Tumor Res. **22**, 63—70 (1987)

Clinical Significance of Abnormal Prothrombin (PIVKA-II) in the Plasma of Patients with Hepatocellular Carcinoma

Toshinori KODAMA[1,2], Toshikazu YOKOI[1], Kazuhiro ARIMA[2], Mitsuo SUGA[2], Kohzoh IMAI[2], Akira YACHI[2], Akira TAKAHASHI[3] and Yoshiyuki SHIMAMURA[3]

1. *Department of Pathology, Cancer Research Institute, Sapporo Medical College*
2. *Department of Internal Medicine (Section 1), Sapporo Medical College*
3. *Matsudo National Hospital*

Department of Pathology, Cancer Research Institute Sapporo Medical College, South 1 West 17, Chuo-Ku, Sapporo 060, Japan

SUMMARY

PIVKA-II (abnormal protein induced by vitamin K absence or antagonist) was measured using an enzyme linked immunosorbent assay (ELISA) in 188 human subjects, of which 82 were patients with hepatocellular carcinoma (HCC). PIVKA-II was positive in 43 out of 82 patients (52%) with hepatocellular carcinoma. In contrast, it was positive in only 2 out of 58 patients (3.4%) with other liver diseases and was not found in 50 normal controls.

The concentration of PIVKA-II in the plasma was not correlated with that of alpha-fetoprotein (AFP). Combination assay of these proteins were positive in 57 out of 82 patients (69.5%) with HCC.

In patients with HCC showing a favorable response to the therapy, the concentration of PIVKA-II was markedly reduced while the patients who showed no improvement by the therapy the concentration increased in parallel with the tumor growth. Therefore, PIVKA-II is a valuable marker for diagnosis and follow-up of the patient with HCC.

Key words: PIVKA-II, Hepatocellular carcinoma, Tumor marker, Lp-TAE

INTRODUCTION

PIVKA-II is an incomplete form of prothrombin that is synthesized in the liver. It contains 10 glutamic acid residues in its amino-terminal domain, which is carboxylated by vitamin K(19). The absence of vitamin K or the prescription of vitamin K antagonists (e. g. Warfarin) inhibits vitamin K dependent carboxylase activity in the liver and PIVKA-II is released into the blood. PIVKA-II does not have coagulation activity(4, 5).

PIVKA-II has been recently found as an useful marker of HCC in laboratory diagnosis(6, 9, 17, 18).

In the clinical field, tumor markers are widely determined for the diagnosis and monitoring of cancer patients(7, 21). However, there has been little information on PIVKA-II response in the early stage of HCC and changes in the concentration after treatment with surgical resection or other effective therapies.

The recent development of several imaging modalities has enabled us to identify small sized HCC in patients(3, 11, 14, 22), and thus gave us the opportunity to observe PIVKA-II response in an early stage and monitoring the management of HCC. This report describes the clinical significance of PIVKA-II in the early diagnosis and management monitoring of HCC patients.

MATERIALS AND METHODS

Samples were collected from 82 patients with HCC from the Department of Internal Medicine (Section 1), Sapporo Medical College Hospital and Matsudo National Hospital, in which 9 patients had undergone hepatic surgery and 20 patients subjected to transcatheter arterial embolization with anticancer drugs containing Lipiodol® (Lp-TAE)(8). Plasma samples were collected from 16 patients with chronic active hepatitis, 26 with liver cirrhosis, 16 with liver tumors other than HCC (five patients with hemangioma, one with hepatoblastoma, one with cholangiocellular carcinoma and 9 with metastatic carcinoma involving the liver). Metastatic carcinomas consist of one pancreatic cancer, four colon cancers, two gastric cancers and two lung cancers. Fifty normal subjects from healthy blood donors were used as control samples.

A "small HCC" was defined as cancer of less than 2 cm in maximal diameter and of no daughter nodules. The size of the tumor was measured in a freshly resected specimen. In those patients without operation, the tumor size was determined by ultrasonography, computed tomography and selective hepatic angiography (10).

Measurement of PIVKA-II and AFP levels

The blood was treated with 3.8% sodium citrate and was centrifuged at 3,000 rpm for 10 minutes. Plasma samples were stored at −20℃ until use. The level of PIVKA-II in the plasma was measured by the ELISA (E-1023 kit, Eizai Lab., Tokyo, Japan) which was a modified method developed by Motohara *et al.*(12, 13, 15). The results were expressed in arbitrary unit (AU), 1 AU corresponds to 1 μg of purified PIVKA-II. The cut-off value was set as 0.13 AU/m*l* according to the manufacture's direction(12). The concentration of AFP in the serum was measured by the use of ELISA kit which was purchased from Eizai Lab., Tokyo, Japan. All

samples for both kits were run in duplicate.

