Interleukin-2 receptor alpha as a biomarker for nonalcoholic fatty liver disease diagnosis

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Abstract

Background: Two recent studies in the adult and pediatric Nonalcoholic Steatohepatitis-Clinical Research Network (NASH-CRN) cohorts have shown that soluble interleukin-2 receptor alpha (IL2RA) levels increased with fibrosis severity. However, no hepatic study has been conducted in Asian morbidly obese patients who underwent bariatric surgery. In this study, we proposed IL2RA as a biomarker for nonalcoholic fatty liver disease (NAFLD) diagnosis and performed immunohistochemistry (IHC) staining of IL2RA.

Methods: This prospective cohort study enrolled 123 morbidly obese patients who underwent bariatric surgery at Taipei Medical University Hospital from October 2016 to June 2018. During bariatric surgery, all patients underwent a wedge liver biopsy under laparoscopic guidance. The diagnoses of NASH and liver fibrosis were made histologically. In IHC of IL2RA, the number of lymphocytes with IL2RA immunoreactivity was counted in five high-power fields (×400, total: 1.19 mm^2).

Results: Among the 123 patients, the mean age was 35.5 years, mean body mass index (BMI) was 40.6 kg/m^2, 87 (70.7%) were female, 25 (20.7%) had diabetes mellitus, and 57 (46.3%; 11 with non-NAFLD and 46 with steatosis) and 66 (53.7%) were included in the non-NASH and NASH groups, respectively. The NASH group had higher IHC of IL2RA than the non-NASH group. In multivariate analysis, IHC of IL2RA (odds ratio, 1.025; 95% confidence interval, 1.006–1.045; $p = 0.011$) and alanine aminotransferase (ALT; odds ratio, 1.045; 95% confidence interval, 1.018–1.073; $p = 0.001$) were the independent factors associated with NASH. The area under the receiver operating curve of IL2RA IHC for NASH was 0.627 at the cutoff value of 82 ($p = 0.0113$).

Conclusion: IL2RA is significantly associated with NASH in morbidly obese patients and would be a useful biomarker for NASH diagnosis.

Keywords: Bariatric surgery; Interleukin-2 receptor alpha; Nonalcoholic steatohepatitis

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world.[1,2] NAFLD is defined as steatosis affecting >5% of hepatocytes in the absence of excessive alcohol consumption, other liver disease, or steatogenic drug use. The histological spectrum of NAFLD includes nonalcoholic fatty liver (NAFL; steatosis without hepatocellular injury), nonalcoholic steatohepatitis (NASH; steatosis with lobular inflammation and hepatocellular ballooning), fibrosis, and cirrhosis. Patients with advanced fibrosis have higher risks of overall mortality and liver-related events such as portal hypertension, hepatic failure, and hepatocellular carcinoma (HCC).[3,4] The development of NAFLD is the result of a combination of genetic, environmental, and metabolic factors, including central obesity, insulin resistance, hypertension, and hypertriglyceridemia.[5] The prevalence rates of NAFLD and NASH in morbidly obese patients undergoing bariatric surgery have been reported up to 74%–90% and 50.8%–71.3% in Taiwan.[6–9]

Currently, approximately 25%–30% of people with NAFLD have been estimated to develop NASH, with hepatic fibrosis development in 40%–50% of patients with NASH.[5,10] A meta-analysis of 40 studies, it has been estimated that NASH increases the risk of liver-related mortality by 5- to 10-fold, mainly depending on the degree of hepatic fibrosis.[11] Although the major risk factors for hepatic fat and hepatic fibrosis development in NAFLD have been well established [eg, age >50 years,

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Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 261-266.

Received October 11, 2020; accepted October 12, 2020.

doi: 10.1097/JCMA.0000000000000469.

obesity, insulin resistance, diabetes mellitus (DM), hypertension, hyperlipidemia, and metabolic syndrome],[12] the pathological mechanisms by which each of these risk factors cause NAFLD progression are unclear. Poor diet (particularly high-fat and high-fructose intakes), physical inactivity, the microbiota, and genetic factors [e.g., patatin-like phospholipase domain-containing protein 3 (PNPLA3),[13] transmembrane 6 superfamily member 2 (TM6SF2),[14] membrane-bound O-acyltransferase domain-containing 7 gene (MBOAT7)[15] polymorphisms, and hydroxysteroid 17-Beta Dehydrogenase 13 (HSD17B13)[16]] may also play a role in NAFLD progression increasing hepatic lipid accumulation and liver fibrosis risk.[17]

