizing this marker, we could differentiate NASH (39/49=80%: ALT (+)) from NAFL (16/19=84%: ALT (-)). Positive pattern recognition system using ALT was better to distinguish between NASH/hepatitis~ early fibrosis and not only NAFL but also NASH/LC.

There are many markers of fibrosis, for example type □ collagen 7S, hyaluronic acid, mac-2 binding protein (M2BPGi), procollagen Type □ peptide (P-□-P) and platelet count. Among these markers, type IV collagen 7S is known to have a characteristic of showing a stable value for a liver fibrosis[25].

But, the statistical results about type □ collagen 7S did not show the significant t test (P value 0.0013) between NAFL vs NASH (Table1). However, the values of $NASH_{S2-3}$ (5.9±1.1ng/ml) and NASHs4 (12.2±2.5ng/ml) were higher than those of NAFL (3.7± 0.4ng/ml) and $NASH_{S0-1}$ (4.4±1.4ng/ml) (Fig 3-a). When the second ROC curve (No.2 in Fig 3-b) depicts the ability to distinguish between NAFL + $NASH_{SO-1}$ and $NASH_{S2-3}$ +NASHs4 and AUROC was 0.896 at a cut off value 5.1 ng/ml. T test revealed a significant difference (p value <0.0001, Table2). Therefore, type □ collagen 7S was a useful marker to distinguish between NAFL + early NASH and fibrosis NASH, but not between NAFL and all NASH.

We identified E(elasticity) value as the third predictor for NASH diagnosis that was obtained by a medical imaging, not a chemical biomarker. The distribution of E values sensitively changed from NAFL (4.9±1.1kPa) to $NASH_{S0-1}$ (8.8±3.0kPa), $NASH_{S2-3}$ (13.3±6.2kPa) and NASHs4(48.5±14.2kPa), shown in Fig 4-a. These results have shown that the liver stiffness measurement expressed with E value would be influenced not only by liver fibrosis but also by chronic inflammation of the liver[26].

Calculating with ROC curve of the E value, the sensitivity, specificity and AUROC were 93.6, 83.3 and 0.926 at a cutoff value 5.5 KPa (Fig 4-b). T test revealed a significant difference (p value <0.0001) between NAFL and all NASH.

Next, we analyzed the relationship between the positive pattern results using three markers and histological classification along natural history of NAFLD.

Fig.5-a showed that the total number of positive pattern cases would be 8 (□□□). When all cases of NAFL and NASH patients were distributed in the combination patters, there were only one case on pattern □ and no case on pattern □. This result showed that the combination (□ collagen (+), E(-)) was unnecessary, and could be deleted. It meant that E value test was more sensitive than type □collagen 7S test.

Fig.5-b shows the relationship between combination patterns and histological classification. The peaks for NAFL, $NASH_{S0-1}$, $NASH_{S2-3}$ and NASHs4 were pattern □, □,□ and □, respectively. The distribution of these four stages were orderly composed in line with natural history (Fig.6). Considering the minor condition □ and □, pattern □ (ALT(+), □ collagen(-), E(-)) showed that the distinction between NAFL and NASH could not be made, since there was only pathologically minimal change under early hepatitis.

NASH post-hepatitis pattern □ (ALT(-), □ collagen(-), E (+)) showed that NASH patients had hepatitis before, but had not when liver function test was done. The reason is that elastography for liver stiffness measurement might make it possible to detect previous traces with hepatitis or minimal fibrosis. Therefore, E value is useful for detecting seronegative conditions of old hepatitis or a little fibrosis.

In conclusion, the diagnostic system for NASH instead of liver biopsy is expected. Considering the natural history, three markers : (1) persistent abnormality of ALT for hepatitis, (2) type □ collagen 7S for liver fibrosis and (3) E value for liver stiffness were selected in order to differentiate NASH from NAFL in this study.

The point is that persistent abnormality of ALT for 3 months, but not higher value of ALT was selected as a marker for inflammation, because temporarily elevated ALT, even if the value was higher, was not immediately influential to fibrosis.

Another important point of this study is that positive pattern recognition taking advantage of three marker's strength was adopted. Because NASH is consisted of many conditions that shows the change of inflammation, fibrosis and stiffness.

Moreover, three markers would change independently, and then the patterns were different in each stage. Different positive patterns were orderly composed (Fig 6) along natural history of NAFL and NASH staging and furthermore, the results showed a strong correlation between positive patterns and histological classification (NAFL, $NASH_{S0-1}$, $NASH_{S2-3}$ and NASHs4).

