Six autoantibodies as potential differential biomarkers of hepatocellular carcinoma vs. liver cirrhosis and chronic hepatitis: A prospective multi-institutional study

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Abstract. Serum autoantibodies respond not only to tumor-associated antigens of hepatocellular carcinoma (HCC) but also to those of liver cirrhosis (LC) and chronic hepatitis (CH). The present prospective multi-institutional study evaluated the diagnostic properties of six autoantibodies in distinguishing HCC from LC and CH. A total of 416 participants were enrolled: 149 With HCC, 76 with LC, 103 with CH and 88 healthy controls. Titers of serum autoantibodies to Sui1, RalA, p62, p53, c-myc and NY-ESO-1 were determined using enzyme-linked immunosorbent assays. All six antibodies were positive for HCC: s-Sui1-Abs (44%), s-RalA-Abs (23%), s-p62-Abs (21%), s-p53-Abs (13%), s-c-myc-Abs (11%) and s-NY-ESO-1-Abs (6%). The positivity rates of all six antibodies combined were 5% for healthy controls, 52% for CH, 58% for LC and 66% for HCC. The positivity rates of s-Sui1-Abs, s-RalA-Abs and s-p53-Abs were higher for HCC compared with those of LC and CH. However, the positivity rates of s-p62-Abs, s-c-myc-Abs and s-NY-ESO-1-Abs for HCC were not higher compared with those for LC and CH. Overall, autoantibodies were useful in differentiating patients with HCC from healthy individuals. However, they were not specific to HCC and were also present in the sera of individuals with CH and LC. These autoantibodies may be induced during the development of HCC. Clinical trial registration number: UMIN000014530 (date of registration 2011/07/11).

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Abbreviations: AFP, α-fetoprotein; CH, chronic hepatitis; ELISA, enzyme-linked immunosorbent assay; HCC, hepatocellular carcinoma; LC, liver cirrhosis; TAA, tumor-associated antigen

Key words: hepatocellular carcinoma, liver cirrhosis, chronic hepatitis, SEREX autoantibody, tumor marker

Introduction

Depending on various disease environments, the presence of abnormal proteins may lead to antigenicity, which can drive the humoral immune response to produce serum autoantibodies. Several recent studies have demonstrated that serum autoantibodies may be useful for detecting various cancers at early stages (1-5). There are various subtype classifications and treatments for HCC, and alternative immunocombined approaches from a molecular pathological point of view have been implemented, but their effects remain unclear (6). Our research group has also evaluated the usefulness of autoantibodies in detecting surgically treatable hepatocellular carcinoma (HCC). Owing to their enhanced signals, autoantibodies may be more useful as immunodiagnostic markers for cancer detection than tumor-associated antigens (TAAs) (7,8). The presence of autoantibodies has been observed not only in cancer but also in autoimmune, inflammatory, and fibrotic diseases (9-12).

Several studies, including those by our research group, have demonstrated potential TAAs in HCC using enzyme-linked immunosorbent assay (ELISA). In a test cohort, Okada *et al* the showed potential benefits of using autoantibodies to detect diseases (1). Moreover, in a previous study of patients with surgically resected HCC, autoantibodies against TAAs, including Sui1, RalA, p62, p53, c-myc, and NY-ESO-1, had

an additive effect with α-fetoprotein (AFP) on the detection of stage I and II HCC (2). However, the clinical significance of autoantibodies in liver cirrhosis (LC) or chronic hepatitis (CH) has not been studied extensively. A single-institutional retrospective study demonstrated the significance of autoantibodies in distinguishing among HCC, LC, and CH; however, no prospective multi-institutional study has been undertaken on this topic (13,14). Patients with CH are at a high risk of progression to LC and HCC, and an early diagnosis of LC is essential for an early diagnosis of HCC.

This prospective multi-institutional study aimed to cross-sectionally validate the positivity rates of six autoantibodies to HCC. We also compared the positivity rates of these six autoantibodies to LC and CH to evaluate whether the three diseases could be differentiated through the measurement of these autoantibodies.

Materials and methods

Patients. Patients enrolled in this study had primary HCC, histologically proven, or either LC or CH according to ultrasound findings. Eligible patients with LC had an aspartate aminotransferase-to-platelet ratio index (APRI) of ≥1.0 or a fibrosis-4 (FIB-4) score of ≥3.25, and eligible patients with CH had an APRI of <1.0 and/or a FIB-4 score of <3.25 (15,16). Patients with active co-cancers (co-cancers or metachronous cancers within 5 years) were excluded to ensure that the previous cancer had no effect on their antibody levels. Each research center conducted central monitoring to confirm data submission, patient eligibility, protocol compliance, safety, and on-schedule research progress. We compared patients' clinicopathological variables, demographic data, and tumor characteristics (positivity and negativity for any of the six autoantibodies). The AFP cutoff value was 10.0 ng/ml. Before enrollment, all participants provided written informed consent to future analyses of their blood samples for research purposes. The protocol for this prospective study was approved by the ethics committee of Toho University, Tokyo, Japan (approval numbers M19213, A18103, A17052, A16035, A16001, 26095, 25024, 24038, and 22047); the Chiba Cancer Center, Chiba, Japan (H30-220, 21-26, and 20-1); and the institutional review boards of each participating hospital (listed in the next section). The study was conducted according to the guidelines of the Declaration of Helsinki and the Japanese Ethical Guidelines for Clinical Research.

Sample collection. Serum samples were obtained from 149 patients with HCC, 76 patients with LC, and 103 patients with CH at the six participating institutions (Chiba University School of Medicine, Chiba Cancer Center, Japan Community Health care Organization Chiba Hospital, Funabashi Municipal Medical Center, Kimitsu Chuo Hospital, and Japan Community Health care Organization Funabashi Central Hospital). Serum samples of 88 healthy controls who had no previous malignant disease and no hepatitis B or hepatitis C infection were also obtained from Biobank Japan. The average age of the healthy control group was 48 years, and the male-to-female ratio was 5:3. All serum samples were stored at -80˚C until analysis.

