

Anti-gp210 antibody in combination with anti-centromere antibody may identify PBC patients who are at high risk for end-stage hepatic failure

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Abstract

Background: PBC patients sustained-positive for anti-gp210 antibodies are at high risk for end-stage hepatic failure. However, 20-25% of PBC patients who progressed to hepatic failure were consistently negative for anti-gp210, while 20-40% of PBC patients consistently positive for anti-gp210 did not progress to hepatic failure (J.Hepatology 2005;42:386-392). Therefore, we evaluated the prognostic utility of autoantibodies against PBC-related autoantigens M2 (MIT3-Gershwin-Leung antigen), sp100, and centromere (CENP A&B) in combination with anti-gp210 antibodies.

Methods: A total of 437 serum samples from 294 definite PBC patients followed in a PBC cohort study by NHOSLJ, consisting of 30 patients who progressed and 264 patients who did not progress to end-stage hepatic failure, were tested for antibodies to gp210, sp100, centromere, and M2(MIT3) by ELISA (INOVA Diagnostics).

Results: In 30 patients with hepatic failure, seropositivity for gp210, sp100, centromere and M2(MIT3) was 22/30(73.3%), 2/30(6.7%), 9/30(30.0%), 27/30(90.0%), respectively. In 264 patients with non-hepatic failure, seropositivity for gp210, sp100, centromere and M2(MIT3) was 62/264(23.5%), 25/264(9.5%), 77/264(29.2%) and 233/264(88.3%), respectively. Seropositivity for gp210 was strikingly higher in the hepatic failure compared to non-hepatic failure group (P<0.00001), while seropositivity for the other 4 antigens was similar between the 2 groups. The incidence of hepatic failure in anti-gp210 positive vs negative, anti-sp100 positive vs negative, anti-centromere positive vs negative and anti-M2(MIT3) positive vs negative patients was 22/64(26.1%) vs 8/210(3.8%) (P<0.00001), 2/27(7.4%) vs 28/267(10.5%) (P=1.0), 9/64(14.1%) vs 21/208(10.1%) (p=1.0) and 27/261(10.3%) vs 3/333(0.9%) (P<1.0). Among 84 anti-gp210 positive patients, the incidence of hepatic failure in anti-gp210 single positive, anti-gp210 + anti-sp100 double positive, and anti-gp210 + anti-centromere double positive-patients was 15/65(23.1%), 1/4(25%) and 6/15(40%), respectively. Thus, anti-gp210 + anti-centromere double positive-PBC patients seemed to have a higher incidence of hepatic failure compared to anti-gp210 single positive patients (P=0.202). However, Kaplan-Meier analysis for OLT free survival revealed that combination of ELISA for anti-gp210 and anti-centromere may not identify PBC patients who are at higher risk for progression to end-stage hepatic failure compared to non-combination (logrank test P=0.3495).

Conclusions: Antibodies to gp210 and sp100 are positive in 20.5% and 7.7%, respectively, of the PBC patients negative for M2(MIT3) antibodies. The frequency of anti-gp210 antibodies is strikingly higher in patients who progressed to end-stage hepatic failure. Thus, ELISA for anti-gp210 is very useful to identify and monitor PBC patients who are at high risk for progression to end-stage hepatic failure.z

Introduction

Anti-nuclear antibodies (ANAs) are positive in 30-50 % of patients with primary biliary cirrhosis(PBC). Among ANAs, anti-gp210 antibodies are specifically detected in 20-30% of PBC patients.

We recently found that PBC patients sustained-positive for anti-gp210 antibodies are at high risk for end-stage hepatic failure by retrospective cohort study of National Hospital Organization Study Group for Liver Disease in Japan (NHOSLJ) (Figure 1)(Nakamura M et al. J.Hepatology 2005;42:386-392).

However, approximately 20% of PBC patients who progressed to end-stage hepatic failure were consistently negative for anti-gp210 antibodies, while 30-40% of PBC patients consistently positive for anti-gp210 antibodies did not progress to end-stage hepatic failure during the observation period.

In order to identify more accurately the PBC patients at high risk for end-stage hepatic failure, we evaluated the prognostic utility of autoantibodies to other PBC-related autoantigens such as M2(MIT3), sp100, and centromere(CENP A&B) in combination with anti-gp210 antibodies.

