

Determination of Serum Carbohydrate-Deficient Transferrin (CDT) by the Nephelometric N Latex CDT Assay in Japanese Habitual Drinkers and Patients with Non-Alcoholic Liver Diseases

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Abstract

Background: Excessive alcohol consumption is a health risk that can lead to a variety of medical and social problems. Carbohydrate Deficient Transferrin (CDT) is now widely used for detection of chronic alcohol abuse and monitoring sobriety in alcoholics. We assessed the diagnostic performance of the direct immunoassay for %CDT (N Latex CDT) in Japanese habitual drinkers at their annual medical check-up. We also tested whether this direct assay is influenced by the presence of non-alcoholic liver disease including non-alcoholic steatohepatitis and HCV-related hepatocellular carcinoma.

Methods: We performed the N Latex CDT assay using a Siemens BN-II nephelometric analyzer to measure CDT concentration. The reference intervals of %CDT and gamma glutamyl transferase (GGT) activities were obtained based on their measurements in 160 apparently healthy nondrinkers. Habitual drinkers were divided into light drinkers (less than 40g ethanol/day) and heavy drinkers (more than 60g ethanol/day). Furthermore, %CDT levels in a total of 184 patients with non-alcoholic chronic liver diseases including Hepatitis C Virus (HCV) related liver cirrhosis, Non-Alcoholic Steatohepatitis (NASH) were determined.

Results: In the heavy drinkers, the positive rates of %CDT and GGT were 58.3% and 41.7%, respectively. The combination assay of %CDT and GGT resulted in 75% sensitivity and 95% specificity. It was notable that 57% of GGT non-responders were detected by CDT measurements. The mean serum level of %CDT was not influenced by the presence of NASH, but was increased in patients with moderate-severe HCV-related liver cirrhosis. The %CDT levels were also high in patients with hepatocellular carcinoma with liver cirrhosis, but were not related to their tumor stages.

Conclusions: The %CDT determined by automated N Latex CDT may be useful biomarker complementary to GGT to detect excessive habitual drinkers in Japan. However, caution should be taken to interpret the results in advanced non-alcoholic liver cirrhosis.

Keywords: Hepatitis C virus; Carbohydrate deficient transferrin; N Latex CDT; Isoelectric focusing; High-performance liquid chromatography

Introduction

Excessive consumption of alcohol is a health risk that can lead to a variety of medical and social problems [1,2]. Heavy drinking not only leads to alcoholism and alcoholic liver disease, but also aggravates many common medical disorders including hypertension, stroke, diabetes mellitus and gout. Although the primary strategy for detecting heavy drinking relies on self-reporting, heavy drinkers tend to underestimate their alcohol consumption. Therefore, objective markers for alcohol consumption are clearly needed.

Stibler and Kjellin [3] first reported that chronic heavy drinking can cause a decrease in transferrin (Tf) sialylation. Transferrin is the most important iron transport protein, synthesized primarily in hepatocytes. Asialo-, monosialo- and disialotransferrin isoforms are collectively known as Carbohydrate-Deficient Transferrin (CDT) [4]. Among various biomarkers of chronic alcohol abuse, CDT is now widely used for early detection of chronic alcohol abuse and monitoring sobriety in alcoholics undergoing treatment [5-8].

Several methods for the measurement of CDT have been developed, including lectin affinity chromatography, gel Isoelectric Focusing

(IEF), anion-exchange chromatography followed by immunochemical determinations, anion-exchange High-Performance Liquid Chromatography (HPLC) (or fast protein liquid chromatography, FPLC), capillary zone electrophoresis, and mass spectrometry, as reviewed elsewhere [9].

More recently, the first direct immunoassay for CDT (N Latex CDT, Siemens Healthcare Diagnostics, Marburg, Germany), based on a specific antibody for immunological epitopes of CDT, has been

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reported [10]. This method allows an automatic calculation of the amount of CDT as a percentage of total transferrin (%CDT).

Although clinical values of this direct immunoassay have been well documented in Caucasian subjects, utility of this new immunoassay in Japanese drinkers has not been tested. Indeed, it is possible that there are differences in basal values of CDT and/or responses to excessive drinking in relation to ethnicity as suggested [11]. Therefore, the first aim of this study was to assess the diagnostic performance of this test in Japanese habitual drinkers as compared with that of Gamma Glutamyltransferase (GGT).

There are reports indicating that CDT measurement is influenced by the presence of liver disease. Murawaki et al. reported that %CDT values were related to the severity of liver cirrhosis rated by the Child's grades and also by the size of liver tumor and their grade of histological differentiation [12]. Also, Perret et al. reported that an increased CDT level may occur in patients with chronic viral hepatitis in the absence of chronic alcohol abuse [13].

