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Evaluation of alpha-fetoprotein as a screening marker for hepatocellular carcinoma in hepatitis prevalent areas

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ABSTRACT

The objective of this study was to establish modified cutoff values of serum alpha-fetoprotein (AFP) according to hepatitis status. While AFP is used as a serum marker in the diagnosis or monitoring of hepatocellular carcinoma (HCC), its use as a screening method to the general population is controversial. We evaluated its screening performance in a hepatitis prevalent East Asian population, and suggest different cutoff values according to the individual's hepatitis status. We evaluated the performance of AFP as a screening test in 48,123 consecutive Koreans during the period from March, 2012 to August, 2013 who underwent routine health checks at a single institution. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with fixed cutoff and with modified cutoffs according the individual's hepatitis status. A total of 24 out of 48,123 subject (0.05%) were newly diagnosed with HCC after screening. Among the 1,874 subject with positive hepatitis B virus surface antigen (HBsAg), 17 (0.91%) developed HCC, compared with two out of 393 (0.51%) individuals with hepatitis C virus antibody (anti-HCV). Five out of 45,855 (0.01%) subject with neither HBsAg nor anti-HCV developed HCC. Compared to the performance of a fixed cutoff, specificity, PPV, and NPV improved without sacrificing sensitivity when applying modified cutoff. In conclusion, our findings suggest that AFP with modified cutoffs according to the individual's hepatitis status might be a useful screening marker for HCC in hepatitis prevalent areas.

Key words. Alpha-fetoprotein. Hepatocellular carcinoma. Liver cancer. Screening.

INTRODUCTION

Hepatocellular carcinoma (HCC) has a high prevalence in Korea. In a recent epidemiologic study of the site of cancer, liver was the fifth leading site of cancer, accounting for 7.9% of all cancer cases and 15.3% of all cancer deaths and ranked as the second most common site for the origin of cancer.[1] Viral hepatitis is also endemic in Korea. Hepatitis B surface antigen (HBsAg) was detected in 6.6-8.6% of the population in the 1980s and 5.7% in the 1990s.[2] On the other hand, the prevalence of hepatitis C is lower than that of hepatitis B. In a nationwide investigation regarding hepatitis serologic markers, when considering the estimated 2009 population of Korea, the age-, sex-, and area-adjusted rate of hepatitis C antibody (anti-HCV) positivity was 0.78%.[3]

Chronic hepatitis B and C are recognized as major factors increasing the risk of HCC,[4-6] and when screening for HCC these factors should be considered. Current guidelines of the American Association for the Study of Liver Diseases (AASLD) for screening and diagnosis of HCC rely on imaging modalities, concluding that the measurement of serum alpha-fetoprotein (AFP) is an inadequate screening test.[7,8]

AFP is a fetal specific glycoprotein that is normally produced primarily by the fetal liver. AFP levels decline rapidly after birth, reaching undetectable levels (less than 10 ng/mL) within several months after birth.[9,10] Although the AFP level is elevated in HCC, elevation of serum AFP level is considered a non-specific finding because it is observed not only in malignant conditions[11,12] but also in benign liver

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conditions.[13-15] For these reasons, use of AFP as a screening test for HCC is controversial.[8,16-18] It is of note that a randomized controlled trial of screening for HCC in a Chinese population using AFP and abdominal ultrasonography indicated that biannual screening reduced HCC mortality by 37%.[18]

There are few studies regarding the effectiveness of AFP as a screening marker for HCC. In the present study, we studied the serum AFP levels of healthy examination subjects and the occurrence of HCC after their examination. We investigated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of AFP according to certain cutoff levels. We also investigated value of modifying the cutoff value of AFP in screening for HCC to increase the PPV without sacrificing sensitivity.

MATERIAL AND METHODS

We analyzed serum AFP levels of all ethnically Korean subjects who visited the Center for Health Promotion at Samsung Medical Center for a routine health check from March 2012 to August 2013. Prior to data collection, this study was approved by the Institutional Review Board of Samsung Medical Center.