RESULTS

The distribution of PIVKA-II levels

The distribution of PIVKA-II levels among the 82 patients with HCC, 58 patients with various liver diseases excluding HCC and 50 normal subjects is shown in Figure 1. Forty-three out of 82 patients (52%) with HCC gave positive results, whereas only 2 out of 56 (3.6%) patients without HCC showed positive. Fifty normal subjects exhibited negative results. The sensitivity of PIVKA-II for HCC was 52% (43/82), the specificity was 97% (56/58) and the predictive accuracy was 96% (43/45) at this cut-off value. Among two cases who showed false positive, one had liver cirrhosis, and another had metastatic liver tumor form pancreas cancer, either cases were not administrated with vitamin K antagonists or inhibitors.

Comparison between plasma PIVKA-II levels and serum AFP levels

The concentration of AFP did not show any correlation to that of PIVKA-II ($n=82$, $r=0.11$) as shown in Figure 2. Thirty-two out of 82 patients with HCC showed the AFP levels lower than 20 ng/m*l*. Higher levels of PIVKA-II were detected in 7 out of these 32 patients. When the positive results of both assays were combined, 57 patients (69.5%) had elevated levels of one antigen or both.

PIVKA-II levels in small HCCs

Twelve patients were diagnosed as a small HCC. PIVKA-II levels of these patients, however, were not increased in their plasma.

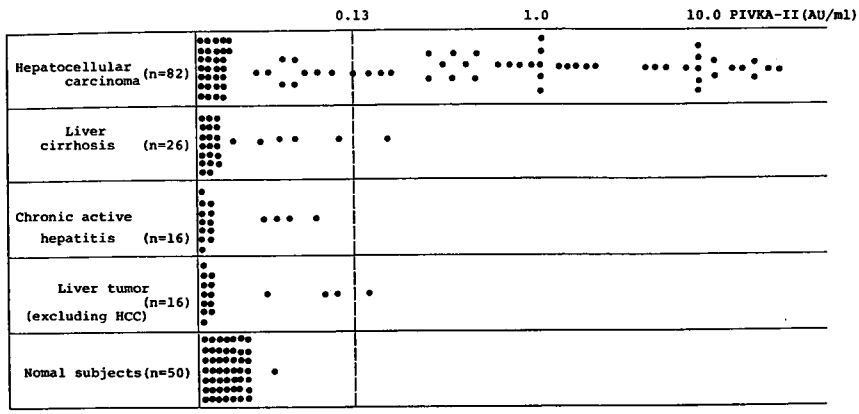

Fig. 1 Concentrations of PIVKA-II in the plasma of hepatocellular carcinomas, other liver diseases and normal controls

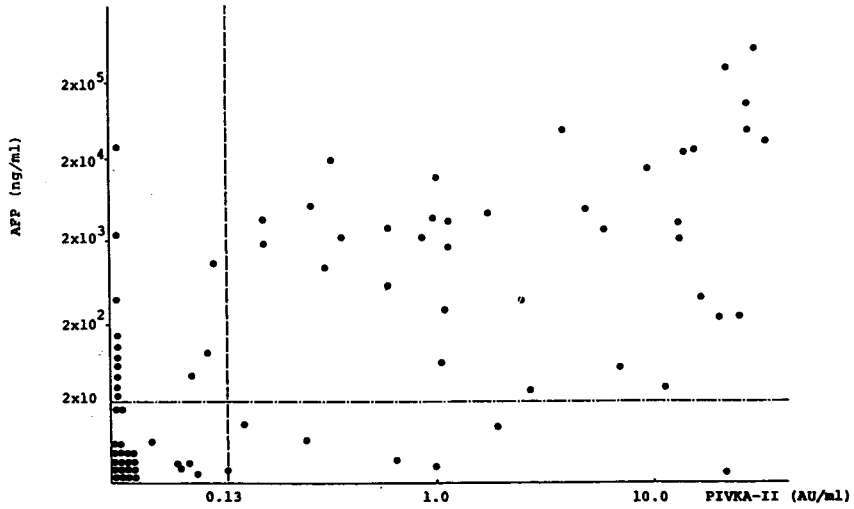

Fig. 2 Concentrations of PIVKA-II and AFP in patients with hepatocellular carcinoma

