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Clinical Utility of Highly Sensitive Measurement of the PIVKA–II in Anticoagulant Therapy Patients Treated with Warfarin

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Abstract： We studied the clinical utility of highly sensitive measurement of the PIVKA–II in anticoagulant therapy in patients receiving warfarin. From May 1997 until January 1999, 131 patients were examined. Group A consisted of 29 patients who were not treated with warfarin. Group B consisted of 102 patients who were given a mean warfarin dose of 2.6±1.1 mg/day. The mean PIVKA–II levels were 25.5±5.5 (mAU/ml) in group A and 17472.0±8766.7 (mAU/ml) in group B. The PIVKA–II levels in group A showed lower values than in group B and the difference was significant ($p<0.0001$). In group B, the PIVKA–II levels correlated with the prothrombin levels ($r=-0.59$, $p<0.0001$). In addition, the PT–INR levels also correlated with the PIVKA–II levels ($r=0.68$, $p<0.0001$), and the TT levels correlated with The PIVKA–II levels ($r=0.69$, $p<0.0001$). The above findings showed PIVKA–II levels to closely correlate with the prothrombin levels, PT–INR and TT. In the relatively narrow PT–INR range of 2.0 to 3.0, the PIVKA–II concentration showed levels of 18383 to 32315 (mAU/ml), while the prothrombin concentration showed levels of 55.4 to 25.3 (μg/ml) and the TT concentration showed of 20.7 to 6.7 (%). In conclusion, the measurement of high sensitivity PIVKA–II is therefore considered to accurately reflect the coagulant system. These findings suggest that the measurement of highly sensitive measurement of the PIVKA–II appears to be an effective monitoring method for patients on warfarin therapy.

Key words：Warfarin, Anticoagulant therapy, PIVKA, Prothrombin level, Carinactivase–1

Introduction

Liver microsomal vitaminK–dependent glutamyl r–carboxylase catalyzes the posttranslational conversion of glutamyl to r–carboxyglutamyl residues in intracellular precursors of a limited number of blood coagulation proteins. The reduced form of Vitamin K functions as a cofactor in this modification, which confers to vitamin K－dependent proteins the Ca^{2+} binding properties required to express their biologic activity.[1] It has been shown that 90% of the vitamin K store in the liver consists of menaquinones.[2] In anticoagulant therapy patients, descarboxylated forms of vitamin K–dependent proteins are observed in human plasma.

The plasma concentration of des–r–carboxy prothrombin is now considered to be a potential complementary diagnostic tool for anticoagulant therapy patients with warfarin. There is some evidence that this abnormal form of prothrombin found in the circulation originates from the liver in anticoagulant therapy patients with warfarin.

Warfarin, an oral anticoagulant drug, is thus considered to protect prothrombin from the r–carboxylase in the region. In addition, this decarboxy prothrombin, called PIVKA–II, has no known biological activity.[3]

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Results of the highly sensitive measurement of the PIVKA-II were compared with the prothrombin levels, the prothrombin time international normalized ratio (PT-INR) and the thrombotest (TT).

Material and methods

This study was conducted from May 1997 until January 1999. One hundred and thirty-one patients were examined. Blood samples were obtained from outpatients and in-hospital patients in Fukuoka University Hospital, who were divided into two groups. Group A consisted of 29 patients who were not treated with warfarin. They comprised 21 males and 8 females with a mean age of 66.4±10.6 years (Table. 1). Group B was consisted with 102 patients who were given a mean warfarin dose of 2.6±1.1 mg/day, and consisted of 47 males and 55 females with a mean age of 60.3±10.5 years (Table. 2).

Blood sample

Three ml of blood were drawn in a vacuum tube with citric acid. The blood was centrifuged for 10 minutes at 3,000 revolutions per minute, and blood the plasma was thus collected.

Table 1. Clinical characteristics of the patients without warfarin (group A)

Total (cases)	29
Mean age (years)	66.4±10.6
Gender	Male ; 21 : female ; 8
CABG (cases)	29

CABG ; Coronary artery bypass grafting

Table 2. Clinical characteristics of the patients with warfarin (group B)

Total (cases)	102
Mean age (years)	60.3±10.6
Gender	Male ; 47 : female ; 55
Dose of warfarin (mg/day)	2.6±1.1
MVR (cases)	47
AVR (cases)	35
DVR (cases)	16
TVR (cases)	1
CABG (cases)	1
ASO (cases)	2

MVR ; mitral valve replacement, AVR ; aortic valve replacement, DVR ; double (mitral and aortic) valves replacement, CABG ; coronary artery bypass grafting, ASO ; arteriosclerosis obliterans

Quantification of PIVKA-II

The PIVKA-II levels were all measured using an electrochemiluminescence immunoassay (ECLIA) (PICOLUMI8220 ; Eisai, Tokyo, Japan). The PIVKA-II reagent used in this study was MU-3 (Eisai, Tokyo, Japan).