Patients with a previous history of liver cancer and subjects that were not ethnically of Korean origin were excluded from this study. Serum AFP was measured by ADVIA Centaur XP immunoassay system (Siemens Healthcare Diagnostics, Erlangen, Germany) and the cutoff provided by the manufacturer was 8.1 ng/mL. HBsAg and anti-HCV antibody were assayed using the Modular E170 (Roche Diagnostics, Basel, Switzerland) and the Centaur XP, respectively. Diagnosis of HCC at our institute is performed by either biopsy or imaging modalities. Data on each subject's status for HCC was obtained from their follow-up visits to our institution and from their registration status with the National Health Insurance Service (NHIS) as a cancer patient. All newly diagnosed cancer patients in Korea are obliged registered within the NHIS as a cancer patient, thus ensuring that the all subjects were followed up for cancer until the end of this study. This system ensured that the data of all subjects who were later diagnosed with HCC after AFP measurement were collected. The follow-up period was at least 1 year after AFP measurement. Assessment for the subject's cancer status was evaluated until August 2014.

Between-group differences were analyzed with the use of the Kruskal-Wallis H test, with a P-value $\leq$ 0.05 considered statistically significant. Post-hoc analysis was performed by applying the Mann-Whitney U test accompanied with Bonferroni's correction, with a P-value $\leq$ 0.017 considered statistically significant. For evaluation of AFP as a screening test, sensitivity, specificity, PPV, and NPV were evaluated according to different cutoffs of AFP. A receiver operator characteristics (ROC) curve was drawn, and the 95% confidence intervals (CI) for area under the ROC curve (AUC) value were calculated. All statistical analysis was performed by SPSS 21 software (IBM Inc., Armonk, NY, USA).

RESULTS

During the study period, a total of 49,381 individuals were evaluated for serum AFP levels, with 33 and 1,226 excluded due to either previous history of HCC or non-Korean ethnicity, respectively. Thus, 48,122 subjects were enrolled for analysis. Serologic studies for HBsAg and anti-HCV antibody showed that 1,873 and 393 were positive for HBsAg or anti-HCV, respectively, with 20 subjects positive for both. The median value for AFP for all subjects was 3.0 ng/mL, with the 1st quartile at 2.1 ng/mL and 3rd quartile at 4.2 ng/mL. For HBsAg (+) subjects (assigned as the hepatitis B group), the 1st, median, and 3rd quartile values were 1.9 ng/mL, 2.8 ng/mL, and 4.0 ng/mL, respectively; these values were slightly lower than those of the whole group. Anti-HCV(+) subjects (assigned as the hepatitis C group) had a median AFP value of 3.0 ng/mL with the 1st and 3rd quartiles being 2.2 ng/mL and 4.3 ng/mL, respectively (Table 1). Subjects that were positive for both HBsAg and anti-HCV were included in both groups for analysis.

Normality test results for each group did not show a Gaussian distribution. Kolmogorov-Smirnov test results for the hepatitis B group, the hepatitis C group, and the HBsAg(-)/ anti-HCV(-) group (assigned as the non-B/non-C group) were all $P < 0.001$.

Kruskal-Wallis H test showed that the AFP test results differed statistically among the three groups ($P < 0.001$); pairwise comparison applying the Mann-Whitney U test showed differences in the AFP results for the hepatitis B group compared with the hepatitis C group ($P = 0.002$) and compared with the non-B/non-C group ($P < 0.001$), whereas the hepatitis C group and the non-B/non-C group did not differ statistically ($P = 0.170$).

We identified 24 subjects who were diagnosed with HCC after measurement of serum AFP.

Table 1. Summary of the alpha-fetoprotein levels of the enrolled subjects.

Group	Subjects (n)	HCC patients (n)	1st quartile	Median	3rd quartile
Hepatitis B	1,873	17	1.9	2.8	4
Hepatitis C	393	2	2.2	3	4.3
Co-infection	20	0	2.3	3	3.7
Non-B/non-C	45,876	5	2.1	3	4.2
Total	48,122	24	2.1	3	4.2

Table 2. List of newly diagnosed hepatocellular carcinoma patients after screening.

Group	Age	Gender	AFP (ng/mL)	Date of screening	Date of diagnosis
Hepatitis B	52	M	200,000	2013-08-05	2013-08-14
	58	M	16,844.2	2012-06-19	2012-07-30
	54	F	6,329.1	2013-07-09	2013-07-31
	64	M	930.8	2013-04-10	2013-04-11
	59	M	206	2012-10-04	2012-10-12
	51	M	50.6	2012-04-25	2012-11-01
	63	M	23.7	2013-03-29	2013-12-01
	67	M	20.1	2013-03-15	2013-04-09
	52	M	17.6	2012-10-04	2012-11-18
	46	M	15.6	2012-10-22	2012-11-01
	54	M	11.5	2012-10-02	2012-10-24
	49	M	10.4	2013-04-24	2013-05-23
	52	M	10	2012-04-27	2013-12-27
	51	M	8.5	2012-11-26	2013-11-26
	53	F	6.8	2012-10-18	2013-08-08
	65	M	3.7	2013-03-28	2013-05-07
	71	M	2.8	2012-04-04	2013-07-01
Hepatitis C	72	M	45.2	2012-11-06	2012-11-16
	67	M	9.3	2012-03-27	2012-04-03
Non-B/non-C	87	M	1,209.1	2012-09-03	2012-09-24
	68	M	176.1	2013-02-28	2013-04-21
	63	M	33.5	2013-05-20	2013-06-03
	52	M	14.7	2013-04-12	2014-01-21
	73	M	6.8	2012-03-26	2013-03-26