Two recent studies in the adult and pediatric NASH-Clinical Research Network (NASH-CRN) cohorts have investigated the relationship between the plasma levels of 32 cytokines as biomarkers and features of NAFLD histology.[18,19] Soluble interleukin-2 receptor alpha (IL2RA) levels increased with fibrosis severity in both cohorts and portal inflammation was found in the pediatric NASH-CRN cohort. That study is the first to document this association in patients with NAFLD.[18] IL2RA, also known as CD25, is constitutively expressed by regulatory T cells (Treg), the defining component of the high-affinity IL-2R complex. Soluble IL2RA is formed by the proteolytic cleavage of the IL-2 receptor from the cell surface of multiple immune cells and is proportional to its membrane-bound form. Soluble IL2RA is a marker of T-cell activation in the plasma. An association between increased IL2RA levels and advanced fibrosis has been documented in non-NAFLD liver disease.[20,21] However, no hepatic study has been conducted in Asian morbidly obese patients who underwent bariatric surgery.

This prospective cohort study investigated IL2RA for predicting NAFLD severity in morbidly obese patients.

2. METHODS

2.1. Study Design and Protocol

This prospective study included 123 morbidly obese patients who underwent laparoscopic sleeve gastrectomy at Taipei Medical University Hospital between October 2016 and October 2018. The inclusion criteria were as follows: aged 20–65 years with body mass index (BMI) over 37.5 kg/m^2, BMI over 32.5 kg/m^2 with comorbidities other than diabetes, and BMI over 27.5 kg/m^2 with poorly controlled diabetes.[22] The exclusion criteria were as follows: the presence of end-organ damage, pregnancy, previous bariatric surgery, prolonged exposure to known hepatotoxins such as alcohol/drugs, and other causes of chronic liver disease, including hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus, human immunodeficiency virus infection, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson disease, or hemochromatosis. During laparoscopic sleeve gastrectomy, all patients underwent a wedge liver biopsy under laparoscopic guidance. Liver-tissue specimens were fixed in 10% formalin, embedded in paraffin, and then stained with hematoxylin and eosin for histopathological analysis. Two experienced pathologists, who were blinded to patient identity and history, coded and read histological slides with a consensus. The SAF (steatosis, activity, fibrosis) score was calculated for each patient for the diagnosis of NASH, as in Bedossa's study.[9,23] Written informed consent was obtained from all patients who agreed to undergo surgery. This study was approved by the Taipei Medical University-Joint Institutional Review Board (TMU-JIRB No. N201601029) (clinical trial number: ClinicalTrials.gov identifier NCT04059029).[9]

2.2. Noninvasive Serum Markers

Venous blood samples were collected after overnight fasting. Fatty liver index (FLI) was calculated using following formula: FLI = (e 0.953 × loge (triglyceride [TG]) + 0.139 × BMI + 0.718 × loge (gamma-glutamyltransferase [GGT]) + 0.053 × waist circumference [WC] – 15.745)/(1 + e 0.953 × loge (TG) + 0.139 × BMI + 0.718 × loge (GGT) + 0.053 × WC – 15.745) × 100.[24] Aspartate aminotransferase/platelet ratio index (APRI) was calculated as the ratio of ([aspartate aminotransferase {AST}/platelet counts [10^9/L]) × 100.[25] The nonalcoholic fatty liver disease fibrosis score (NAFLD-FS) was calculated using the following formula: –1.675 + (0.037 × age [years]) + (0.094 × BMI) + (1.13 × hyperglycemia or diabetes [yes = 1, no = 0]) + (0.99 × AST/alanine aminotransferase [ALT]) – (0.013 × platelet [109/L]) – (0.66 × albumin [g/dL]).[26] The fibrosis-4 score (FIB-4 score) was calculated using following formula: [age (years) × AST (U/L) /platelet (10^9/L) × √ALT (U/L)].[27]

2.3. Ultrasonographic and Transient Elastography Examination

For liver stiffness measurement (LSM) and Controlled Attenuation Parameter (CAP) through TE (FibroScan®), Ultrasonographic (US) fatty score and US fibrosis score through abdominal sonography were calculated, as described elsewhere.[9]