Quantification of the prothrombin level

In 1996, Yamada et al.[4] reported carinactivase-1 (CA-1), Ca^{2+}-dependent prothrombin activator, which allows us to determines the prothrombin levels. This activator was isolated from the venom of *Echis carinatus leucogaster* and activates the Gla-domain form of prothrombin.

Ten-µl aliquots of blood plasma (tenfold-diluted with 20 mM Tris-HCl, 140 mM NaCl, pH7.5 TBS containing 1 mg/ml bovine serum albumin (TBS /BSA)) were mixed with 80 µl of containing 3 mM $CaCl_2$, and 0.31 mM Boc-Val-Pro-Arg-pNA and incubated at 37℃ for an appropriate length of time (usually 5 minutes for routine assays). Next, 10 µl of 2.5 nM CA-1 were added. The amount of thrombin generated was quantified by measuring the initial velocity of p-nitroaniline liberation at 405 nm with a kinetic plate reader (MICROPLATE READER, BIO-RAD, Tokyo, Japan), with pure human prothrombin as the standard.

Quantification of PT-INR and TT

The PT and TT were all measured using the CA-5000 (Sysmex, Tokyo, Japan). The PT reagent used in this study was Thromborel-S (ISI : 1.08) (BEHRING). In addition, the TT reagent was Trombotest owren (NYCOMED).

Statistical analysis

A statistical analysis was performed using Mann-Whitney's U-test. Statistically significant differences were assumed at $P<0.05$. The mean value was taken as the mean±standard deviation.

Results

The mean of PIVKA-II levels were 25.5±5.5 (mAU/ml) in group A and 17472.0±8766.7 (mAU/ml) in group B. The PIVKA-II levels in

group A showed a lower value than in group B and the difference was significant ($p<0.0001$). The mean prothrombin levels were 113.5±19.3 (μg/ml) in group A and 57.4±20.9 (μg/ml) in group B. The prothrombin levels in group A were significantly higher than in group B ($p<0.0001$). The PT values were significantly lower in group A than in group B, the TT values were significantly higher in group A than in group B (Table. 3).

We next examined that relationship between the PIVKA-II levels and another coagulant test (prothrombin levels, PT-INR and TT) in Group B. The PIVKA-II levels correlated with the prothrombin levels ($y=-245.9x+31581.5$, $r=-0.59$, $p<0.0001$) (Fig. 1). In addition, the PT-INR correlated with the PIVKA-II levels ($y=13931.9x+9480.6$, $r=0.68$, $p<0.0001$) (Fig. 2), the TT correlated with the PIVKA-II levels ($y=-507.4x+28896.4$, $r=0.69$, $p<0.0001$) (Fig. 3). Regarding the PIVKA-II levels, the above findings showed that the PIVKA-II levels correlated closely with the prothrombin levels, PT-INR and TT.

In this study, we prepared a chart with the PT-INR equivalents to the PIVKA-II levels, the prothrombin levels and TT. An example of this chart is shown in Table 4.

It is said that the therapeutic range for PT-INR range from 2.0-3.0 in anticoagulant therapy with warfarin. In this study, in the relatively narrow range of PT-INR of 2.0 to 3.0, the PIVKA-II showed of 18383 to 32315(mAU/ml), the prothrombin levels showed of 55.4 to 25.3 (μg/ml) and TT showed of 20.7 to 6.7 (%).

Table 3. Comparison of the results of the two groups

	Group A (n=29)	Group B (n=102)	P-Value
PIVKA-II (mAU/ml)	25.5±5.5	17472.0±8766.7	<0.0001
Prothrombin level (μg/ml)	113.5±19.3	57.4±20.9	<0.0001
PT-INR	1.0±0.1	1.9±0.4	<0.0001
TT (%)	123.6±21.8	22.5±12.0	<0.0001

PIVKA-II ; protein induced vitamin-K absence-Ⅱ, PT-INR ; prothrombin time international normalized ratio, TT ; Thrombotest