Patient variables. The HCC stage in each affected patient at the time of the study was pathologically determined according to the TNM Classification of Malignant Tumours, 8th edition (17). Preoperative resectability and local or distant tumor enlargement were determined via computed tomography. Tumors associated with distant metastasis, including peritoneal dissemination, were considered nonresectable. The hepatectomy procedure was performed according to the treatment method described in the Japanese guidelines (18,19).

Isolation, purification, and amplification of TAAs followed by ELISA of serum antibodies. Full-length complementary DNA of the TAAs Sui1 (GenBank accession number: JN545747), RalA (BM 560822), p62 (AF057352), p53 (AB082923), c-myc (K02276), and NY-ESO-1 (NM 001327) were amplified through polymerase chain reaction as previously described (1,2). The recombinant proteins were expressed in *Escherichia coli* BL21-CodonPlus (DE3)-RIL cells (Agilent Technologies). Each TAA extract was added to Ni Sepharose 6 Fast Flow (GE Healthcare UK), and the column was washed with 50 mmol/L imidazole in phosphate-buffered saline. Purified TAA recombinant proteins were eluted with 200 mmol/L imidazole in PBS. DNA sequencing confirmed that the correct gene was inserted into the constructed plasmid. Serum samples collected from patients and controls were analyzed through ELISA as previously described (2). Serum AFP was measured through enzyme-linked fluorescent assay as previously described (20).

Titration of autoantibodies. Using the serum from the patients with HCC, LC, and CH and from the healthy controls, we measured the titers (means ± standard deviations) of autoantibodies against the six TAAs. The cutoff value for positive reactivity of each autoantibody was an optical density greater than the mean plus three standard deviations observed in the controls. We calculated the specificity of the assay as the percentage of the controls in whom the reactivity was negative. We also assessed the significance of differences in each of the six autoantibody titers. Various serum markers, clinicopathological characteristics, and AFP concentrations were included in the analysis. Additionally, we estimated the clinical utility of the combination of the six autoantibodies in diagnosing HCC, LC, and CH.

Statistical analysis. Statistical analyses were performed using the JMP statistical software (version 12; SAS Institute). We used Fisher's exact test to determine whether the proportions of positive results differed significantly between the patients with cancer and the healthy controls and to determine associations between individual and combined antibody assay results and clinical parameters. For all tests, a P value of <0.05 (two-tailed) was considered statistically significant.

Results

Serum anti-TAA antibody titers. The serum titers of the autoantibodies against TAAs were higher in patients with HCC, LC, and CH than in healthy controls (P<0.05 for all). The cutoff titers were 8.0, 15.0, 6.0, 8.0, 4.0, and 10.0 U/ml for s-Sui1-Abs, s-RalA-Abs, s-p62-Abs, s-p53-Abs, s-c-myc-Abs, and s-NY-ESO-1-Abs, respectively (Fig. 1).