2.4. Immunohistochemistry

Formalin-fixed tissue sections (4 μm) were deparaffinized and rehydrated in graded alcohols and xylene. After retrieval through the autoclaved retrieval technique (10 mM citric acid buffer; 10—20 min) and inhibition of endogenous peroxidase activity (0.3% H_2O_2; 5 minutes), the sections were incubated with primary antibody (1:100 dilutions; overnight at 4°C) for rabbit polyclonal IL2RA (Atlas Antibodies Cat no. HPA054622, RRID: AB_2682546, Sweden).[28] Negative controls without primary antibodies were used. Subsequently, the sections were incubated for 30 minutes with secondary antibody (1:100 dilution), reincubated with 100 μg/mL peroxidase-conjugated streptavidin, and colonized with 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB) (0.05 M Tris—HCl buffer with 0.03% H_2O_2). The sections were counterstained with hematoxylin. The number of lymphocytes with IL2RA immunoreactivity was counted in 5 high-power fields (×400, total: 1.19 mm^2) were counted (Fig. 1).[29]

2.5. Statistical Analysis

All statistical analyses were performed using the Statistical Program for Social Sciences (SPSS 19.0 for Windows, SPSS. IBM

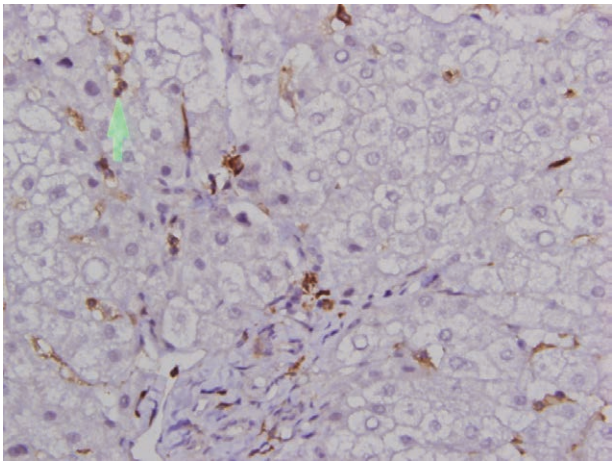

Fig. 1 The immunohistochemistry staining of IL2RA in liver tissue from a patient with NASH in our cohort. The number of lymphocytes with IL2RA immunoreactivity in five high-power fields were counted (arrow) (Original magnification: ×400). NASH = non-alcoholic steatohepatitis; IL2RA = interleukin-2 receptor alpha.

Corp. Armonk, NY). Continuous variables were compared using the Mann-Whitney *U* test. Categorical variables were compared using Pearson chi-squared analysis or Fisher's exact test. A *p* value of <0.05 was considered statistically significant. Variables that were statistically significant ($p < 0.05$) or close to it ($p < 0.1$) in univariate analysis were included in multivariate analysis using a forward stepwise logistic regression model. The accuracy of IL2RA immunohistochemistry (IHC) for NASH was determined by testing sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) and by calculating the area under the curve from corresponding receiver operating curve (AUROC). The AUROC was expressed as plots of test sensitivity versus 1—specificity. The AUROC cutoff value was determined using MedCalc (version 4.20, MedCalc Software, Mariakerke, Belgium).

3. RESULTS

3.1. Characteristics of Patients in the Non-NASH and NASH Groups

Among the 123 patients, the mean age was 35.5 years, mean BMI was 40.6 kg/m^2, 87 (70.7%) were female, 25 (20.7%) had diabetes mellitus (DM), and 57 (46.3%; 11 with non-NAFLD and 46 with steatosis) and 66 (53.7%) were included in the non-NASH and NASH groups, respectively; and 28 (22.8%), 14 (11.4%), and 2 (1.6%) patients had fibrosis stage 2, 3, and 4, respectively. The mean LSM value was 8.3 ± 5.9 kPa. As presented in Table 1, the NASH group had higher WC, fasting glucose, TG, AST, ALT, GGT, IL2RA IHC, APRI, FIB-4 score, FLI, LSM, CAP, and US fatty and fibrosis score, and a higher proportion of F2-F4 than the non-NASH group (Fig. 2).