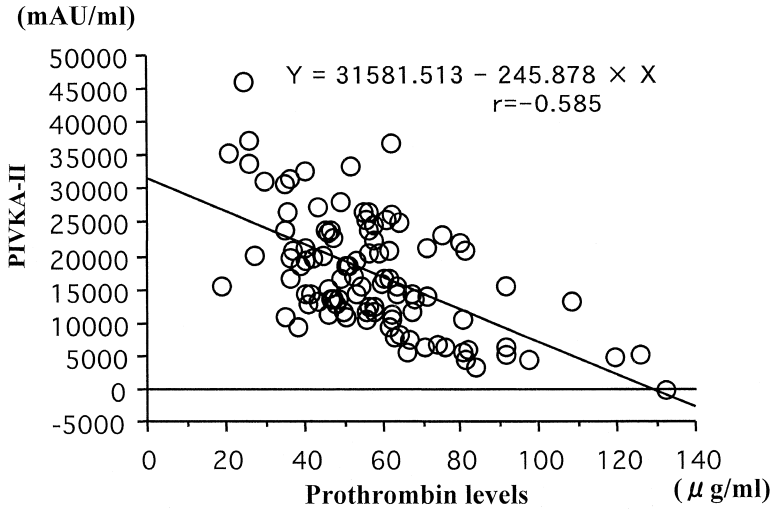

Fig. 1. The relationship between the PIVKA-II levels and the prothrombin levels in group B. The PIVKA-II levels correlated with the prothrombin levels ($y=-245.9x+31581.5$, $r=-0.59$, $p<0.0001$)

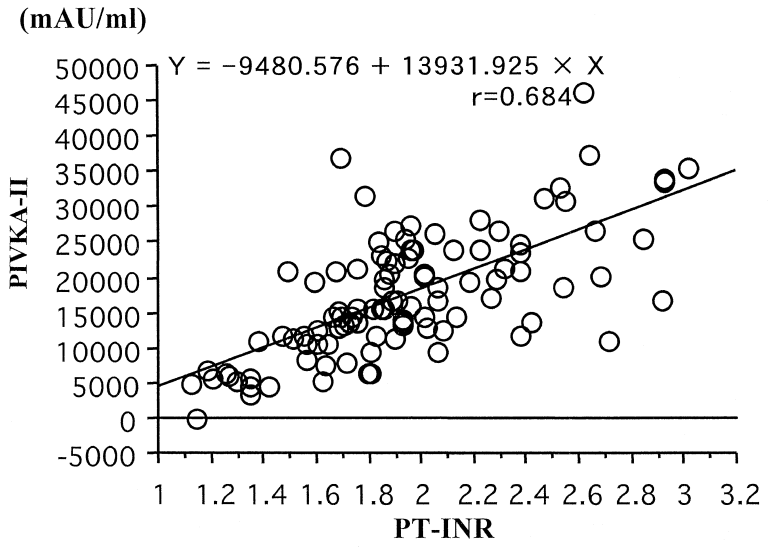

Fig. 2. The relationship between the PIVKA-II levels and PT-INR in group B. The PT-INR correlated with the PIVKA-II levels (y= 13931.9x+9480.6, r=0.68, p<0.0001)

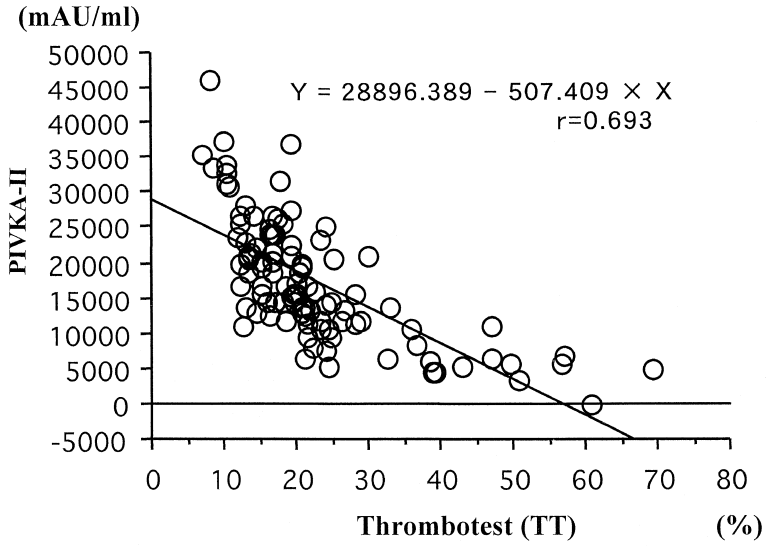

Fig. 3. The relationship between the PIVKA-II levels and TT in group B. The TT correlated with the PIVKA-II levels (y=−507.4x+ 28896.4, r=0.69, p<0.0001)

Table 4. Control area for anticoagulant therapy with warfarin

PT-INR	2.0-3.0
TT (%)	20.3-6.7
Prothrombin level (μm/ml)	55.4-25.3
PIVKA-II (mAU/ml)	18383-32315

PT-INR ; prothrombin time international normalized ratio, TT ; Thrombotest, PIVKA-II ; protein induced vitamin-K absence-II