Our study has several limitations. First, the total number of NAFLD patients was limited, and the percentage of NAFL patients and NASHs4 was 29% and 3%. We included patients that had a NASH like characteristic, and some referral bias could not be ruled out. As liver biopsy might have been considered for NAFLD patients who were likely to have NASH, selection bias could have existed. Other limitations are that we could include a small number of patients with NASH s4 (liver cirrhosis), and it could not determine that pattern was the major one for NASHs4. We are ready to organize clinical trials to carry out by cooperative groups and liver centers and to include a large number of patients who take part in many locations. Though there are limitations, it should be emphasized that we would like to propose the idea of a simple system. The positive pattern recognition system based on natural history, could predict NASH in NAFLD patients and the combination patterns showed a strong correlation with histological NASH staging.

List Of Abbreviations

NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; NAFL: nonalcoholic fatty liver; AST: aspartate aminotransferase; ALT: alanine aminotransferase; E value: elasticity value; CAP: controlled attenuation parameter; HCC: hepatocellular carcinoma; LC: liver cirrhosis; kPa: kilopascals; AUROC: area under the receiver operating characteristic; NPV: negative predictive value; PPV: positive predictive value;

Declarations

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Authors' contributions

MT: study concept, design and analysis, drafting of the manuscript, SS: critical revision of the manuscript for important intellectual content, approval of the final version, NA: study concept and design, statistical analysis, TT: acquisition of data, TI: critical revision of the manuscript for important content, MY: critical revision of the manuscript for important content, HI: critical revision of the manuscript for important content, YK: acquisition of data, HN: study supervision.

All authors read and approved of the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Tenshi Hospital. Patients provided written informed consent before enrolment. The study was conducted in accordance with the Declaration of Helsinki and informed consent was obtained from

all patients before liver biopsy and blood sampling.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1,2,3 is only available as a download in the Supplemental Files section.