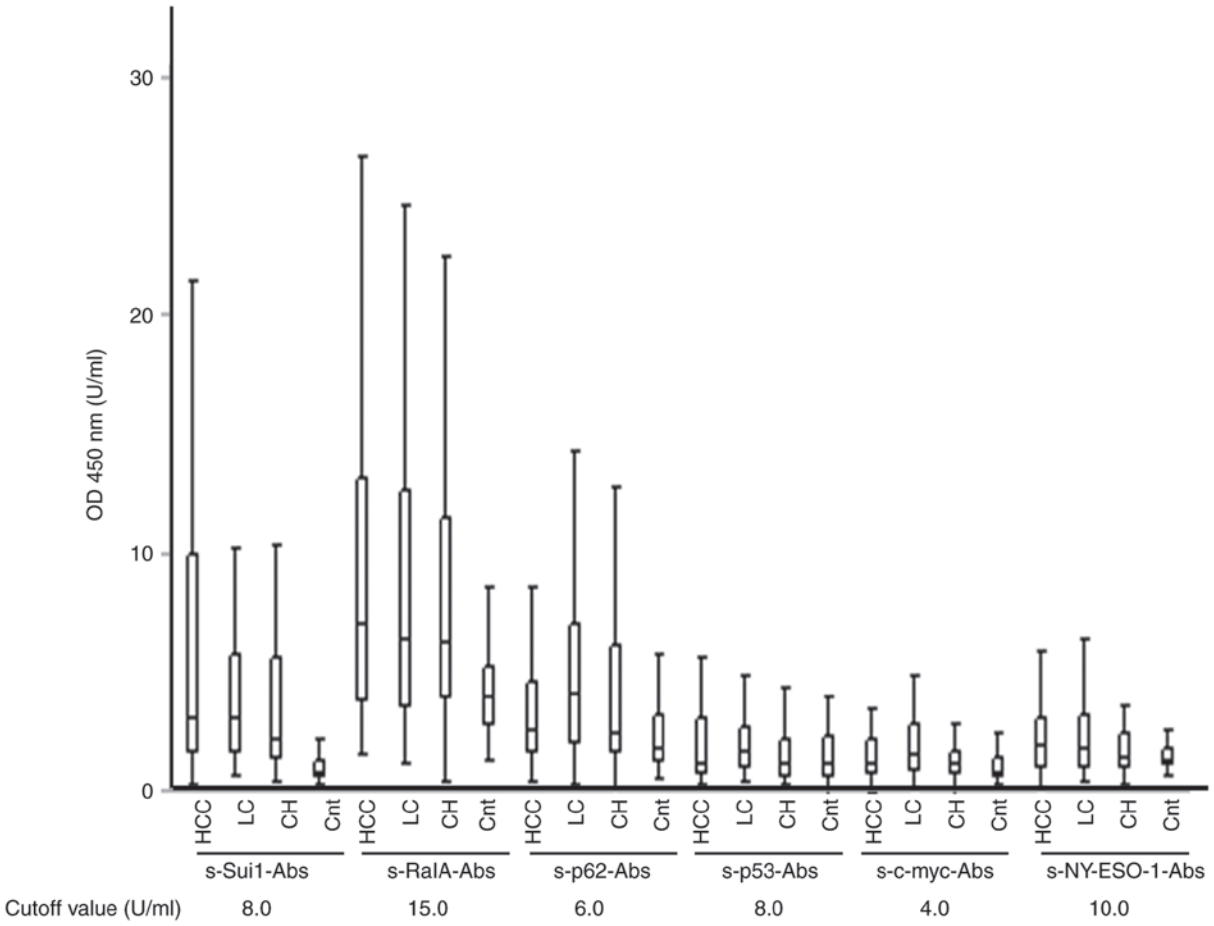

Figure 1. Autoantibody titers for tumor-associated antigens in individual patients and healthy controls as determined using enzyme-linked immunosorbent assay. Box plots of OD values of autoantibodies in serum from 149 patients with HCC, 76 patients with LC, 103 patients with CH and 88 healthy cnts. OD, optical density; HCC, hepatocellular carcinoma; LC, liver cirrhosis; CH, chronic hepatitis; Cnt, control.

Clinical characteristics and autoantibody status of patients with HCC. Table I lists the clinical characteristics of patients with HCC. Gender, age, hepatitis virus infection type, liver disease type (CH vs. LC), tumor size, tumor number, and TNM classification were not significantly associated with autoantibody status. Autoantibody status was also not associated with AFP status.

Positivity rates of each autoantibody and AFP in patients with HCC at different TNM stages. The overall positivity rate of the serum autoantibodies against the TAA panel (66%) was higher than that of AFP (48%, P<0.05). Fig. 2 shows the positivity rates of each autoantibody and AFP in patients with HCC at different TNM stages. The corresponding positivity rates for the autoantibody panel and AFP were 66 and 34%, respectively (P=0.001), for TNM stage I disease; 64 and 48%, respectively (P=0.047), for stage II; 69 and 75%, respectively (P=0.694), for stage III; and 75 and 63%, respectively (P=0.588), for stage IV. The combination of the positivity rates of the autoantibody panel and AFP was significantly greater than the positivity rate of AFP alone (P<0.001). The positivity rates of AFP and AFP + serum autoantibodies were 34 and 78%, respectively (P<0.001), for stage I disease; 48 and 84%, respectively (P<0.001), for stage II; 75 and 88%, respectively (P=0.361), for stage III; and 63 and 75%, respectively (P=0.588), for stage IV.

Ability of autoantibody response to TAAs to diagnose HCC. Table II lists the positivity rates, specificities, positive predictive values, negative predictive values, and accuracy of serum autoantibodies in detecting HCC. Of the 149 patients with HCC, 66 (44%) had s-Sui1-Abs; 34 (23%) had s-RalA-Abs; 32 (21%) had s-p62-Abs; 20 (13%) had s-p53-Abs; 17 (11%) had s-c-myc-Abs; and 9 (6%) had s-NY-ESO-1-Abs. All autoantibodies had a specificity of >95%. When the assay results for all six autoantibodies were combined, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy increased to 66% (95% confidence interval [CI], 62-67%), 96% (95% CI, 90-98%), 96% (95% CI, 91-98%), 62% (95% CI, 58-64%), and 77% (95% CI, 73-79%), respectively.

Clinical characteristics and autoantibody status in patients with HCC, LC, and CH. Table III lists the clinical characteristics of patients with HCC, LC, and CH. Male sex, age of ≥65, APRI of ≥1.0, FIB-4 of ≥3.25, hepatitis virus infection, and autoantibody positivity rates were significantly associated with HCC. Autoantibody positivity rates in patients with HCC were significantly higher than those in patients with CH (P=0.033).

Positivity of autoantibodies in patients with HCC, LC, and CH and in healthy controls. Table IV and Fig. 3 present the autoantibody positivity rates in patients with HCC, LC, and

Table I. Patients' clinical characteristics and serum tumor markers according to status of autoantibody panel in 149 patients with HCC.

Panel	Total	Autoantibody panel (+)	Autoantibody panel (-)	P-value[a]
Number	149	98	51	
Sex, n (%)				
Male	105	67 (68)	38 (75)	0.432
Female	44	31 (32)	13 (25)	
Age, n (%)				
<65	28	16 (16)	12 (24)	0.291
≥65	121	82 (84)	39 (76)	
Hepatitis B virus infection, n (%)				
Negative	133	88 (90)	45 (88)	0.771
Positive	16	10 (10)	6 (12)	
Hepatitis C virus infection, n (%)				
Negative	50	30 (31)	20 (39)	0.293
Positive	99	68 (69)	31 (61)	
Liver disease, n (%)				
Chronic hepatitis	29	20 (20)	9 (18)	0.686
Liver cirrhosis	120	78 (80)	42 (82)	
Tumor size, n (%)				
<36 mm	109	70 (71)	39 (76)	0.500
≥36 mm	40	28 (29)	12 (24)	
Tumor number, n (%)				
1	52	33 (34)	19 (37)	0.664
≥2	97	65 (66)	32 (63)	
TNM stage, n (%)				
I	50	33 (34)	17 (33)	0.967
II, III, IV	99	65 (66)	34 (67)	
AFP, n (%)				
<10 ng/ml	79	52 (53)	27 (53)	0.989
≥10 ng/ml	70	46 (47)	24 (47)	

[a]Fisher's exact test. AFP, α-fetoprotein; TNM, tumor node metastasis.