Of these, 17 were positive for HBsAg, 2 were positive for anti-HCV, and 5 showed negative results for both. There was no HCC patient with both HBsAg and anti-HCV. The characteristics of the 24 patients are summarized in table 2. When the cutoff provided by the manufacturer (8.1 ng/mL) was applied to all subjects, i.e., regardless of HBsAg and anti-HCV status, a total of 1,203 subjects showed AFP values exceeding the cutoff. Of the 24 newly diagnosed HCC patients, 20 showed serum AFP levels exceeding the cutoff. The sensitivity, specificity, PPV, and NPV of AFP with this cutoff were 82.33, 99.99, 1.66, and 99.99%, respectively (Table 3). The ROC curve showed an AUC value of 0.956 (95% CI, 0.906-1.000), as shown on the upper left of figure 1.

The cut-off level that shows the highest Youden's index is equivalent to the point with the tallest height above the chance line in ROC analysis. In our study, an AFP level of 6.7 ng/mL had the highest Youden's index for the hepatitis B and the non-B/non-C group. In the hepatitis C group, 9.2 ng/mL had the highest Youden's index. This approach decreases the cutoff level in the hepatitis B and non-B/non-C group, and demonstrates higher sensitivity with lower PPV. However, when we considered modifying the cutoff value of serum AFP levels to improve PPV without sacrificing sensitivity, the values that showed the highest Youden's index in the hepatitis B, hepatitis C, and non-B/non-C groups, were 8.4 ng/mL, 9.2 ng/mL, and 14.6 ng/mL respectively.

Table 3. Statistical data when a fixed cutoff value (8.1 ng/mL) is applied.

Group	No. exceeding cutoff	No. of HCC patients exceeding cutoff	Sensitivity	Specificity	PPV
Hepatitis B	100	14	82.35	99.83	14.00
Hepatitis C	15	2	100	100	13.33
Non-B/non-C	1,088	4	80.00	~100	0.38
Total	1,203	20	83.33	99.99	1.66

Table 4. Statistical data when adjusted cutoff value according to hepatitis status is applied.

Group (cutoff, ng/mL)	No. exceeding cutoff	No. of HCC patients exceeding cutoff	Sensitivity	Specificity	PPV
Hepatitis B (8.4)	95	14	82.35	99.83	14.74
Hepatitis C (9.2)	14	2	100	100	14.29
Non-B/non-C (14.6)	116	4	80.00	~100	3.45