3.2. Factors Associated With NASH

Univariate analysis revealed that WC, homeostasis model assessment of insulin resistance (HOMA-IR), total bilirubin, AST, ALT, GGT, IL2RA IHC, LSM, US fibrosis, and fatty score were associated with NASH. Multivariate analysis revealed that IHC of IL2RA (odds ratio, 1.025; 95% confidence interval, 1.006–1.045; $p = 0.011$) and ALT (odds ratio, 1.045; 95% confidence interval, 1.018–1.073; $p = 0.001$) were the independent factors associated with NASH (Table 2).

3.3. IHC of IL2RA for NASH Diagnosis

The area under receiver operating curve (AUROC) of IL2RA IHC for NASH diagnosis was 0.627 at the cutoff value of 82 ($p = 0.0113$). IL2RA IHC exhibited sensitivity of 34.3%, specificity of 96.4%, PPV of 92.0%, and NPV of 54.6% for NASH diagnosis (Fig. 3).

Table 1.

Demographic data of non-NASH and NASH groups

	A11 (n = 123)	Non-NASH (n = 57)	NASH (n = 66)	*p*
Age, y[a]	35.5 ± 8.0	35.2 ± 8.6	35.8 ± 7.6	0.544
Sex (M/F) (%)	36/87 (29.3/70.7)	15/42 (26.3/73.7)	21/45 (31.8/68.2)	0.504
Smoking (yes/no) (%)	26/95 (21.5/78.5)	14/43 (24.6/75.4)	12/52 (18.8/81.3)	0.437
BMI, kg/m^2[a]	40.6 ± 5.4	40.1 ± 4.9	41.1 ± 5.7	0.484
WC, cm[a]	118.2 ± 12.7	115.6 ± 13.8	120.5 ± 11.2	0.044
HTN (yes/no) (%)	32/89 (26.4/73.6)	11/46 (19.3/80.7)	21/43 (32.8/67.2)	0.092
DM (yes/no) (%)	25/96 (20.7/79.3)	10/47 (17.5/82.5)	15/49 (23.4/76.6)	0.424
Fasting glucose, mg/dL[a]	117.9 ± 53.5	108.1 ± 39.1	126.5 ± 62.5	0.015
HOMA-IR[a]	5.3 ± 9.1	3.3 ± 3.7	7.1 ± 11.7	0.058
Cholesterol, mg/dL[a]	193.5 ± 38.2	190.5 ± 34.3	196.1 ± 41.3	0.247
LDL, mg/dL[a]	130.3 ± 34.9	128.1 ± 33.3	132.4 ± 36.4	0.357
TG, mg/dL[a]	173.8 ± 133.3	152.2 ± 111.6	192.7 ± 148.1	0.030
Total bilirubin, mg/dL[a]	0.6 ± 0.3	0.5 ± 0.3	0.6 ± 0.3	0.098
AST, U/L[a]	36.0 ± 29.6	24.6 ± 16.7	45.9 ± 34.5	<0.001
ALT, U/L[a]	53.6 ± 40.9	37.5 ± 31.7	67.6 ± 42.9	<0.001
GGT, U/L[a]	39.0 ± 28.6	23.6 ± 29.2	47.1 ± 30.1	<0.001
Creatinine, mg/dL[a]	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.2	0.335
Albumin, g/dL[a]	4.5 ± 0.3	4.5 ± 0.3	4.5 ± 0.3	0.270
Platelet, 1000/mm^3[a]	286.5 ± 67.6	294.0 ± 72.8	280.1 ± 62.6	0.361
F2-F4/F0-F1 (%)	44/79 (35.8/64.2)	45/12 (78.9/21.1)	34/32 (51.5/48.5)	0.002
IL2RA IHC[a]	64.2 ± 44.7	49.6 ± 25.3	76.2 ± 53.0	0.001
Noninvasive serum markers				
APRI[a]	0.34 ± 0.32	0.23 ± 0.22	0.44 ± 0.35	<0.001
NAFLD-FS[a]	2.53 ± 1.55	2.49 ± 1.67	2.57 ± 1.45	0.563
FIB-4 score[a]	0.63 ± 0.37	0.52 ± 0.27	0.72 ± 0.41	0.001
FLI[a]	91.84 ± 16.71	91.43 ± 9.49	92.2 ± 21.0	0.024
Imaging techniques				
LSM (E score), kPa[a]	8.3 ± 5.9	7.0 ± 5.5	9.4 ± 6.0	<0.001
CAP, dB/m	314.9 ± 59.0	303.5 ± 49.7	321.9 ± 63.5	0.035
US fatty score[a]	6.5 ± 2.2	5.8 ± 2.6	7.1 ± 1.6	0.005
US fibrosis score[a]	5.1 ± 0.8	4.9 ± 0.8	5.2 ± 0.9	0.049
SAPI[a]	0.85 ± 0.27	0.84 ± 0.28	0.86 ± 0.26	0.488

[a]Expressed as mean ± standard deviation.

