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# The CA–1 Test as a New Method for Monitoring Liver Dysfunction

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**Abstract：** Recent studies have focused on the fact that liver dysfunction is associated with the prothrombin time (PT) and hepaplastintest (HPT). We developed a new test to determine the prothrombin levels using the Carinactivase–1 (CA–1) test for liver dysfunction. Total plasma samples were assayed for the CA–1 test, PT and HPT. This prospective randomized study was carried out in 47 samples. The samples were divided into 2 groups. Group 1 included 20 samples and group 2 included 27 samples. Group 1 consisted of samples from individuals with no liver dysfunction, while Group 2 comprised samples form patients with liver dysfunction. The mean prothrombin levels(CA–1 score)were measured using the CA–1 test in groups 1 and 2. The mean value was 119.4 μg/ml in group 1 and 95.8 μg/ml in group 2. The CA–1 score of group 2 decreased more significantly than in group 1 ($p<0.05$). Even the prothrombin time international normalized ratio (PT–INR) decreased more significantly in group 2 than in group 1. Therefore, the HPT was not significantly different between groups 1 and 2. Consequently, the CA–1 test is a quantitative analysis. In contrast, the PT and HPT are qualitative analyses. Therefore, the CA–1 test is considered to be superior to the PT and HPT. The CA–1 test is therefore considered to be more useful for monitoring liver dysfunction than HPT.

**Key words：Liver dysfunction, Prothrombin time, Carinactivase–1, Hepaplastin test**

## Introduction

The prothrombin time international normalized ratio (PT–INR) and Hepaplastin test (HPT) are major methods for monitoring liver dysfunction. However, as the titer of reagents used to measure the PT–INR and the HPT differs from manufacturer to manufacturer, and thus the determined values based on such measurements are not absolute. Therefore, the establishment of new and more accurate monitoring methods is required. With this purpose in mind, we tried to establish a new test to determine the prothrombin levels using a $Ca^{2+}$–dependent prothrombin activator, which was designated as the Carinactivase–1(CA–1) test.

## Patients and methods

### Patients

This prospective randomized study was carried out using 47 samples obtained from the Fukuoka University Hospital Department of Cardiovascular Surgery between May 1997 and December 1998. The samples were prospectively randomized to receive either a non–liver dysfunction or a liver dysfunction group.

Group 1 included 20 samples while group 2 included 27 samples. Group 1 had no samples from individuals with liver dysfunction. (mean age 63.2 years, gender 12 males and 8 females). Group 2 had samples from patients with liver dysfunction. (mean age 65.9 years, gender 15 males and 12 fe-

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males) (Table 1)

**Methods**

All samples were measured using the CA-1 test, PT-INR and HPT. For comparisons of the two-groups, we performed statistical analysis.

**Liver dysfunction**

In this study, the liver dysfunction definition is the aspartate aminotransferase (AST)≧35 IU/L and/or alanine aminotransferase (ALT)≧35 IU/L and/or γ-glutamyl transpeptidase (γ-GTP)≧49 IU/L. Neither abdominal echography nor the CT scanning data were considered in this study.

**CA-1 test**

Carinactivase-1 (CA-1), $Ca^{2+}$-dependent prothrombin activator which was isolated from the venom of *Echis carinatus leucogaster*, was used in this study. The CA-1 test is performed as follows: Blood samples (3 ml) were withdrawn from all patients and then were kept in a vacuum tube containing citric acid as anticoagulant. The blood was centrifuged for 10 min at 3000 r.p.m., and the plasma separated. Aliquots of plasma (10 μl) were diluted 10-fold with 20 mM Tris-HCl 140 mM NaCl pH 7.5 (Tris-buffered saline; TBS) containing 1 mg/ml bovine serum albumin (TBS/BSA), and then were mixed with 80 μl of 3 mM $CaCl_2$ and 0.31 mM t-butoxycarbonyl (Boc)-Val-Pro-Arg-p-nitroanilide (pNA) (Seikagaku Corporation, Tokyo, Japan) and incubated at 37℃ for an appropriate time (5 min). 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 at 405 nm, using kinetic plate reader, with pure human prothrombin as a standard. This method was reported by Yamada *et.al*. for the first time in 1996.[1)]

**PT and HPT**

The PT and HPT were all measured using the CA-5000 (Sysmex, Kobe, Japan). The PT-INR reagent used in this study was Thromborel-S (ISI: 1.08) (Sysmex, Kobe, Japan). The HPT reagent was determined using the Hepaplastin test (Eisai, Tokyo, Japan).

**Statistical analysis**

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

**Table 1**

| | Group 1 | Group 2 |
|---|---|---|
| Cases | 20 | 27 |
| Male/female | 12/8 | 15/12 |
| Mean age (years) | 63.2±13.0 | 65.9±14.1 |

Group 1 had no samples from individuals with liver dysfunction.
Group 2 had samples from patients with liver dysfunction.

## Results

After comparing the demographics of the samples in group 1 and group 2, no significant difference was observed in the two populations (Table 1).

Table 2 shows the pre-measure CA-1 test variables. Even the AST, ALT and γ-GTP decreased more significantly in group 2 than in group 1.

Table 3 shows the CA-1 test variables. The mean prothrombin levels (CA-1 score) of group 1 was 119.4±44.3 μg/ml. In contrast, the mean CA-

**Table 2**

| | Group 1 | Group 2 | p-value |
|---|---|---|---|
| AST (IU/L) | 19.7±5.3 | 83.1±115.6 | <0.0001 |
| ALT (IU/L) | 13.6±6.3 | 33.0±29.6 | 0.0002 |
| r-GTP (IU/L) | 23.2±9.9 | 82.5±18.4 | 0.0002 |

Group 1 had no samples from individuals with liver dysfunction.
Group 2 had samples from patients with liver dysfunction.
AST: Aspartate aminotransferase, ALT: alanine aminotransferase, γ-GTP: γ-glutamyl transpeptidase.