On the other hand, Viitala reported that CDT levels in non-alcoholic liver diseases determined by two different methods are not different from those in healthy non-drinking controls [14]. Therefore, the second aim of this study was to test the effects of a variety of non-alcoholic liver diseases including liver cancer on serum CDT levels.

Materials and Methods

Determination of CDT levels

N Latex CDT assay: N Latex CDT (Siemens Healthcare Diagnostics, Marburg, Germany) is a monoclonal antibody-based direct immunoassay of transferrin glycoforms lacking one or two complete N-glycans [i.e., disialo-, monosialo-, and asialotransferrins (CDT glycoforms)]. This immunoassay can quantify %CDT in serum without any preanalytical sample pretreatment [10].

We used the nephelometric system BN II™ (Siemens Healthcare Diagnostics, USA) for N Latex CDT. The simultaneous measurement of total Tf with a polyclonal antibody-based assay (N Antiserum to Human Transferrin; Siemens Healthcare Diagnostics) performed on the same instrument allowed an automatic calculation of the CDT value as a percentage of total transferrin (%CDT) [10].

Serum samples were separated by centrifugation at 1,500×g for 10 min at 4°C and were stored in aliquots at -80°C until analysis.

GGT measurements

Serum gamma glutamyltransferase (GGT) activities were measured using established procedures on a BioMajesty JCA-BM2250 auto analyzer (JEOL Ltd, Tokyo, Japan).

Subjects studied

All of the subjects provided written informed consent, and the Ethics Committee of Chiba University School of Medicine approved this study.

Habitual drinkers

Blood samples were obtained from 290 apparently healthy subjects (207 men and 83 women) with various drinking habits who visited the Kashiwado Clinic in Port-Square of Kashiwado Memorial Foundation (Chiba, Japan) for their annual medical check-up. Their mean age was 46.4 ± 9.5 years (range, 23-73 years). All subjects were interviewed by experts to obtain their drinking histories concerning the amount of

alcoholic beverages consumed (calculated as grams of pure ethanol per day), the duration of drinking, and the frequency of drinking. For the comparative studies, we chose 70 moderate drinkers (less than 40 g/day, 59 men and 11 women), and 60 heavy drinkers (more than 60 g/day for more than 5 years, 48 men and 12 women). As a reference population, 160 nondrinkers (100 men and 60 women) were included. They had all been screened for Hepatitis B Virus (HBV) and hepatitis C virus (HCV) and were included in this study after they had been confirmed to be negative for both.

Non-alcoholic liver diseases

A total of 174 patients with non-alcoholic chronic liver diseases (95 men and 79 women) who visited Chiba University Hospital were included in the study: 20 patients with biopsy-proven Chronic Hepatitis C (CH), 84 patients with hepatitis C cirrhosis (LC) due to HCV infection, and 70 patients with HCV-related Hepatocellular Carcinoma (HCC). Most HCC patients had liver cirrhosis as an underlying lesion. Diagnosis of liver cirrhosis was made based on either by biopsy findings or typical radiological findings. Severity of cirrhosis was assessed by the Child-Pugh classification [15]. Daily alcohol intake was none or less than 20 g in all of these subjects. Staging of the liver tumors was based on the International Union against Cancer (UICC) TNM classification.

Statistical analysis

Statistical significance was evaluated by the Mann-Whitney test. P values < 0.05 were considered significant. The numerical data are presented as the mean ± Standard Deviation (SD). Reference intervals were calculated by Stat-Flex version 5.0 (Artech Co., Ltd., Osaka, Japan).

Results

%CDT in habitual drinkers visited for medical check-up

Reference intervals for %CDT and GGT were obtained by the non-parametric method based on data obtained from 160 nondrinkers. The upper limits of the 95% confidence interval for %CDT and GGT were 1.74 and 61 U/l, respectively; these values were considered as cut-off levels in the present study.

The mean %CDT was 1.35 ± 0.19 (range, 0.83-2.26) in the nondrinkers, 1.55 ± 0.23 (range, 1.14-2.21) in the moderate drinkers and 2.04 ± 0.72 (range, 1.04-4.73) in the heavy drinkers. The mean serum GGT was 25.6 ± 14.2 U/l (range, 8-66) in the nondrinkers, 57.6 ± 51.0 (range, 10-303) in the moderate drinkers and 65.9 ± 46.9 U/l (range, 17-215) in the heavy drinkers (Figure 1). The positive rates of %CDT and GGT in moderate drinkers were 21.4% and 28.6% respectively and were 58.3% and 41.7%, respectively in heavy drinkers. The combination assay of %CDT and GGT (namely either %CDT or GGT positive) resulted in 75% sensitivity and 95% specificity in heavy drinkers. Thus, 57% of GGT non-responders among the heavy drinkers could be detected by %CDT measurement (Figure 2).