Figures

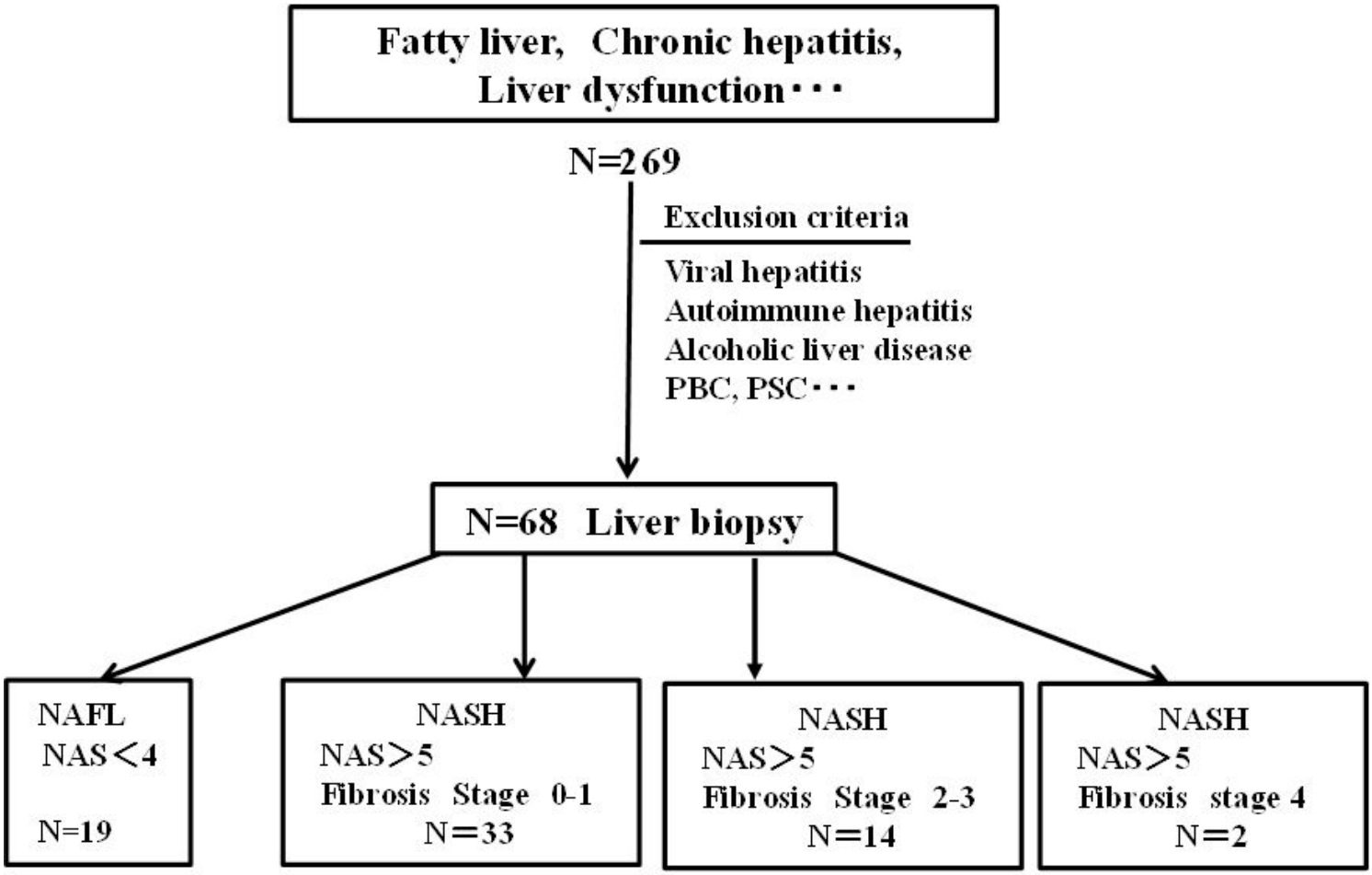

Figure 1

A schematic representation of the study design.