**Table 3**

| | Group 1 | Group 2 | p-value |
|---|---|---|---|
| CA-1 score (μg/ml) | 119.4±44.3 | 95.8±29.4 | 0.0402 |
| PT-INR | 1.0±0.1 | 1.1±0.1 | 0.0486 |
| HPT (%) | 101.8±25.4 | 94.4±32.9 | NS |

Group 1 had no samples from individuals with liver dysfunction.
Group 2 had samples from patients with liver dysfunction.
CA-1 score : Normal prothrombin levels, PT-INR : Prothrombin time-international normalized ratio, HPT : Hepaplastin test.

1 score of group 2 was 95.8±29.4 μg/ml. The CA-1 score of group 2 decreased more significantly than in group 1 based on the above findings.

Table 3 shows the PT-INR variables. The mean PT-INR of group 1 was 1.0±0.1. In contrast, the mean PT-INR of group 2 was 1.1±0.1. The PT-INR of group 2 decreased more significantly than in group 1 based on the above findings.

Table 3 showed the HPT variable. The mean HPT of group 1 was 101.8±25.4%. In contrast, the mean HPT of group 2 was 94.4±32.9%. No significant differences in the HPT variables were observed between the 2 groups.

## Discussion

This prospective randomized study was carried out in 47 samples. The mean prothrombin levels (CA-1 score) were measured using the CA-1 test. The mean value of CA-1 score was 119.4 μg/ml in the control group and 95.8 μg/ml in the liver dysfunction group. The prothrombin levels of the liver dysfunction group decreased more significantly than in the control group.

Therefore, no significant difference in the HPT level was observed between the 2 groups.

Prothrombin is coagulation factors that is made in liver. Prothrombin has 72kDa polypeptide and single chain.[2] The volume of prothrombin from the liver demonstrates a higher quantity than another vitamin-K dependent coagulation factors.[2] Measuring the prothrombin level is therefore considered to be a good measurement of the liver function. The CA-1 test activates only the prothrombin levels[1]. This test is not only a qualitative analysis but also a quantitative analysis. The CA-1 test requires only 10 μl of diluted blood plasma.[3] Each examination took only about 30 minutes, and neither EDTA nor heparin affected the normal prothrombin level obtained by the CA-1 test. Therefore, the CA-1 test is thus considered to be an accurate monitoring system.[2]

In the present study, the PT and HPT were measured to demonstrate their levels in the liver.

PT examines coagulant factors Ⅱ, Ⅴ, Ⅶ, Ⅹ and fibrinogen. In addition, the HPT examines the Vitamin K-dependent coagulant factors Ⅱ, Ⅶ, and Ⅹ. It is said that the prothrombin induced by vitamin K absence (PIVKA) does not affect HPT, and it is also considered to be superior to PT for monitoring liver dysfunction.[4][5] However, as the titer of reagents used to measure the PT-INR and the HPT differ from manufacturer to manufacturer. The determined values based on such measurements are not absolute. Therefore, both the PT and HPT are not accurate monitoring systems. In addition the PT and HPT can not measure the coagulation activity of plasma sample anticoagulated by heparin and EDTA.[5] Therefore, the HPT can not monitor anticoagulant therapy patients treated by heparin. In contrast, the CA-1 test can measure such patients. In this study, the CA-1 score of the liver dysfunction group decreased more significantly than in the control group. In contrast, no significant differences in the HPT variables were observed between the 2 groups. Consequently, the CA-1 test is therefore considered to be superior to the PT and HPT.

Camacho-Lobato L et al[6] reported that they observed a prolonged prothrombin fragment 1+2 in early liver dysfunction in schistosomiasis as the albumin levels tended to be normal. However, the prothrombin fragment 1+2 needs a long measurement time, while, in addition, this method is also difficult to perform and expensive.[7] In addition, the thrombin-antithrombin Ⅲ complex (TAT) is

used to analyze for liver dysfunction.

However, the TAT takes three hours to perform and it is also expensive.[8)]

Singer AM[9)] reported about“ increased transaminase levels in patients with acetoaminophen–induced liver dysfunction ”. In such cases, the CA–1 test is therefore considered to be an appropriate analytical test.

Furie et al.[10)] developed a new radioimmunoassay to analyze normal prothrombin levels.

Kornberg et al[11)] developed an assay for the prothrombin levels by using ELISA. These studies clearly indicated that the prothrombin levels reflected the clotting activities of plasma samples. However, an assay to determine the prothrombin levels is difficult because it requires a $Ca^{2+}$–dependent anti–human prothrombin antibody which recognizes the $Ca^{2+}$–bound conformation of the Gla–domain. In contrast, the CA–1 test is a highly sensitive chromogenic microplate assay that easily quantifies normal prothrombin.[1)]

## Conclusions

This study shows that the CA–1 test can be used to easily and rapidly determine the prothrombin levels in plasma specimens from liver dysfunction patients. The sensitivity of the CA–1 test is higher than the HPT. Neither EDTA nor heparin affected the normal prothrombin levels obtained by the CA–1 test. In addition, the CA–1 test is also a more accurate monitoring system than PT and HPT. Based on these preliminary data, we therefore recommend the use of the CA–1 test to measure the prothrombin levels as an effective new method for monitoring liver dysfunction.

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