IL2RA IHC = interleukin-2 receptor alpha immunohistochemistry; NASH = nonalcoholic steatohepatitis; LSM = liver stiffness measurement; CAP = controlled attenuation parameter; HTN = hypertension; DM = diabetes mellitus; BMI = body mass index; SD = standard deviation; M = male; F = female; WC = waist circumference; LDL = low-density lipoprotein; TG = triglyceride; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyltransferase; HOMA-IR = the homeostasis model assessment of insulin resistance; APRI = aspartate aminotransferase/platelet ratio index; FLI = fatty liver index; NAFLD-FS = nonalcoholic fatty liver disease fibrosis score; FIB-4 score = fibrosis-4 score; US = ultrasonographic; SAPI = splenic arterial pulsatility index.

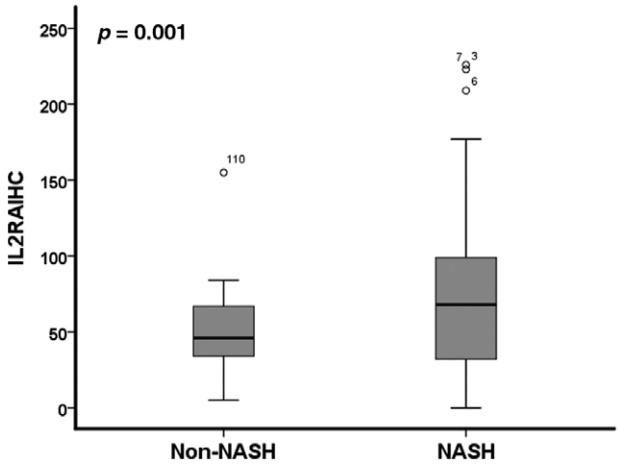

Fig. 2 Box and whisker graph showing the relationship between IL2RA IHC and the NASH cohort. The bottom and top of each box represent the 25th and 75th percentiles, giving the interquartile range. The line through the box indicates the median value, and the error bar indicates 10th and 90th percentiles. NASH = non-alcoholic steatohepatitis; IL2RA IHC = interleukin-2 receptor alpha immunohistochemistry.

4. DISCUSSION

This study is the first to report the data of IHC of IL2RA for NASH diagnosis in morbidly obese Asian patients who underwent bariatric surgery. Liver biopsy should be considered routine during bariatric surgery given the high prevalence of NASH (53.7%) and significant liver fibrosis (35.8%) among our morbidly obese cohort. In the multivariable model, IHC of IL2RA and ALT were the independently associated with NASH. The AUROC of IL2RA IHC for NASH diagnosis was 0.627 at the cutoff value of 82.

Currently, liver biopsy is the gold standard for diagnosing and differentiating the NAFLD spectrum. Nevertheless, several limitations exist.[30] First, it is expensive and invasive with a small risk of complications and has low patient acceptance. Second, it cannot be used for routine screening and repeated assessments to monitor disease progression or the treatment response. Third, a sampling error or the interobserver and intraobserver variability for NASH diagnosis has been reported.[31] Hence, many serum biomarkers have been investigated for NASH diagnosis.[26,32,33] Serum ALT levels remain the most commonly used marker of NASH. However, in previous studies, the current threshold for upper limit of normal (ULN) of serum ALT levels (40 IU/L) has been challenged for patients with chronic hepatitis due to the risk of liver disease progression, including viral hepatitis, metabolic syndrome, and fatty liver.[34–36] Our previous study in 34 346 consecutive subjects who completed a health check-up demonstrated that the optimal threshold of ULN for ALT is 21 IU/L for men and 17 IU/L for women for better discrimination between healthy and unhealthy status.[2] Hence, patients with NASH may have normal ALT levels. Other serum biomarkers have been investigated to differentiate NASH from NAFL, including apoptosis markers (cytokeratin-18 fragments, and soluble FAS), inflammatory markers (tumor necrosis factor-alpha, interleukin 6 and 8, C-reactive protein, and CC-chemokine ligand 2), adipokines (adiponectin, leptin, resistin, and visfatin), oxidative stress markers (fibroblast growth factor 21, thioredoxin, copper-to-zinc superoxide dismutase, glutathione peroxidase, and vitamin E), and panel markers (NASHTest and NASH Diagnostics

Table 2.