Changes of PIVKA-II in the plasma before and after therapy

The levels of PIVKA-II were measured in 29 patients at before and after anti-cancer therapy. PIVKA-II were positive in 15 cases at the time of prior-treatments. Seven of these cases were subjected to surgical resection of HCC, and 8 cases received Lp-TAE. PIVKA-II levels fell rapidly to normal range within 4 weeks in all of the patients that were treated with surgical resection. All of them showed no clinical signs of recurrence after operation. PIVKA-II levels decreased in 5 patients treated by Lp-TAE in whom a decrease of tumor masses was observed with ultrasonography, computed tomography and/or selective hepatic angography. In 3 patients with no clinical evidence of favorable response to Lp-TAE, PIVKA-II levels increased in parallel with the tumor growth. Therefore it is suggested that the PIVKA-II reflects the clinical efficiency of the treatment (Fig. 3).

Three representative cases of the above-description are shown in Figure 4

Case 1 was a 59-years-old male and the TNM classification was T_2, N_0 and M_0. He underwent an operation on HCC at the 6th week after Lp-TAE. Although the concentrations of PIVKA-II and AFP were very high before Lp-TAE, they decreased after Lp-TAE. At the operation, the legion of HCC was found to be completely necrotic (Fig 4A).

Case 2 was a 76-year-old female and the TNM classification was T_3, N_0, M_0. This patient underwent a surgical resection of HCC at the 4th week after Lp-TAE. The level of PIVKA-II, but not of AFP, was positive before Lp-TAE and it decreased gradually after Lp-TAE and retured to normal range after surgical

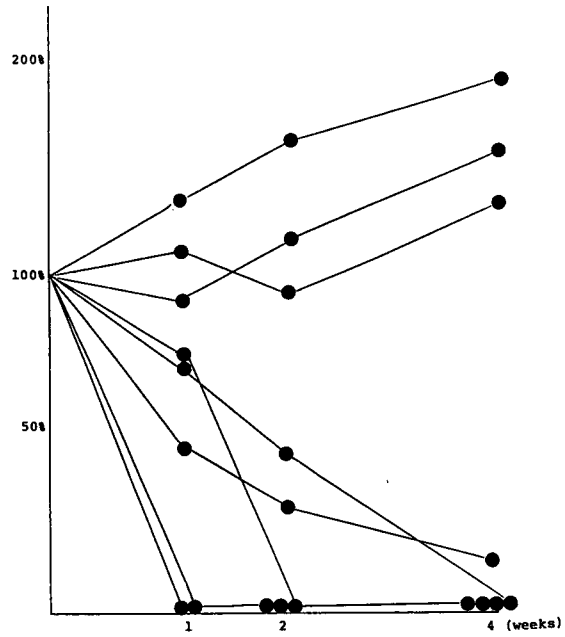

Fig. 3 Changes in PIVKA-II levels before and after Lp-TAE assuming that pretreatment value is 100%

resection. At the operation, the area of HCC was partially necrotized (Fig 4B).

Case 3 was a 72-year-old male and the TNM classification was T_4, N_0, M_0. Before the initial Lp-TAE, only the level of PIVKA-II was positive and it gradually decreased after Lp-TAE but did not show normal values. After an elapse of 2 months from the initial Lp-TAE, levels of PIVKA-II and AFP gradually increased, which appeared to reflect the growth of tumor (Fig. 4C).

DISCUSSION

HCC is one of the most prevalent malignant tumors in Japan and certain other countries and its prognosis is extremely poor. Therefore, the development of clinical diagnosis of HCC in addition to AFP have been awaited. HCC is known to produce aberrant and ectopic proteins, some of them may be useful for the diagnostics of HCC.