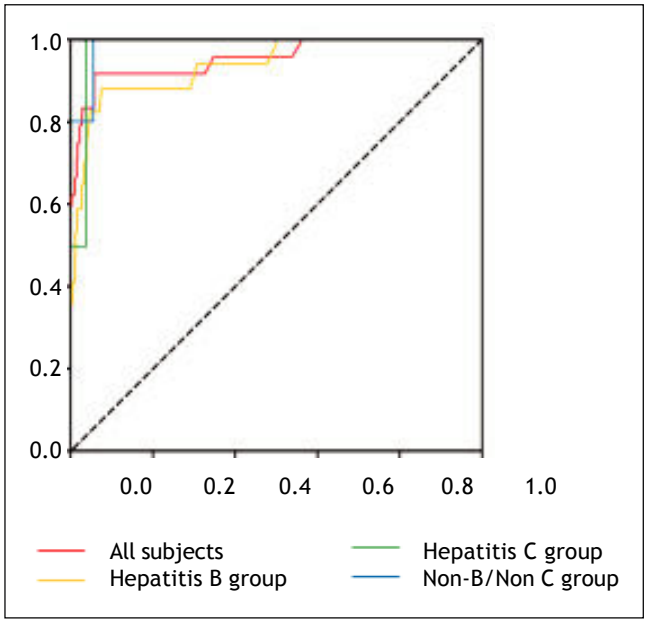

***Figure 1**. Receiver operator characteristics curve for each group.*

When a new cut-off value of 8.4 ng/mL was applied to the HBsAg(+) group, the results showed 82.53% sensitivity with a slightly improved PPV compared to the previous cut-off value of 8.1 ng/mL (14.74% from the previous 14.00%). For subjects in the anti-HCV(+) group, a cut-off value of 9.2 ng/mL showed 100% sensitivity, 100% specificity, and improved PPV (14.29 from 13.33%). The largest improvement could be observed when applying a 14.6 ng/mL cut-off value to the non-B/non-C group; with the same 80% level of sensitivity, PPV increased to 3.45% from the previous value of 0.38%, with approximately 100% specificity (Table 4).

The AUC of AFP for each group was 0.941 (95% CI, 0.880-1.000) for the hepatitis B group, 0.985 (95% CI, 0.963-1.000) for the hepatitis C group, and 0.989 (95% CI, 0.971-1.000) for the non-B/non-C group (Figure 1). Considering that the AUC was 0.956 for all subjects regardless of each subject's hepatitis status, the AUC increased in the latter two groups. However, since the CI range overlaps, this change is statistically questionable.

DISCUSSION

Our study suggests that evaluation of AFP levels in a general population should be approached according to each subject's hepatitis status; subjects without any hepatitis would be the control healthy group, with the highest cut-off value, and groups with hepatitis B and hepatitis C having lower cut-off values compared to the control group. This is in contrast to previous studies,[8,16,19] and our investigation demonstrates that with the appropriate cutoff value, screening for HCC with serum AFB level can show reasonable sensitivity and specificity. However, as HCC itself has a low prevalence,[1] screening with AFP showed a low PPV. This may raise ques-

tions regarding the effectiveness of serum AFP as a screening test. Since elevated serum AFP levels are not observed solely in HCC patients, we speculated that there might be limitations in increasing the PPV; however, when we applied different cutoff values according to each individual's hepatitis status, PPV could be raised without sacrificing sensitivity. In this study, we demonstrated that such an approach is especially effective in individuals that are not HBsAg(+) or anti-HCV(+). The benefits of this approach will be mostly in the reduction of medical costs and the decrease in false positive screening results in individuals with little reason to suspect HCC.

Approaches using serum AFP as a screening test were reported previously,[19] and serum AFP cutoff levels as high as 200 ng/mL have been suggested by Zhou, *et al.*;[20] when applied to our data, an AFP level of 200 ng/mL would result in an overall sensitivity of 25 and 50% sensitivity. Such results are hard to accept as good levels of sensitivity for a screening test. If AFP is to be used as a screening test, the sensitivity should be increased, but doing so will also increase false positive results; thus we suggest a discriminated strategy when using serum AFP level as a screening test for HCC. Health examination subjects should first be evaluated for the possibility of chronic hepatitis B or C. As our results show that subjects with hepatitis B are more likely to have HCC at lower serum AFP levels, subjects should first be evaluated for their serum HBsAg and positive subjects should be assigned to the group with the lowest AFP cutoff level. The remaining subjects should be checked for anti-HCV, and positive subjects should be assigned to a different group with an appropriate AFP cutoff level. Finally, subjects with no reason to suspect hepatitis B or C should be classified as a third group with AFP cutoff levels set to a higher level than for the former two groups.

In reality, tests for HBsAg, anti-HCV, and AFP will be performed simultaneously, and interpretation of the AFP results should be done according to the subject's hepatitis status. A diagram for this strategy is illustrated on figure 2. This strategy can also be justified on a statistical basis. Kruskal-Wallis H test showed that the three groups differed statistically in AFP test results. Post-hoc analysis by applying Mann-Whitney U test accompanied with Bonferroni correction showed that the mean level for AFP was the lowest in the hepatitis B group. Thus, it can be speculated that the lowest cutoff level should be applied to the hepatitis B group. While the low cutoff for the hepatitis B group can be approached with statistics, the hepatitis C group did not differ from the non-B/non-C group. Yet, the application of a lower cutoff value to the hepatitis C group compared to the non-B/non-C group was not fully explained in this case. A study done on Japanese hepatitis C patients spots some light on this matter. In this study, the 10-year cumulative risk of HCC was 6.0% in the patients with AFP 6 ng/mL, 24.6% in the patients with AFP 6-20 ng/mL, and 47.3% in patients with AFP levels exceeding 20 ng/mL.[21] This can be interpreted as that AFP levels exceeding 6 ng/mL is with increased risk of HCC; our study suggests 9.2 ng/mL in a health examination environment. On the other hand, the non-B/non-C group is the group without risk factors; thus the application of the highest cutoff level can be justified.