CH and in healthy controls. Among the 149 patients with HCC, autoantibodies against Sui1 were most common in 66 patients (44%); among the 76 patients with LC, autoantibodies against p62 were most common in 23 patients (30%); and among the 103 patients with CH, autoantibodies against p62 were most common in 26 patients (25%). The positivity rates of autoantibodies in patients with HCC, LC, and CH and in healthy controls were 66, 58, 52, and 5%, respectively. Among patients with HCC, LC, and CH, the autoantibodies s-Sui1-Abs, s-RalA-Abs, and s-p53-Abs were more common than other autoantibodies.

Association between hepatitis virus infection and s-Sui1-autoantibody. Table SI presents the association between hepatitis virus infection and s-Sui1-Abs positivity in patients with HCC, LC, and CH. There was no association between hepatitis B virus infection and s-Sui1-Abs. In patients with hepatitis C virus, the s-Sui1-Abs positivity rate gradually increased in CH, LC, and HCC. Patients with hepatitis C HCC were significantly more positive for s-Sui1-Abs than those with not hepatitis C HCC.

Discussion

The combination of positivity rates of autoantibodies against the whole TAA panel and AFP was significantly higher than that of AFP alone in stage I and II disease. The positivity rates of the autoantibodies against the whole TAA panel were significantly higher than those of AFP in stage I and II disease. The positivity rates of the autoantibodies against the whole TAA panel gradually increased (in order) in patients with CH, LC, and HCC.

The fact that autoantibodies were effective in identifying HCC in two different prospective studies indicates that HCC induced serum immunoglobulin G autoantibodies against TAAs from an early stage (2). However, unlike other types

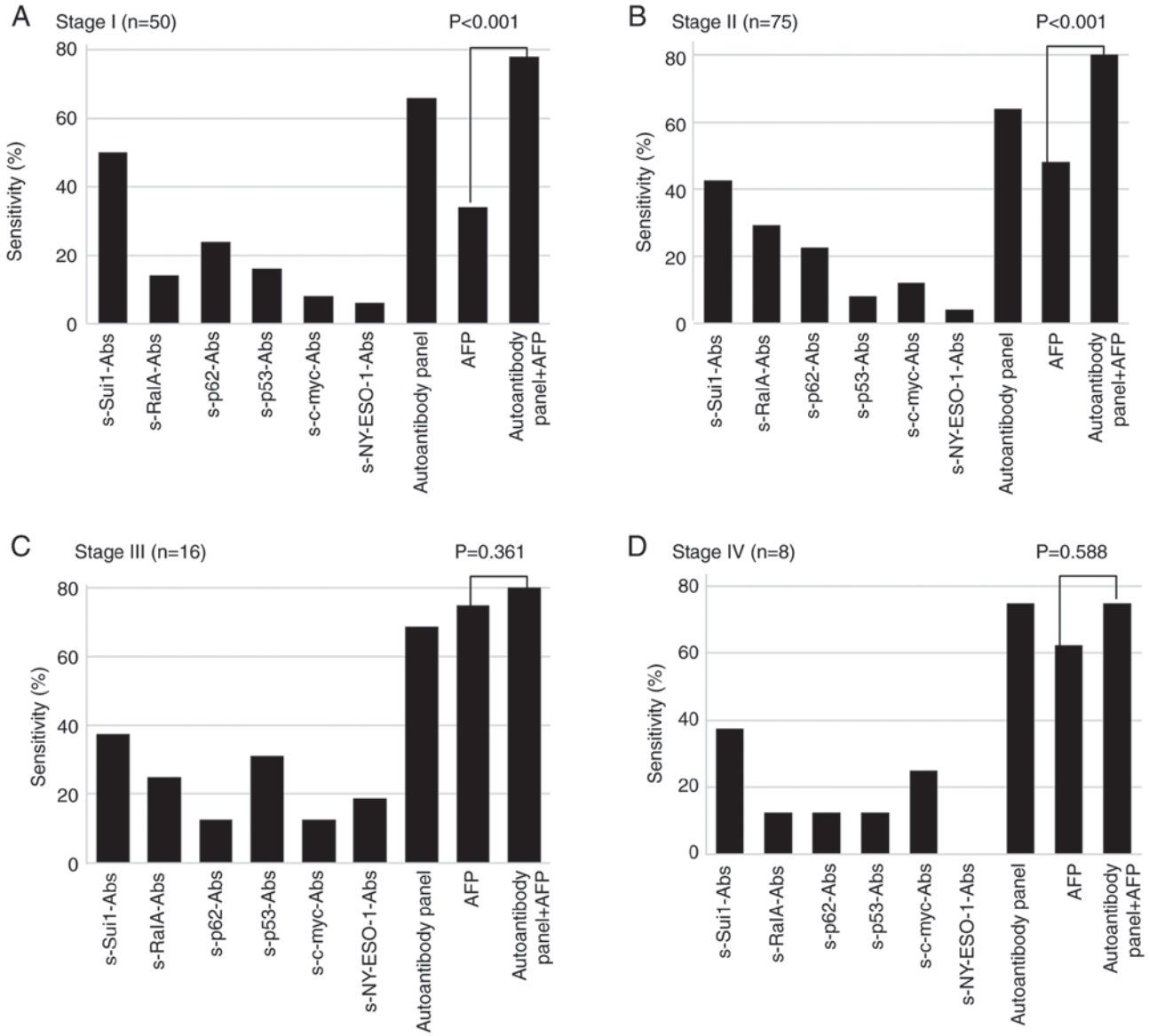

Figure 2. Sensitivity of each autoantibody according to tumor stages: (A) stage I, (B) stage II, (C) stage III and (D) stage IV. AFP, α-fetoprotein.

of cancer, HCC comprises multistage carcinogenic states that include CH and LC, and previous reports did not reveal at what stage serum autoantibodies would appear. Zheng *et al* reported that serum anti-SMP30 autoantibody titers were significantly higher in patients with HCC than in healthy controls or in patients with CH or LC (14). Furthermore, He *et al* reported that serum anti-ACY1 autoantibody titers were significantly higher in patients with LC than in healthy controls or in patients with CH (13). In this study, we found that serum autoantibody titers were elevated not only in patients with HCC but also in those with CH and LC.