Methodology

A total of 437 serum samples from 294 definite PBC patients followed in a PBC cohort study by NHOSLJ, consisting of 30 patients who progressed and 264 patients who did not progress to end-stage hepatic failure, were tested for antibodies to gp210, sp100, centromere, and M2(MIT3) IgG by Quanta Lite™ ELISA kits (INOVA Diagnostics, San Diego, CA).

Some serum samples were also tested for anti-mitochondrial or anti-nuclear antibodies by indirect immunofluorescence test using HEp-2 cells or by western blotting using recombinant full length gp210 proteins and lysates of nuclear or non-nuclear fractions derived from HepG2 cells.

Table 1
Autoantibodies detected in PBC patients

anti-mitochondrial (AMAs)	antigens
anti-PDC	PDC-E2 (74 kD)
	PDC-E3BP (60 kD)
	PDC-E1 (44 kD)
anti-OGDC	OGDC-E2 (46 kD)
anti-BOADC	BOADC-E2 (62 kD)
anti-nuclear (ANAs)	
perinuclear (rim-like)	gp210 (nuclear pore)
(nuclear dot)	p82 (nuclear pore)
	Lamin B Receptor (58 kD)
	sp100 (100 kD)
	PML, SUMO
centromere	CENP-A(17 kD), CENP-B (90 kD)

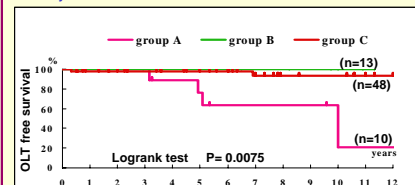
Table 2

Seropositivity to gp210, sp100, centromere A&B or M2(MIT3) in PBC patients who progressed or did not progress to end-stage hepatic-failure

	seropositivity to			
	gp210*	sp100	centromere A&B	M2 (MIT3)
Hepatic failure (n=30)	22/30 (73.3%)	2/30 (6.7%)	9/30 (30.0%)	27/30 (90.0%)
Non-hepatic failure (n=264)	62/264 (23.5%)	25/264 (9.5%)	77/264 (29.2%)	233/264 (88.3%)

*P<0.00001
 Serum antibodies determined by Quanta Lite™ ELISA kits (INOVA Diagnostics)

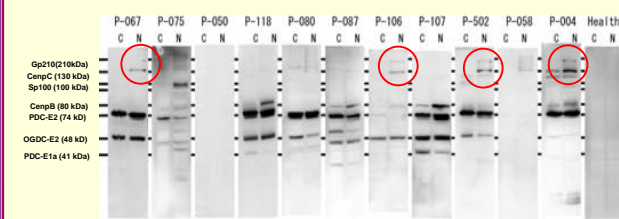
Figure 1
Kaplan-Meier analysis for OLT free survival depending on anti-gp210 antibody status in PBC



The risk for progression to end-stage hepatic failure is 12 times higher in group A as compared to group B + C. Serum antibodies were determined by home-made ELISA as described elsewhere (Nakamura M et al. J.Hepatology 2005;42:386-392)
 group A = anti-gp210 antibody titer sustained high
 group B = anti-gp210 antibody titer decreased to normal level after UDCA treatment
 group C = anti-gp210 antibody titer initially negative
 OLT:orthotopic liver transplantation

Figure 4

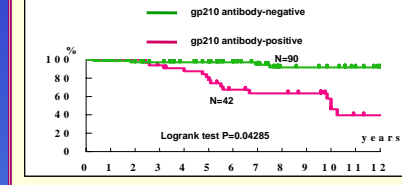
The presence of anti-gp210 antibodies which can be detected by western blotting but not by ELISA in PBC patients who progressed to end-stage hepatic failure



ELISA法	P-067	P-075	P-050	P-118	P-080	P-087	P-106	P-107	P-502	P-058	P-004	Healthy
GP210	11.2	132.4	117.4	164.8	368.5	111.7	32.7	2.8	3.9	20.3	2.4	-
Sp100	3.0	6.3	2.2	2.2	11.8	9.7	2.8	3.6	1.1	2.3	2.5	-
centromere	14.2	6.4	114.9	106.2	7.2	4.6	10.2	6.2	102.5	4.6	102.9	-
M2	154.5	6.2	2.7	101.3	72.3	71.0	73.8	97.2	32.9	70.1	13.6	-
M2EP	60.1	85.7	15.5	189.3	181.5	198.6	110.3	150.0	196.5	2.8	183.7	-

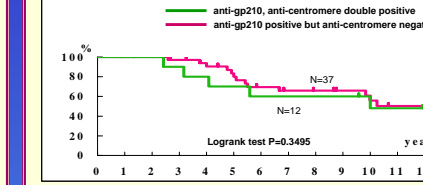
Anti-gp210 antibodies which can be detected only by western blotting are likely to react to N-terminal glycosylated epitope of gp210.