Discussion

Warfarin is widely used for anticoagulant therapy patients. Warfarin blocks the carboxylation system, thus preventing the formation of r-carboxyglutamic acid residues. For coagulant factor II, VII, IX and X, this decreases their capacity to bind with phospholipids for activation.[5] Both the prothrombin time (PT) and TT have so far been used to monitor the therapeutic level of war-

farin. However, both hemorrhaging and thrombosis still often occur despite such careful monitoring.[6)7)] The PT examines coagulant factors II, V, VII, X and fibrinogen. In addition, the TT and the Hepaplastin test (HPT) examine vitamin K-dependent coagulant factors II, VII and X. The activity of coagulant factor IX can not be determined by these two measurements. Therefore, Xi reported that coagulation factors VII, IX and X may have an effect on the prothrombinase activity when these concentrations are lower than 5%, 20% and 30%, respectively.[8)] Kornberg reported that the prothrombin concentrations more accurately reflect the antithrombotic effect of warfarin than the PT.[9)] Furie et al. has recently developed a new assay method to determine the prothrombin levels using a radioimmunoassay.[10)11)] However, this method is very time consuming and thus is not yet pratical for clinical use. In 1996, Yamada et al.[4)] reported the use of carinactivase-1 (CA-1), a Ca^{2+}- dependent prothrombin activator, to determine the prothrombin levels. This activator was isolated from the venom of *Echis carinatus leucogaster* and activates a Gla-domain form of prothrombin. However, this method is also not pratical for clinical use. PIVKA-II is an abnormal prothrombin. After starting anticoagulant therapy with warfarin, the prothrombin levelis considered to decrease while the PIVKA-II levels are thought to increase. In this study, the PIVKA-II correlated with prothrombin levels, as well as the PT-INR and TT. The PT-INR ranged from 2.0-3.0 and these levels are considered to correlate with the ranges of 18383-32315 (mAU/ml) for the PIVKA-II levels and 55.4-25.3 (μg/ml) for the prothrombin levels and 20.7-6.7 (%) for the TT. The measurement of high sensitivity PIVKA-II is therefore considered to accurately reflect the state of the human coagulant system. This method is also easy to perform and thus is considered to be pratical for clinical use. Basis on these preliminary data, we consider the measurement of high sensitivity PIVKA-II levels to be an effective monitoring method for patients on warfarin therapy.

Conclusions

We studied the clinical utility of measuring the high sensitivity PIVKA-II levels in anticoagulant therapy patients receiving warfarin. The PIVKA-II levels closely correlated with the prothrombin levels, the PT-INR and TT. The measurement of high sensitivity PIVKA-II is therefore considered to accurately examine the human coagulant system. We therefore consider the highly sensitive measurement of the PIVKA-II levels to be an effective monitoring method for patients receiving warfarin therapy.

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References

1) Huisse MG, Leclercq M, Belghiti F, Huisse MG, Leclercq M, Belghiti J, Flejou JF, Suttie JW, Bezeaud A, Stafford DW, Guillin MC: Mechanism of the abnormal vitamin K-dependent r-carboxylation process In human hepatocellular carcinomas. Cancer 74: 1533-1541, 1994.
2) Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, Coleman MS, Furie B: Des-r-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinomas. N Engl Med 310: 1427-1431, 1984.
3) Yamada D, Morita T: CA-1 methods, a novel assay for quantification of normal prothrombin using a Ca^{2+}-dependent prothrombin activator, carinactivase-1. Thrombosis Research 94: 221-226, 1999.
4) Yamada D, Sekiya F, Morita T: Isolation and characterization of carinactivase, a novel prothrombin activator in *Echis carinatus venom* with a unique catalytic mechanism. J. Biochem 271: 5200-5207, 1996.
5) Wessler S, Gitel SN: Warfarin. N Engl Med 311: 645-652, 1984.
6) Robert AO, Paul MA, Lois SL: Studies on the Coumarin anticoagulant drug: A comparison of the pharmacodynamics of dicumarol and warfarin in man. Tromb Diathsis Haemorr. 1: 1-22, 1964.
7) Shetty HGM, Fennerty AG, Routledge PA: Clinical pharmacokinetic consideration in the control of oral anticoagulant therapy. Clin. Pharmacokinetics 16: 238-253, 1989.
8) Xi M, Beguin S, Hemker HC: The relative importance of the factor II, VII, IX and X for the prothrombinase activity in plasma of orally anticoagulated patients.

Thromb Haemostasis. 62：788–791, 1989.
9）Kornberg A, Francis CW, Pellegrini VD Jr, Gabriel KR, Marder VJ.：Comparison of native prothrombin antigen the prothrombin time for monotoring oral anticoagulant prophylaxis. Circulation 88：454–460, 1993.
10）Furie B, Liebman HA, Blanchard RA, Coleman MS, Kruger SF, Furie BC.：Comparison of the native prothrombin antigen and prothrombin time for monitoring oral anticoagulant therapy. Blood. 64：445–451, 1984.
11）Furie B, Diuguid CF, Jacobs M, Diuguid DL, Furie BC.：Randomized prospective trial comparing the native prothrombin antigen with the prothrombin time for monitoring oral anticoagulant therapy. Blood. 75：344–349, 1990.

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