Sui1 may be a potential biomarker for HCC according to Zhou *et al*, who reported that the positivity rate of s-Sui1-Abs in patients with HCC was 15.5%, which was higher than that in patients with LC (3.3%), those with CH (0%), and healthy participants (0%) (21). Our findings of relatively high positivity rates in patients with HCC, LC, and CH confirmed their data. The differences may be explained partly by the difference in the proportions of patients with hepatitis B virus- or hepatitis C virus-related carcinogenesis. Compared with other serum antibodies, only s-Sui1-Abs exhibited significantly higher positivity rates in patients with HCC than in patients with LC and CH. Moreover, the Suil positivity rate in this study differed from that in previous studies (2). This may be partially explained by the difference in the cutoff values calculated from two cohorts of healthy subjects (8.0 U/ml vs. 4.4 U/ml) and the difference in the proportion of early-stage cancer in this study.

RalA has also been reported to be a potential biomarker for HCC as well as for esophageal, gastric, colorectal, breast, and ovarian carcinomas (2,5,22-25). This TAA has not been reported to be associated with fibrosis or chronic inflammation. Because guanosine triphosphatase is aberrantly induced during tumorigenesis by oncogenic *Ras*, s-RalA-Abs positivity may indicate HCC development in patients with LC and CH.

Previous studies have reported that s-p62-Abs are positive not only for cancers such as HCC, colorectal cancer, and breast cancer but also for conditions characterized by chronic inflammation and fibrosis, such as primary biliary

Table II. Autoantibody responses to tumor-associated antigens in 149 patients with hepatocellular carcinoma.

Group	s-Sui1-Abs	s-RalA-Abs	s-p62-Abs	s-p53-Abs	s-c-myc-Abs	s-NY-ESO-1-Abs	Autoantibody panel
Sensitivity	44 (42-45)	23 (20-23)	22 (20-23)	14 (11-14)	11 (9-13)	6 (4-6)	66 (62-67)
Specificity	99 (94-100)	99 (94-100)	99 (94-100)	99 (94-100)	97 (92-99)	99 (94-100)	96 (90-98)
PPV	99 (93-100)	97 (86-100)	97 (86-100)	95 (78-99)	85 (65-95)	90 (60-98)	96 (91-98)
NPV	51 (49-52)	43 (41-44)	43 (41-44)	40 (38-41)	39 (37-40)	38 (37-39)	62 (58-64)
Accuracy	65 (62-66)	51 (48-52)	50 (47-51)	45 (42-46)	43 (40-45)	40 (38-41)	77 (73-79)

All values are given in percentages of positivity (95% confidence intervals in parentheses) in each group. PPV, positive predictive value; NPV, negative predictive value; Autoantibody panel, autoantibody positivity to any one of the six antigens.

Table III. Clinical characteristics according to status of autoantibody panel in patients with HCC, LC and CH.

Panel	HCC (n=149)	LC (n=76)	P-value[a]	CH (n=103)	P-value[b]
Gender, n (%)					
Male	105 (70)	41 (54)	0.015	50 (49)	<0.001
Female	44 (30)	35 (46)		53 (51)	
Age, n (%)					
<65	28 (19)	32 (42)	<0.001	59 (57)	<0.001
≥65	121 (81)	44 (58)		44 (43)	
APRI, n (%)					
<1.0	55 (37)	21 (28)	0.160	103 (100)	<0.001
≥1.0	94 (63)	55 (72)		0 (0)	
FIB-4, n (%)					
<3.25	30 (20)	5 (7)	0.005	103 (100)	<0.001
≥3.25	119 (80)	71 (93)		0 (0)	
Hepatitis B virus infection, n (%)					
Negative	133 (89)	74 (97)	0.021	67 (65)	<0.001
Positive	16 (11)	2 (3)		36 (35)	
Hepatitis C virus infection, n (%)					
Negative	50 (34)	28 (37)	0.625	51 (50)	0.011
Positive	99 (66)	48 (63)		52 (50)	
Autoantibody panel					
Negative	51 (34)	32 (42)	0.248	49 (48)	0.033
Positive	98 (62)	44 (58)		54 (52)	

[a]Fisher's exact test, HCC vs. LC; [b]Fisher's exact test, HCC vs. CH. APRI, aspartate aminotransferase to platelet ratio index; CH, chronic hepatitis; FIB-4, fibrosis-4; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

cholangitis (2,5,26,27). Even in our study, the positivity rate of s-p62-Abs was not significantly higher in patients with HCC than in those with LC and CH. Thus, s-p62-Abs is not a tumor-specific autoantibody.

NY-ESO-1 has been reported to be effective in identifying cancers such as gastric cancer, esophageal cancer, rectal cancer, HCC, and lung cancer; however, the identification of inflammation and fibrosis has not been reported (2,4,5,28). Because there are few reports on the positivity rate of s-NY-ESO-1-Abs in precancerous conditions including LC and CH, the mechanism of positive conversion during the carcinogenic process remains unclear. However, considering that there is a report of s-NY-ESO-1-Abs positivity in CH, s-NY-ESO-1-Abs positivity in chronic liver disease may indicate the presence of microcancer that is not represented in the image or the presence of precancerous conditions (29). In addition, it has been reported that p53 antibody is associated with autoimmune hepatitis; therefore, the possibility that s-NY-ESO-1-Abs positivity occurs as a result of concomitant autoimmune hepatitis cannot be denied (30,31). It may be necessary to carefully search for tumors if s-NY-ESO-1-Abs is positive in patients with LC or CH.

Our study had some limitations. First, we did not analyze the prognosis of the patients or changes in their autoantibody titers. A previous study showed that only s-NY-ESO-1-Abs

Table IV. Positivity of autoantibodies to tumor-associated antigens in patients with HCC, LC, and CH and in controls.