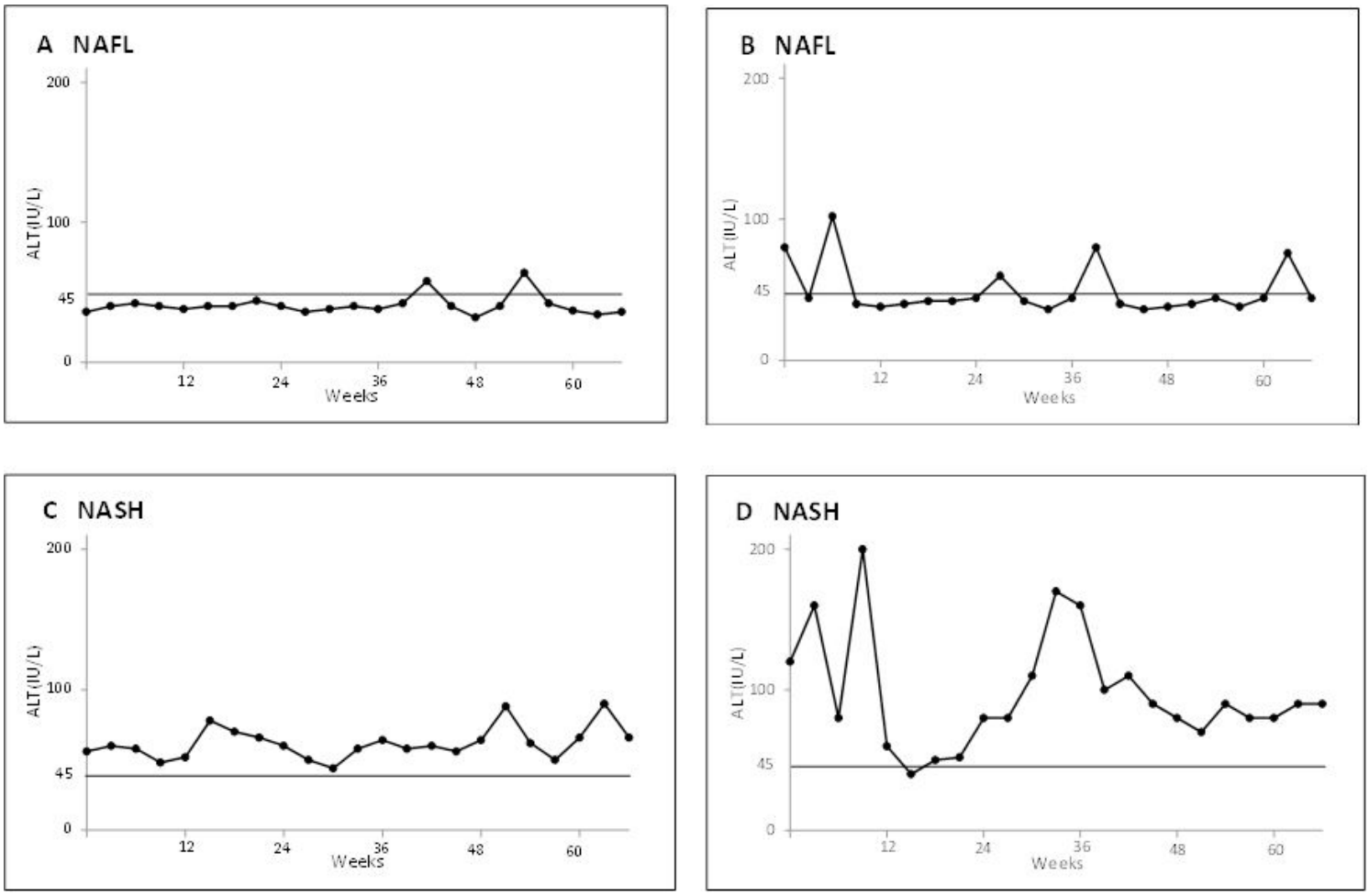

Figure 2

Representative clinical course of alanine amino-transferase (ALT) levels. NAFL patients showed only temporary higher values than optimal level of ALT 45 IU/L (A, B). However, NASH patients showed a persistent abnormality of ALT (more than 45 IU/L) for long-term period (C, D).

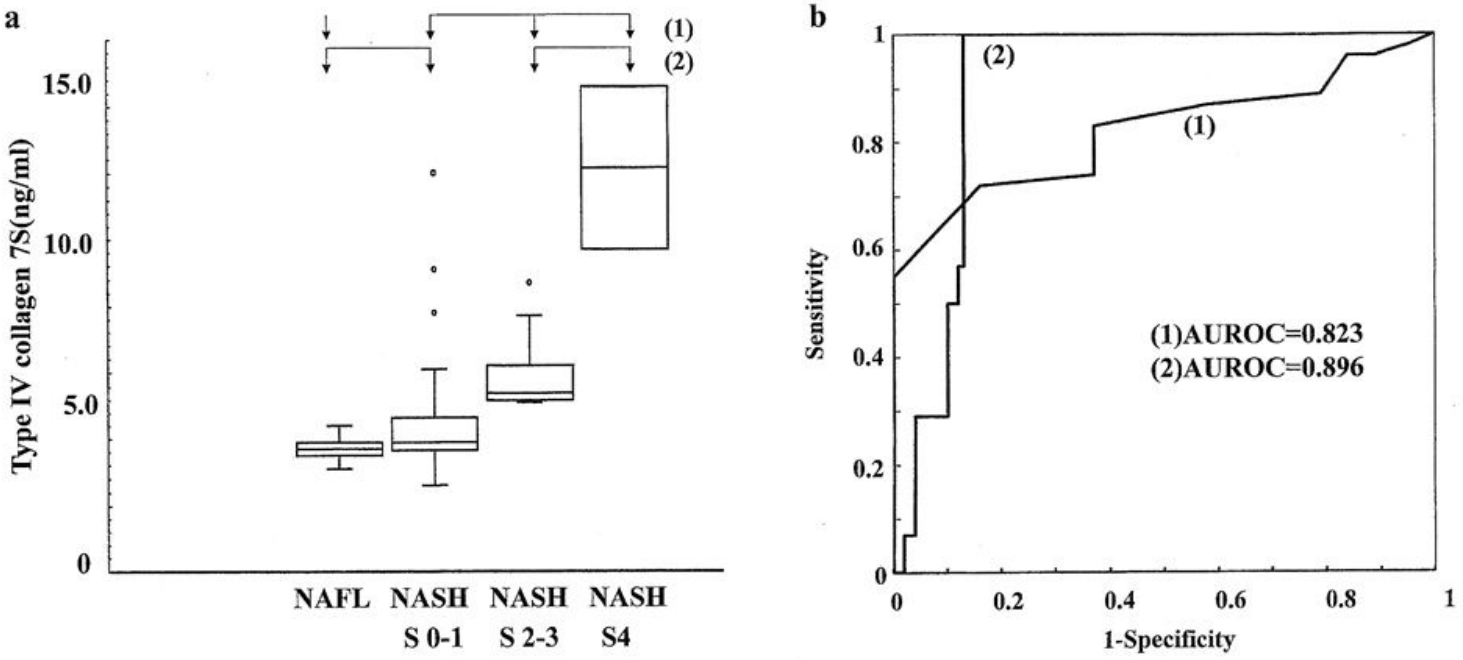

Figure 3

(a) Boxplots (median, upper and lower quartiles, range and outliers(circles)) of type IV collagen 7s. A stepwise increase in type IV collagen 7s was observed from NAFL to NASH s4. Note: (1): p< 0.0013 between NAFL and all NASH (2): p<0.0001 between NAFL / NASH s0-1 and NASH s2-3 / NASH s4. (b) Receiver-operating characteristic (ROC) analysis of type IV collagen 7S. The area under the ROC curve (AUROC) was calculated as (1) 0.823 and (2) 0.896.