An American study showed that non-B/non-C patients with HCC is closely linked to alcohol liver disease or nonalcoholic steatohepatitis.[22] Further history taking to health examination accompanied with abdominal ultrasound may be helpful in these cases. However, speculation regarding whether if HBsAg negative state is truly hepatitis B negative have been reviewed before, and in many cases,

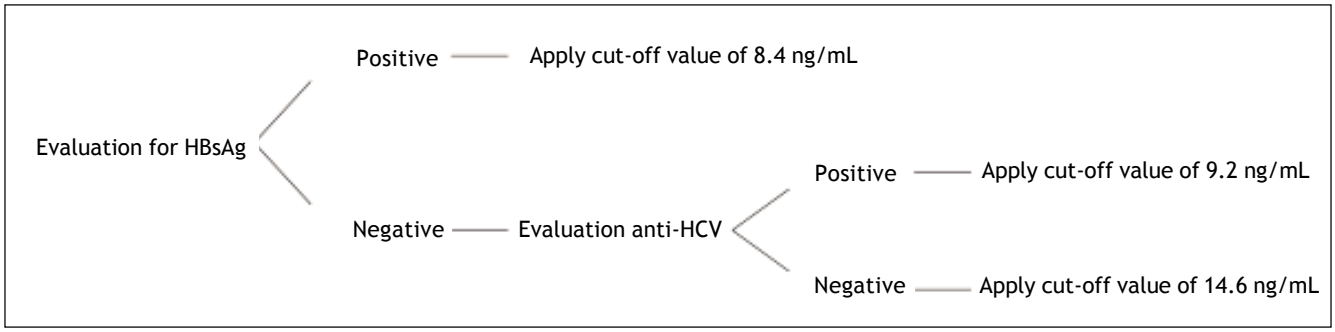

***Figure 2**. Suggested strategy for the use of alpha-fetoprotein as a screening test.*

HBsAg negative may not necessarily mean that the subject is free of hepatitis B infection.[23] Employment of tests regarding liver tissue hepatitis B core antigen or hepatitis B virus DNA could have been complementary. Such test may be required to investigate the true status of the subjects classified as non-B/non-C to fully validate our results. Hypothetically, if the single case of the 73 year old male with an AFP level of 6.8 ng/mL proves to be a case of occult hepatitis B infection, such results would further solidify our results. However, other studies regarding hepatitis B infection were not done to our subjects, and such speculation could not be validated at the moment.

Our study integrated data from the Korean NHIS, and the cancer status at the end of this study can be considered reliable. We consider that no cases with low AFP levels that were later diagnosed with HCC went unnoticed. However, if an individual refused further medical treatment and did not register as a cancer patient, our data could have been biased.

The location of our institute may limit the general application of our results as a result of geographic and socioeconomic factors. However, it should be noted that the health examination program in our institute is open to the general public therefore there is no apparent reason to suspect selection bias of the subjects of our study. Also, the number of newly diagnosed HCC patients was too small in the anti-HCV positive and HBsAg negative/anti-HCV negative groups and a larger study is required to validate the exact cutoff value to be applied to these groups, in addition to validating our findings. Other factors, the most noted is liver cirrhosis is also known to be a major risk factor in HCC.[16,22] Evaluation for liver cirrhosis was not put into this study, and while this could have been evaluated with routine abdominal sonography, not adjusting the subjects for their cirrhosis status can be a shortcoming of this study.

Nevertheless, our approach suggests that when measurement of serum AFP level is applied as a screening test in a HCC prevalent community such as the Korean population, the cut-off level can be increased in subjects that are not affected with hepatitis B or C, reducing false positive results and unnecessary further work-up for HCC.

ABBREVIATIONS

- **AASLD:** American Association for the Study of Liver Diseases.
- **AFP:** alpha-fetoprotein.
- **anti-HCV:** hepatitis C virus antibody.
- **HBsAg:** hepatitis B virus surface antigen.
- **HCC:** hepatocellular carcinoma.
- **KNHIS:** Korean National Health Insurance Service.
- **NPV:** negative predictive value.
  PPV: positive predictive value.

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