Factors associated with NASH (n = 123)

		Univariate analysis		Multivariate analysis	
Variable	**Case No.**	**Odds ratio (95% CI)**	***p***	**Odds ratio (95% CI)**	***p***
Age	123	1.008 (0.964–1.054)	0.724		
Sex (M/F)	36/87	0.739 (0.665–3.236)	0.343		
Smoking (yes/no)	26/95	0.679 (0.284–1.623)	0.384		
BMI	123	1.035 (0.967–1.107)	0.327		
WC	115	1.033 (1.002–1.065)	0.037		
HTN (yes/no)	32/89	1.952 (0.843–4.521)	0.118		
DM (yes/no)	25/96	1.380 (0.564–3.376)	0.480		
HOMA-IR	92	1.108 (1.007–1.220)	0.036		
Cholesterol	122	1.003 (0.994–1.013)	0.488		
LDL	117	1.003 (0.993–1.014)	0.537		
TG	122	1.003 (0.999–1.006)	0.153		
Total bilirubin	75	8.043 (1.158–55.875)	0.035		
AST	123	1.048 (1.020–1.077)	0.001		
ALT	123	1.028 (1.013–1.043)	<0.001	1.045 (1.018–1.073)	0.001
GGT	71	1.042 (1.011–1.075)	0.009		
Creatinine	122	0.995 (0.243–4.082)	0.994		
Albumin	77	1.795 (0.362–8.901)	0.474		
Platelet (per 1000/mm^3)	123	0.996 (0.990–1.001)	0.139		
IL2RA IHC	122	1.017 (1.006–1.029)	0.002	1.025 (1.006–1.045)	0.011
LSM	121	1.184 (1.054–1.330)	0.004		
CAP	71	1.007 (0.998–1.017)	0.124		
US fibrosis score	122	1.862 (1.117–3.103)	0.017		
US fatty score	122	1.358 (1.125–1.638)	0.001		
SAPI	122	1.169 (0.309–4.419)	0.818		

IL2RA IHC = interleukin-2 receptor alpha immunohistochemistry; NASH = non-alcoholic steatohepatitis; LSM = liver stiffness measurement; CAP = controlled attenuation parameter; HTN = hypertension; DM = diabetes mellitus; BMI = body mass index; SD = standard deviation; M = male; F = female; WC = waist circumference; LDL = low-density lipoprotein; TG = triglyceride; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyltransferase; HOMA-IR = the homeostasis model assessment of insulin resistance; LSM = liver stiffness measurement; US = ultrasonographic; SAPI = splenic arterial pulsatility index; APRI = aspartate aminotransferase/platelet ratio index; FIB-4 score = fibrosis-4 score; NAFLD-FS = nonalcoholic fatty liver disease fibrosis score; FLI = fatty liver index; CI = confidence interval

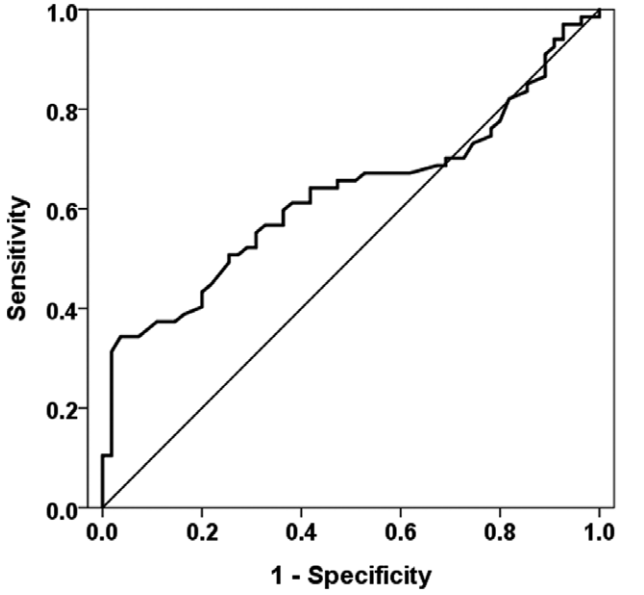

Fig. 3 Area under receiver operating curve of IL2RA for NASH. NASH = non-alcoholic steatohepatitis; IL2RA = interleukin-2 receptor alpha.