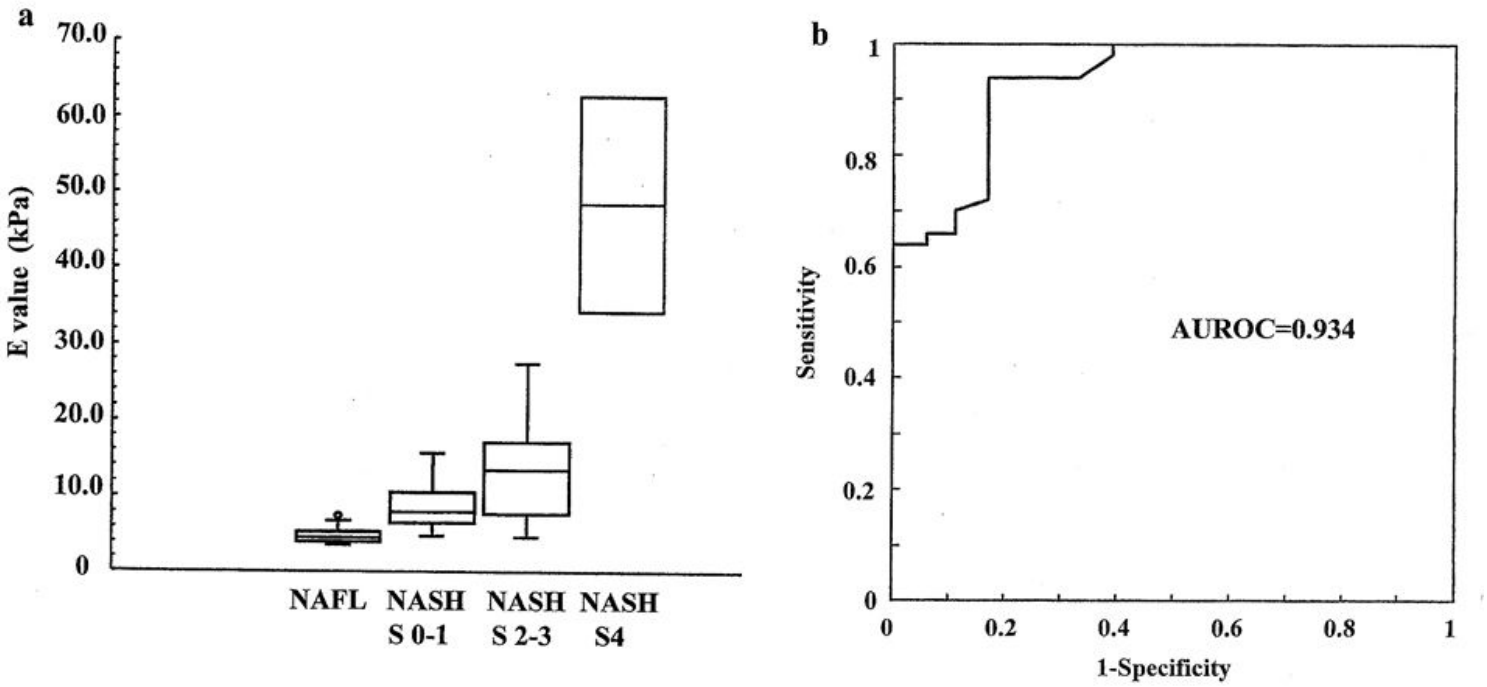

Figure 4

(a) Boxplots (median, upper and lower quartiles, range and outliers(circles)) of E value. A stepwise increase in E value was observed from NAFL to NASH s4. Note: p<0.0001 between NAFL and all NASH. (b) Receiver-operating characteristic (ROC) analysis of E value. The area under the ROC curve (AUROC) was calculated as 0.934.