Recently, Liebman *et al.*(9) and Fujiyama *et al.*(6) have reported that approximately two-thirds of the patients with HCC showed abnormal levels of PIVKA-II in the plasma. In the present study, we found that PIVKA-II was positive in 52% of 82 patients with HCC. Only two patients without HCC were positive for PIVKA-II. Therefore, it is suspected that the measurement of PIVKA-II will an aid in discrimi-

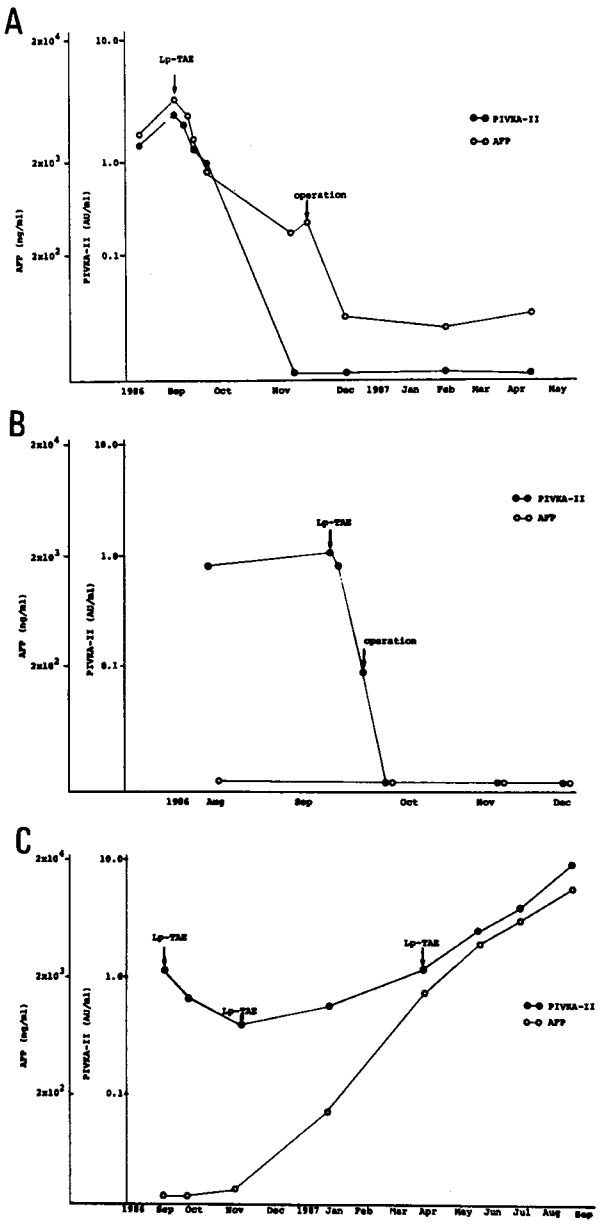

Fig. 4 A ; Serial levels of PIVKA-II and AFP in a 59-yr-old male whth hepatocellular carcinoma during treatment with Lp-TAE and operation
B ; Serial levels of PIVKA-II and AFP in a 76-yr-old female with hepatocellular carcinoma during treatment with Lp-TAE and operation
C ; Serial levels of PIVKA-II and AFP in a 72-yr-old male with hepatocellular carcinoma durdng treatments with Lp-TAE

nating HCC from other chronic liver diseases together with other liver tumors. In the presence of a steadily rising PIVKA-II level in patients with chronic liver diseases may be suspected to have HCC.

The AFP has been widely utilized as a tumor marker in HCC. Previous studies have indicated that approximately 70% of the patients with HCC show abnormal levels of AFP. However, AFP in the serum is also known to increase in the patients with various liver diseases and other malignant diseases(1, 2, 20). In this study we have shown that the values of PIVKA-II have no correlation with AFP. False-positive in PIVKA-II was only 3.6% in the patients without HCC who were not administrated with vitamin K antagonists. Therefore, the combination of these markers might be very useful for diagnosing and monitoring the patients with HCC.

On the other hand, PIVKA-II may not be useful for diagnosis of small HCC, since none of small HCCs tested showed an elevation of PIVKA-II. However, in 7 out of 9 patients who received a surgical resection, PIVKA-II increased at the time of diagnosis, suggesting that PIVKA-II may be a useful tumor marker for monitoring of HCC.

We have performed Lp-TAE in the treatment of patients who were judged to have unresectable HCC. Estimation of the efficiency of Lp-TAE was difficult from the view point of tumor size, since most of the cases treated with Lp-TAE did not show an apparent reduction of tumor size by the measurement of various image modalities within 4 weeks. PIVKA-II would be a useful tumor marker in monitoring patients with HCC, who did not show an increase in serum AFP, since PIVKA-II levels decreased rapidly to a normal range after effective treatments and increased in parallel with the tumor growth.

In conclusion, monitoring of PIVKA-II concentration in the plasma may be valuable in diagnosis and treatments of patients with HCC.

ACKNOWLEDGEMENT

This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare of Japan for the Comprehensive ten years Strategy for Cancer Control (Akira Yachi, M. D.).