Panel).[26] Cytokeratin-18 is by far the only most widely validated biomarker for NASH, but its use is limited in clinical practice due to the lack of a commercially available clinical test, limited sensitivity at the individual level, and variability cutoffs for diagnostic accuracy across studies. In summary, none of the current serum biomarkers is acceptable for NASH diagnosis with high sensitivity and specificity.

Recently, glycoprotein-based biomarkers (glyco-biomarker) have been emerged as novel disease biomarkers. Several recent studies have shown that Mac-2 Binding Protein (M2BP) is a promising biomarker for predicting the severity of liver fibrosis in different chronic liver diseases,[37] including HBV,[38] HCV,[39] NAFLD,[40,41] and primary biliary cholangitis,[42] and they may also predict HCC development.[43] M2BP is a glycoprotein that is almost undetectable in normal liver but becomes easily detected in patients with hepatocyte injury as liver fibrosis progresses; therefore, it is considered a biomarker for liver injury and fibrosis. One recent study demonstrated that serum M2BP levels were negatively correlated with the degree of M2BP IHC in the liver from patients with NAFLD.[41] These phenomena may be due to the balance between the production and secretion of M2BP. This implies that serum biomarkers are not able to differentiate NASH from simple steatosis; thus, hepatic biomarkers should still be considered.

Previous studies have shown that soluble IL-2R is elevated in patients with chronic liver diseases, cirrhosis, and HCC.[44,45] Circulating inflammatory cells, including activated B cells, monocytes, eosinophil granulocytes, and natural killer cells also express IL2RA.[21] Functional IL2 receptors include IL2RA (CD25), IL2 receptor-beta, and IL2 receptor-gamma. Nonactivated T lymphocytes only express IL2 receptor-beta and IL2 receptor gamma and have a low affinity for IL2, whereas activated T lymphocytes express IL2RA and have a high affinity for IL2. Hence, IL2RA is a surrogate marker of T-lymphocytes activation.[46] Furthermore, immunohistochemistry can identify a field effect of injury that may not be as easily detected by routine staining procedures. In this study, the lymphocytes with IL2RA immunoreactivity were used to quantify IHC. Our study is the first to document an association between increased IHC of IL2RA in the liver and NASH diagnosis. Because the inter-observer and intraobserver bias in assessing NAFLD has been reported to be as high as 10%–20%, IHC of IL2RA can be useful in NASH diagnosis.[31]

The strengths of this study are in the prospective cohort design and that a planned protocol with detailed data was followed. Procedures were performed by an experienced operator to ensure good specimen quality, and 2 experienced, blinded pathologists read histological slides with a consensus. However, this study had a few limitations. First, the molecular mechanisms underlying the induction of IL2RA during NAFLD progression should be investigated. Second, the hepatic IL2RA study was conducted in morbidly obese patients undergoing bariatric surgery, with a predominantly young females study population. External validation studies are needed to examine whether IL2RA can be applied to the overall NAFLD population.

In conclusion, IHC of IL2RA is significantly associated with NASH in morbidly obese patients, and IL2RA would be a useful single biomarker for NASH diagnosis. Further large cohort long-term follow-up cohort studies are warranted to investigate the predicted biomarkers of NAFLD improvement in morbidly obese patients undergoing bariatric surgery.

ACKNOWLEDGMENTS

Declaration of funding interests: This study was supported by grants from the Ministry of Science and Technology (MOST 105-2314-B-038-006; MOST 106-2314-B-038-030; MOST 107-2314-B-038-064; and MOST 108-2314-B-038-055). Part of this study was presented as an oral presentation at the 29th Asian Pacific Association for the Study of the Liver. March 4–8, 2020, Bali, Indonesia (with travel grant).

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