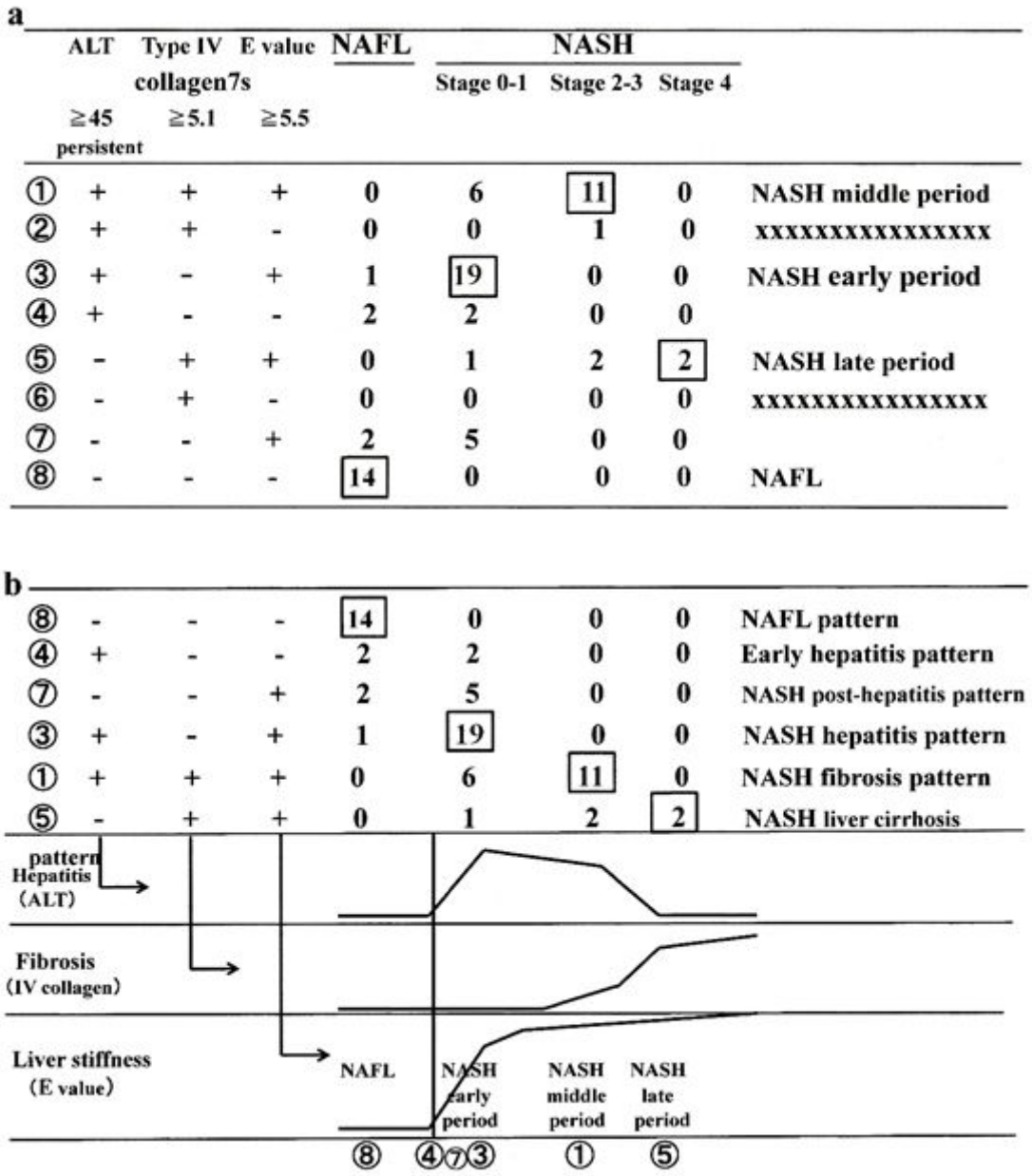

Figure 5

(a) The distribution map of NAFL and NASH patients in eight combination patterns using three markers. All NAFL patients distributed in combination□, most of NASH s0-1 ones in combination□, most of NASH s2-3 ones in combination□ and all of NASH s4 ones distributed in combination□. As most patients were not distributed in combination□ or □, □ and □ could be deleted from the table. (b) The case distributions to see the relationship between combination patterns and histological classification. According to natural history of NAFLD, most of NAFL, NASH s0-1, NASH s2-3 and NASH s4 cases distributed in□ (all negative), □(ALT(+), IV collagen(-), E(+)), □(all positive) patterns and □ (ALT(-), IV collagen(+), E(+)), respectively. These results showed a strong correlation between positive patterns and NAFL and NASH staging classification.