Group	s-Sui1-Abs	s-RalA-Abs	s-p62-Abs	s-p53-Abs	s-c-myc-Abs	s-NY-ESO-1-Abs	Autoantibody panel
Control (n=88) (%)	2 (2)	1 (1)	1 (1)	1 (1)	3 (3)	1 (1)	4 (5)
CH (n=103) (%)	18 (17)	17 (17)	26 (25)	7 (7)	8 (8)	8 (8)	54 (52)
LC (n=76) (%)	13 (17)	12 (16)	23 (30)	7 (9)	11 (15)	5 (7)	44 (58)
HCC (n=149) (%)	66 (44)	34 (23)	32 (21)	20 (13)	17 (11)	9 (6)	98 (66)

CH, chronic hepatitis; HCC, hepatocellular carcinoma; LC, liver cirrhosis.

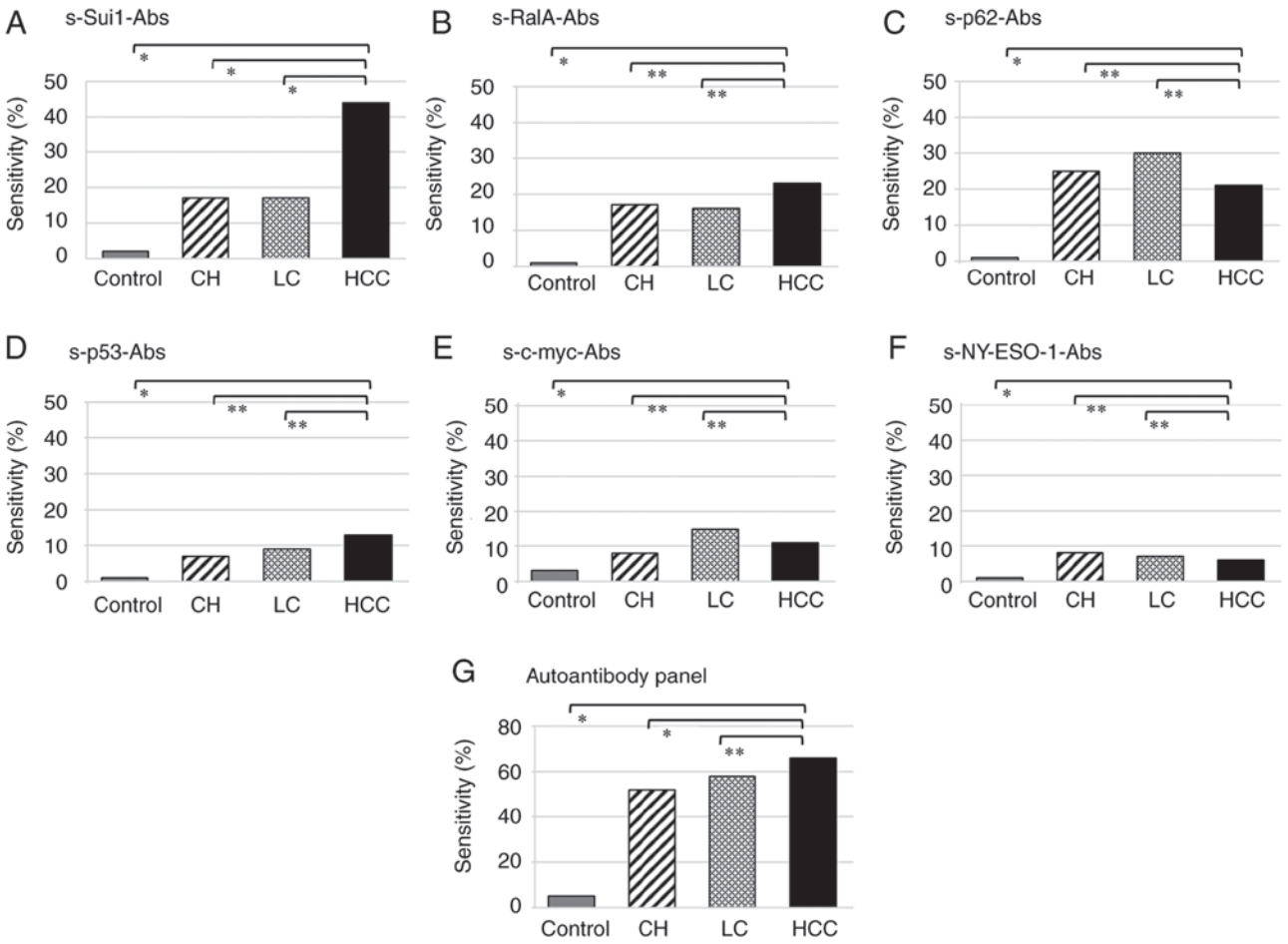

Figure 3. Sensitivity of each serum autoantibody in patients with HCC, LC and CH and in healthy controls. (A) s-Sui1-Abs, (B) s-RalA-Abs, (C) s-p62-Abs, (D) s-p53-Abs, (E) s-c-myc-Abs, (F) s-NY-ESO-1-Abs and (G) autoantibody panel. *P≤0.05 and **P>0.05. HCC, hepatocellular carcinoma; LC, liver cirrhosis; CH, chronic hepatitis.

was associated with poor overall survival in patients with HCC after radical resection. Second, we could not assess the risk of carcinogenesis in patients with autoantibody-positive CH or LC. Third, the cases in this study were not surgically resected, so we could not analyze tumor tissues immunohistochemically. Serum autoantibodies are usually associated with TAA expression in tumor tissue (32,33). Similar to serum autoantibodies, Sui1, RalA, and p53 are reported to have higher expression rates in HCC than in normal liver, CH, or LC, as revealed through immunohistochemical studies (21,34,35). Fourth, no pathological data were available for this study. The diagnoses of CH and LC were based on ultrasound and hematological findings. Finally, we were unable to investigate the genetic mutations analysis of the cases in this study, including HCC. A correlation between the protein expression of p53 and genetic mutations of p53 has been demonstrated in human solid tumors, including HCC (36-39). We could not clarify the association between s-p53-Abs and *p53* mutation, but we speculate that the two are strongly correlated.