The authors are indebted to Dr. H. Tsukada for his valuable discussion and reading of the manuscript.

REFERENCES

1. ABELEV, G. I., PEROVA, S. D., KHRAMKOVA, N. I., POSTNIKOVA, Z. A. and IRLIN, I. S.: Transplantation **1**, 174-180 (1963).
2. ALPERT, M. E., URIEL, J. and de NECHAUD, B.: N. Engl. J. Med. **278**, 984-986 (1968).
3. CHEN, D. S., SHEU, J. C., SUNG, J. L., LAI, M. Y., LEE, C. S., SU, C. T., TSANG, Y. M.,

HOW, S. W., WANG, T. H., YU, J. Y. YANG, T. H., WANG, G. Y. and HSU, C. Y.: Gastroenterology **83**, 1109-1119 (1982).
4. ESNOUF, M. P. and PROWSE, C. V.: Biochim. Biophys. Acta **490**, 471-476 (1977).
5. FRIEDMAN, P. A., ROSEMNBERG, R. D., HAUSHKA, P. V. and FITZ-JAMES, A.: Biochim. Biophys. Acta **494**, 271-276 (1977).
6. FUJIYAMA, S., MORISHITA, T., HASHIGUCHI, O., AKAHASHI, M., SHIRAOKU, H., SAGARA, K., SATO, T., MOTOHARA, K. and MATSUDA, I.: Igaku no Ayumi **134**, 1107-1109 (1985).
7. JOHNSON, P. J. and WILLIAMS, R.: JNCI **64**, 1329-1332 (1980).
8. KANEMATSU, T., INOKUCHI, K., FURUTA, T., TAKENAKA, K., SONODA, T., EZAKI, T., YANAGA, K., SUGIMACHI, K., TAMURA, S. and MATSUURA, K.: Kanzo. **26**, 472-478 (1985).
9. LIEBRAN, H. A., FURIE, B. C., TONG, M. J., BLANCHARD, R. A., LO, K., LEE, S., COLEMAN, M. S. and FURIE, B.: N. Engl. J. Med. **310**, 1427-1431 (1984).
10. Liver Cancer Study Group of Japan, The general rules for the clinical and pathological study of primary liver cancer. 2nd ed. Kanahara, Tokyo (1987).
11. MAZIMA, Y., SAKAMI, T., SAKAI, T., SANO, Y., KUBO, Y. and TANIGAWA, I.: JSUM proceeding 39-40 (1983).
12. MOTOHARA, K., ENDO, F. and MATSUDA, I.: Lancet **2**, 242-244 (1985).
13. MOTOHARA, K., KUROKI, Y., KAN, H., ENDO, F. and MATSUDA, I.: Pediatr. Res. **19**, 354-357 (1985).
14. NAKATSUKA, H., KAMINOU, T., TAKEMOTO, K., TAKASHIMA, S., KOBAYASHI, N., NAKAMURA, K., ONOYAMA, Y. and KURIOKA, N.: Kanzo **26**, 1217-1223 (1985).
15. NARAKI, T., WATANABE, K., SHIMOZURU, Y., NAMBA, Y., MORIMOTO, T., MOTOHARA, K. and MATSUDA, I.: Clin. Immunol. **18**, 479-492 (1986).
16. NELSESTUEN, G., ZYKOVICZ, T. and HORWARD, J.: J. Biol. Chem. **249**, 6347-6350 (1974).
17. OKUDA, H., NAKANISHI, T., FURUKAWA, R., HASHIMOTO, E. and OBATA, H.: Kanzo **27**, 1697-1702 (1986).
18. SOULIER, J. P., DANIELLE, G. and LEFRERE, J. J.: Gastroenterology **9**, 1258-1262 (1986).
19. STENFLO, J., FEMLUND, P., ESAN, W., and ROEPSTORRF, P.: Proc. Natl. Acad. Sci. USA **71**, 2730-2733 (1972).
20. YACHI, A., KODAMA, T. and SUGA, M.: Shokaki Geka **8**, 1867-1872 (1985).
21. YACHI, A., IMAI, K., and KODAMA, T.: Soshiran (in press) (1988).
22. YUMOTO, Y., JINNO, K., TOKUYAMA, K., ARAKI, Y., ISHIMITSU, T., MAEDA, H., KONNO, T., IWAMOTO, S., OHNISHI, K. and OKUDA, K.: Radiology **154**, 19-24 (1985).