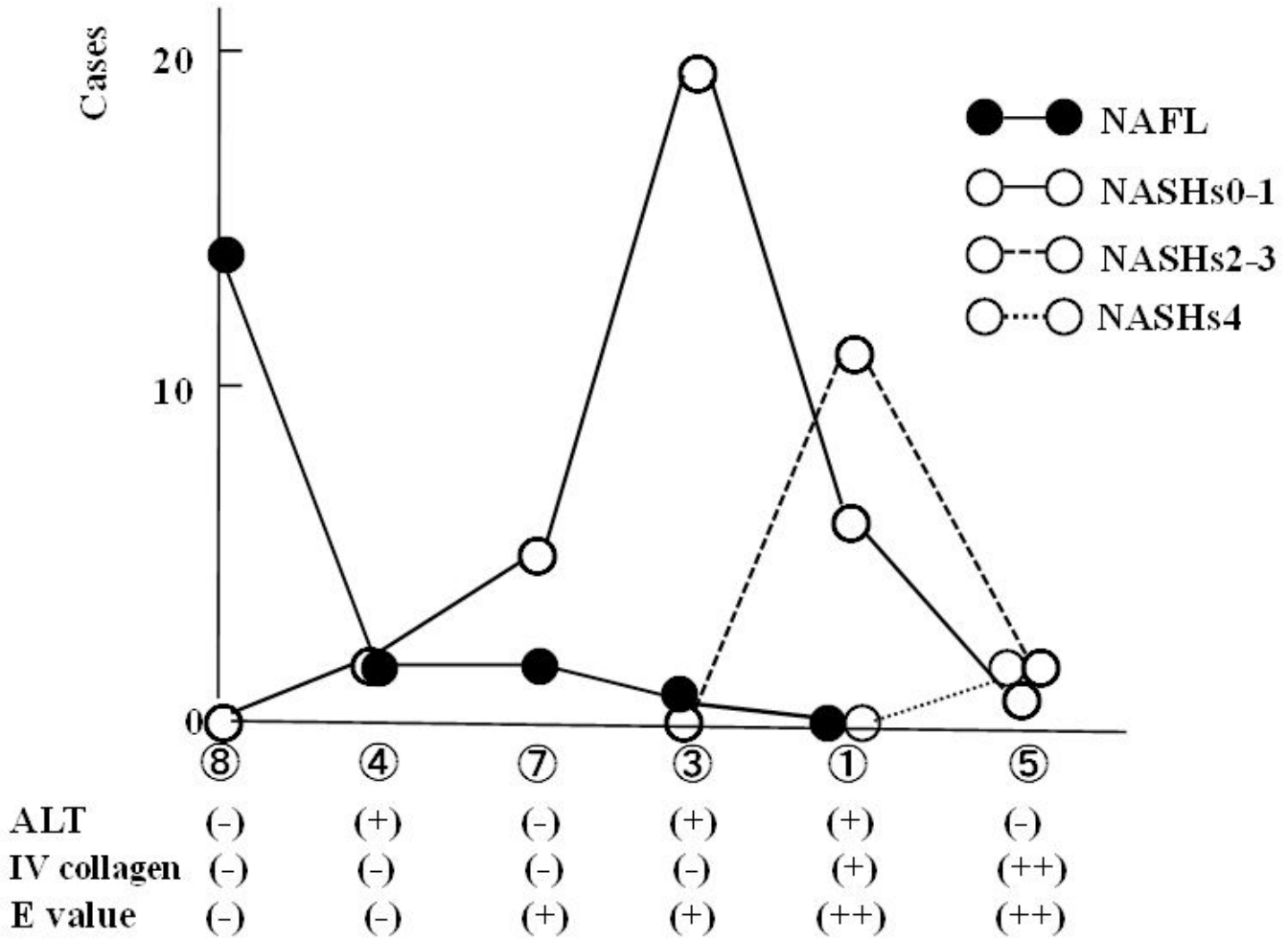

Figure 6

The distribution of NAFLD patients in combination patterns. Combination patterns were supposed to be lined up from□□□□□ to□ as shown in Fig.6. NAFLD patients were composed of NAFL,NASH s0-1,NASH s2-3 and NASH s4. Frequency distribution of cases as following: NAFL cases □(peak), □,□ and □NASH s0-1 cases □,□, □(peak),□ and □NASH s2-3 cases □(peak) and □NASH s4 cases □ (peak) Four types of NAFLD cases lined up in order and properly distributed in the combination patterns.