Figure 2
Kaplan-Meier analysis for OLT free survival in PBC



The incidence of the progression to end-stage hepatic failure is significantly higher in patients positive for anti-gp210 antibodies compared to those negative for anti-gp210 antibodies as determined by gp210 ELISA kit (INOVA).

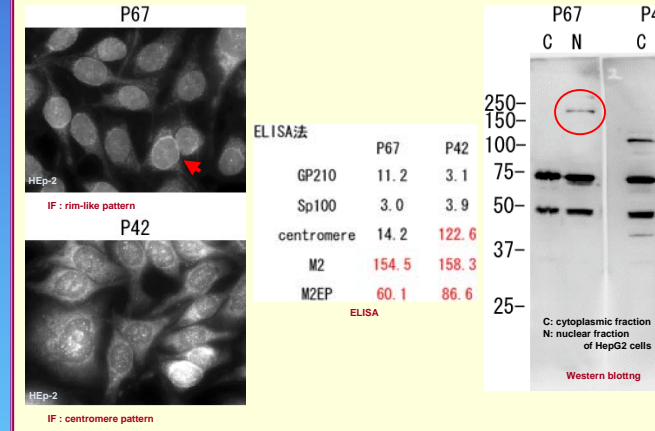
Figure 3
Kaplan-Meier analysis for OLT free survival in PBC



Combination of ELISA for anti-gp210 and anti-centromere may not identify PBC patients who are at higher risk for progression to end-stage hepatic failure compared to non-combination for anti-gp210 and anti-centromere.

Figure 6

Serum sample showing IF- and western blotting - positive but ELISA-negative for anti-gp210 antibodies



Summary and Conclusions

- gp210-ELISA is very useful to identify PBC patients who are at high risk for the progression to end-stage hepatic failure, whereas sp100, centromere and M2(MIT3) do not show a significant utility for this purpose.
- Although the anti-gp210 + anti-centromere double positive-PBC patients seemed to have a higher incidence of hepatic failure compared to anti-gp210 single positive patients (P=0.202), the Kaplan-Meier analysis for OLT free survival revealed that combination of gp210-ELISA and centromere ELISA may not have an additional impact on the identification of PBC patients who are at higher risk for end-stage hepatic failure (logrank test P=0.3495).
- Since there exists some PBC sera in which anti-gp210 antibodies can be detected only by western blotting but not by gp210-ELISA, an efficient and accurate assay system for antibodies reacting to N-terminal glycosylated region of gp210 maybe required.

Table 3

The number of PBC patients who progressed to end-stage hepatic failure in patients positive or negative for antibodies to gp210, sp100, centromere A&B or M2(MIT3)

Antibody to	Number of patients who progressed to end-stage hepatic failure in:		P
	antibody-positive	antibody-negative	
gp210	22/84 (26.1%)	8/210 (3.8%)	p<0.0001
sp100	2/27 (7.4%)	28/267 (10.5%)	p=1.0
Centromere	9/86 (10.5%)	21/208 (10.1%)	p=1.0
M2(MIT3)	27/216 (10.3%)	3/33 (9.1%)	p=1.0

Serum antibodies determined by Quanta Lite™ ELISA kits (INOVA Diagnostics)

Table 4

Prognostic utility of anti-centromere or anti-sp100 antibodies in combination with anti-gp210 antibodies in PBC

Combination of autoantibodies	n	Number of patients who progressed to end-stage hepatic failure
gp210 positive (total)	84	22/84 (26.1%)
gp210 single positive	65	15/65 (23.1%)
gp210+sp100 double positive	4	1/4 (25.0%)
gp210+centromere A&B double positive	15	6/15 (40.0%)
gp210+sp100+centromere A&B Triple positive	0	-