In conclusion, serum autoantibodies, including s-Sui1-Abs, s-RalA-Abs, s-p62-Abs, s-p53-Abs, s-c-myc-Abs, and s-NY-ESO-1-Abs, may be useful in differentiating patients with

HCC from healthy individuals. They are, however, not specific to HCC and were also found to be positive in patients with CH and LC. Possibly, the production of these autoantibodies is induced during the development of HCC.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

RO and HS designed and performed the experiments. YO collected clinicopathological data. RO, OY, NK, FI, IH, NS, HM, RA and KK analyzed the results. RO and YO generated the data, prepared the panels and assembled the figures and tables. RO and HS wrote the manuscript. RO and HS confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The protocol of the present study, involving human clinical samples, was approved by the Ethics Committee of Toho University of Medicine, Tokyo, Japan (approval nos. M19213, A18103, A17052, A16035, A16001, 26095, 25024, 24038 and 22047); the Chiba Cancer Center, Chiba, Japan (approval nos. H30-220, 21-26 and 20-1); and the institutional review boards of each participating hospital. Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Okada R, Shimada H, Tagawa M, Matsushita K, Otsuka Y, Kuwajima A and Kaneko H: Profiling of serum autoantibodies in Japanese patients with hepatocellular carcinoma. Toho J Med 3: 84-92, 2017.
2. Okada R, Otsuka Y, Wakabayashi T, Shinoda M, Aoki T, Murakami M, Arizumi S, Yamamoto M, Aramaki O, Takayama T, *et al*: Six autoantibodies as potential serum biomarkers of hepatocellular carcinoma: A prospective multicenter study. Int J Cancer 147: 2578-2586, 2020.
3. Hoshino I, Nagata M, Takiguchi N, Nabeya Y, Ikeda A, Yokoi S, Kuwajima A, Tagawa M, Matsushita K, Satoshi Y and Hideaki S: Panel of autoantibodies against multiple tumor-associated antigens for detecting gastric cancer. Cancer Sci 108: 308-315, 2017.
4. Hoshino I, Nabeya Y, Takiguchi N, Gunji H, Ishige F, Iwatate Y, Shiratori F, Yajima S, Okada R and Shimada H: Prognostic impact of p53 and/or NY-ESO-1 autoantibody induction in patients with gastroenterological cancers. Ann Gastroenterol Surg 4: 275-282, 2020.
5. Ushigome M, Nabeya Y, Soda H, Takiguchi N, Kuwajima A, Tagawa M, Matsushita K, Koike J, Funahashi K and Shimada H: Multi-panel assay of serum autoantibodies in colorectal cancer. Int J Clin Oncol 23: 917-923, 2018.
6. Kabashima A, Shimada S, Shimokawa M, Akiyama Y, Tanabe M and Tanaka S: Molecular and immunological paradigms of hepatocellular carcinoma: Special reference to therapeutic approaches. J Hepatobiliary Pancreat Sci 28: 62-75, 2021.
7. Tan HT, Low J, Lim SG and Chung MC: Serum autoantibodies as biomarkers for early cancer detection. FEBS J 276: 6880-6904, 2009.
8. Lacombe J, Mangé A and Solassol J: Use of autoantibodies to detect the onset of breast cancer. J Immunol Res 2014: 574981, 2014.
9. Di Sabatino A, Biagi F, Lenzi M, Frulloni L, Lenti MV, Giuffrida P and Corazza GR: Clinical usefulness of serum antibodies as biomarkers of gastrointestinal and liver diseases. Dig Liver Dis 49: 947-956, 2017.
10. Pongratz G, Frieser R, Brinks R, Schneider M, Hartung W, Fleck M and Ehrenstein B: Association between autoantibody level and disease activity in rheumatoid arthritis is dependent on baseline inflammation. Clin Exp Rheumatol 38: 691-698, 2020.
11. Jodeleit H, Milchram L, Soldo R, Beikircher G, Schönthaler S, Al-Amodi O, Wolf E, Beigel F, Weinhäusel A, Siebeck M and Gropp R: Autoantibodies as diagnostic markers and potential drivers of inflammation in ulcerative colitis. PLoS One 15: e0228615, 2020.
12. Peng B, Huang X, Nakayasu ES, Petersen JR, Qiu S, Almeida IC and Zhang JY: Using immunoproteomics to identify alpha-enolase as an autoantigen in liver fibrosis. J Proteome Res 12: 1789-1796, 2013.
13. He X, Hong Y, Wang X, Zhang X, Long J, Li H, Zhang B, Chen S, Liu Q, Li H, *et al*: Identification and clinical significance of an elevated level of serum aminoacylase-1 autoantibody in patients with hepatitis B virus-related liver cirrhosis. Mol Med Rep 14: 4255-4262, 2016.
14. Zheng SX, Xiang BD, Long JM, Qu C, Mo ZJ, Li K, Zhuang Y, Lv ZL and Zhou SF: Diagnostic value of serum SMP30 and Anti-SMP30 antibody in hepatocellular carcinoma. Lab Med 49: 203-210, 2018.
15. Kruger FC, Daniels CR, Kidd M, Swart G, Brundyn K, van Rensburg C and Kotze M: APRI: A simple bedside marker for advanced fibrosis that can avoid liver biopsy in patients with NAFLD/NASH. S Afr Med J 101: 477-480, 2011.
16. Sumida Y, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, *et al*: Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. BMC Gastroenterol 12: 2, 2012.
17. Brierley JD, Gospodarowicz MK and Wittekind CH (eds.): TNM classification of malignant tumors. UICC International Union Against Cancer, 8th edition, New York, USA pp 90-93, 2016.
18. Makuuchi M and Kokudo N: Clinical practice guidelines for hepatocellular carcinoma: The first evidence based guidelines from Japan. World J Gastroenterol 12: 828-829, 2006.
19. Kubota K, Makuuchi M, Kusaka K, Kobayashi T, Miki K, Hasegawa K, Harihara Y and Takayama T: Measurement of liver volume and hepatic functional reserve as a guide to decision-making in resectional surgery for hepatic tumors. Hepatology 26: 1176-1181, 1997.
20. Jang ES, Jeong SH, Kim JW, hoi YS, Leissner P and Brechot C: Diagnostic performance of alpha-fetoprotein, protein induced by vitamin K absence, osteopontin, Dickkopf-1 and Its combinations for hepatocellular carcinoma. PLoS One 11: e 0151069, 2016.
21. Zhou JW, Li Y, Yue LX, Luo CL, Chen Y and Zhang JY: Autoantibody response to Sui1 and its tissue-specific expression in hepatocellular carcinoma. Tumour Biol 37: 2547-2553, 2016.
22. Nanami T, Hoshino I, Ito M, Yajima S, Oshima Y, Suzuki T, Shiratori F, Nabeya Y, Funahashi K and Shimada H: Prevalence of autoantibodies against Ras-like GTPases, RalA, in patients with gastric cancer. Mol Clin Oncol 13: 28, 2020.