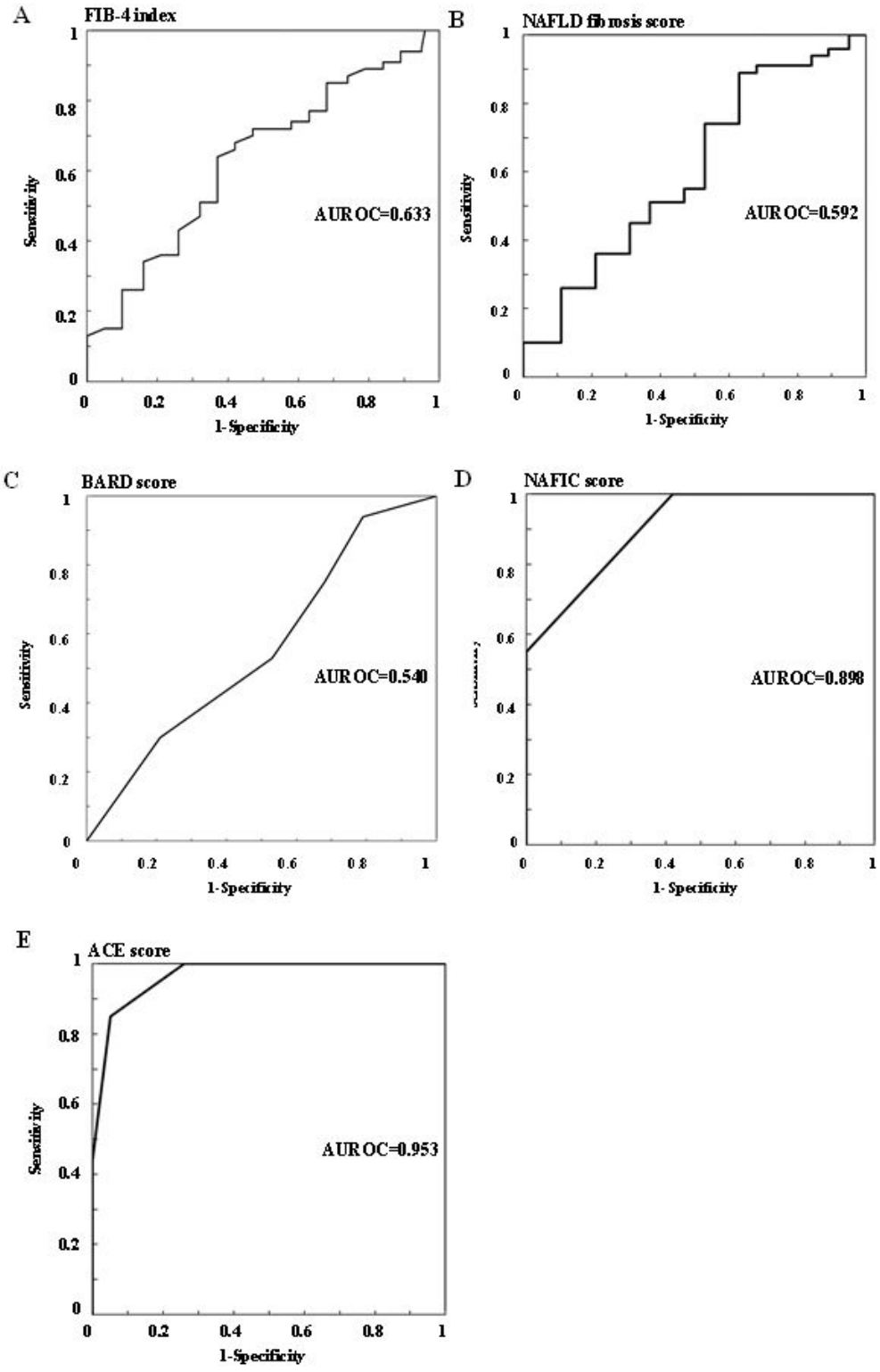

Figure 7

Comparison of ROCs among five scores. (A) FIB4 index, (B) NAFLD fibrosis score, (C) BARD,(D) NAFIC score, and (E) ACE score. The AUROCs were calculated as 0.633 (FIB4 index), 0.592 (NAFLD fibrosis score), 0.540 (BARD), 0.898 (NAFIC score) and 0.953 (ACE score). ACE score was superior to other scores to distinguish NASH and NAFL.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.jpg
- Table2.jpg
- Table3.jpg