23. Nanami T, Shimada H, Yajima S, Oshima Y, Matsushita K, Nomura F, Nagata M, Tagawa M, Otsuka S, Kuwajima A and Kaneko H: Clinical significance of serum autoantibodies against Ras-like GTPases, RalA, in patients with esophageal squamous cell carcinoma. Esophagus 13: 167-172, 2016.
24. Kubota Y, Ogata H, Otsuka S, Kuwajima A, Saito F and Shimada H: Presence of autoantibodies against Ras-like GTPases in Serum-in stage I/II breast cancer. Toho J Med 3: 125-130, 2017.
25. Sun H, Shi JX, Zhang HF, Xing MT, Li P, Dai LP, Luo CL, Wang X, Wang P, Ye H, *et al*: Serum autoantibodies against a panel of 15 tumor-associated antigens in the detection of ovarian cancer. Tumour Biol 39: 1010428317699132, 2017.
26. Liu W, Li Y, Wang B, Dai L, Qian W and Zhang JY: Autoimmune response to IGF2 mRNA-binding protein 2 (IMP2/p62) in breast cancer. Scand J Immunol 81: 502-507, 2015.
27. Bauer A and Habior A: Detection of autoantibodies against nucleoporin p62 in Sera of patients with primary biliary cholangitis. Ann Lab Med 39: 291-298, 2019.
28. Yang J, Jiao S, Kang J, Li R and Zhang G: Application of serum NY-ESO-1 antibody assay for early SCLC diagnosis. Int J Clin Exp Pathol 8: 14959-14964, 2015.
29. Zhang Z, Li FF, Lu MD, Zhang SX and Li YX: Anti-NY-ESO-1 autoantibody may be a tumor marker for intrahepatic cholangiocarcinoma. Oncotarget 8: 103283-103289, 2017.
30. Herkel J, Modrow JP, Bamberger S, Kanzler S, Rotter V, Cohen IR and Lohse AW: Prevalence of autoantibodies to the p53 protein in autoimmune hepatitis. Autoimmunity 35: 493-496, 2002.
31. Himoto T, Yoneyama H, Kurokohchi K, Inukai M, Masugata H, Goda F, Haba R, Watanabe S, Senda S and Masaki T: Clinical significance of autoantibodies to p53 protein in patients with autoimmune liver diseases. Can J Gastroenterol 26: 125-129, 2012.
32. Okada R, Shimada H, Otsuka Y, Tsuchiya M, Ishii J, Katagiri T, Maeda T, Kubota Y, Nemoto T and Kaneko H: Serum p53 antibody as a potential tumor marker in extrahepatic cholangiocarcinoma. Surg Today 47: 1492-1499, 2017.
33. Ito M, Hiwasa T, Oshima Y, Yajima S, Suzuki T, Nanami T, Sumazaki M, Shiratori F, Funahashi K, Li SY, *et al*: Association of serum anti-PCSK9 antibody levels with favorable postoperative prognosis in esophageal cancer. Front Oncol 11: 708039, 2021.
34. Wang Z, Gou W, Liu M, Sang W, Chu H and Zhang W: Expression of P53 and HSP70 in chronic hepatitis, liver cirrhosis, and early and advanced hepatocellular carcinoma tissues and their diagnostic value in hepatocellular carcinoma: An immunohistochemical study. Med Sci Monit 21: 3209-3215, 2015.
35. Wang K, Chen Y, Liu S, Qiu S, Gao S, Huang X, Zhang J, Peng X, Qiani W and Zhang JY: Immunogenicity of Ra1A and its tissue-specific expression in hepatocellular carcinoma. Int J Immunopathol Pharmacol 22: 735-743, 2009.
36. Liu J, Li W, Deng M, Liu D, Ma Q and Feng X: Immunohistochemical determination of p53 protein overexpression for predicting p53 gene mutations in hepatocellular carcinoma: A meta-analysis. PLoS One 11: e0159636, 2016.
37. Chen L, Liu Y, Zhang Q, Zhang M, Han X, Li Q, Xie T, Wu Q and Sui X: p53/PCDH17/Beclin-1 proteins as prognostic predictors for urinary bladder cancer. J Cancer 10: 6207-6216, 2019.
38. Bian C, Li Z, Xu Y, Wang J, Xu L and Shen H: Clinical outcome and expression of mutant P53, P16, and Smad4 in lung adenocarcinoma: A prospective study. World J Surg Oncol 13: 128, 2015.
39. Kashofer K and Regauer S: Analysis of full coding sequence of the TP53 gene in invasive vulvar cancers: Implications for therapy. Gynecol Oncol 146: 314-318, 2017.