% CDT in patients with non-alcoholic liver diseases

%CDT values in non-alcoholic liver diseases are presented in Figure 3. They were 1.54 ± 0.12 (range, 1.32-1.79; p < 0.0001) in chronic viral hepatitis, 1.54 ± 0.28 (range, 1.01-2.75; p < 0.0001) in HCV-related liver cirrhosis and 1.57 ± 0.31 (range, 0.92-2.44; p < 0.0001) in HCC. The elevation of serum %CDT exceeding the cut-off level (1.74%) was found in 20 (23%) of the 84 patients with LC and 20 (28%) of the 70 patients with HCC (Figure 3).

Serum %CDT values in patients with LC and HCC were compared according to the Child-Pugh classification. The serum %CDT values increased depending on the degree of liver cirrhosis based on the Child-Pugh classification, even in nondrinkers. The mean serum %CDT in the Child C subgroup was significantly higher than that in the Child A subgroup in LC patients ($p = 0.0009$) and also in HCC patients ($p = 0.002$) (Figure 4).

Figure 5 presents the %CDT levels in HCC patients according to tumor stage. There were no significant differences among the four groups (Figure 5).

Discussion

In the present study, we assessed the diagnostic performance of the direct immunoassay for %CDT (N Latex CDT) in Japanese habitual drinkers who presented for their annual medical check-up. We also tested whether this direct assay is influenced by the presence of non-alcoholic liver disease, including HCV-related liver cirrhosis and HCC.

We first determined %CDT values in 160 nondrinkers (100 men and 60 women) in order to obtain the reference intervals of this immunoassay in Japanese subjects. Since the %CDT values obtained in apparently healthy nondrinkers were not in a normal distribution, the

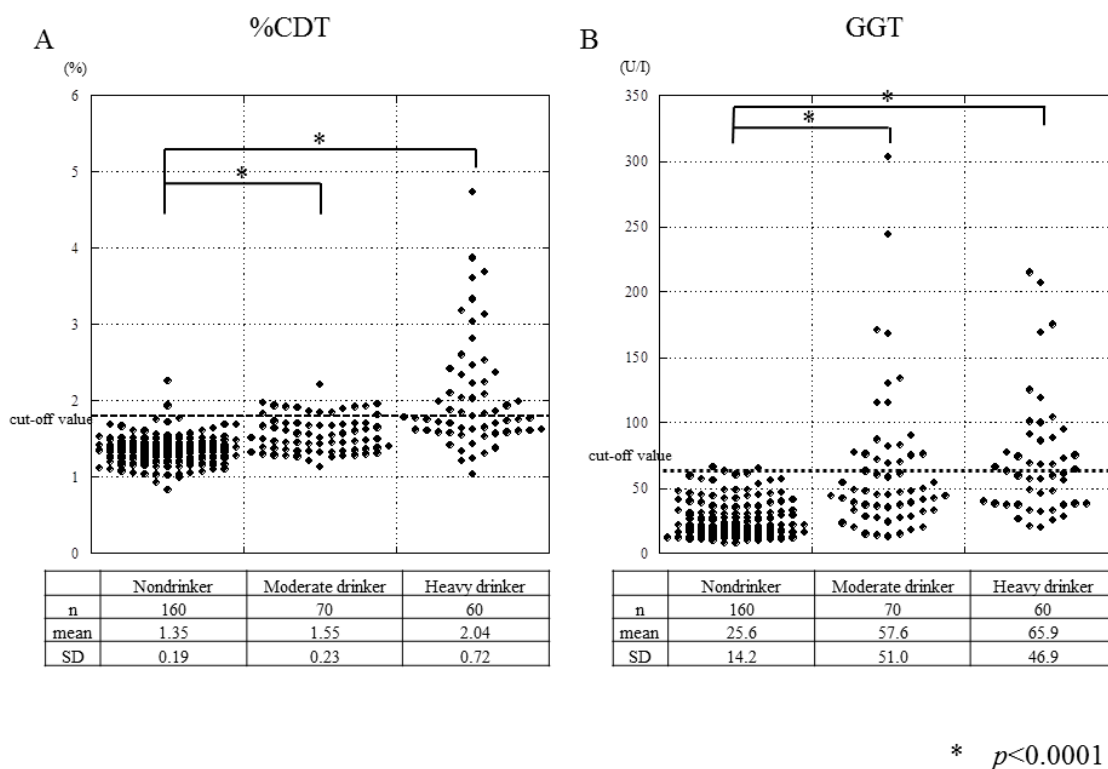


Figure 1: % CDT levels in habitual drinkers who presented for medical check-up
Dot plots of %CDT (A) and GGT (B) values among 160 nondrinkers, 70 moderate drinkers (less than 40 g/day), and 60 heavy drinkers (more than 60 g/day). The positivities of %CDT and GGT in heavy drinkers were 58% and 41%, respectively; the cutoff values of %CDT and GGT were set at 1.74 and 61, respectively. * $p < 0.0001$.

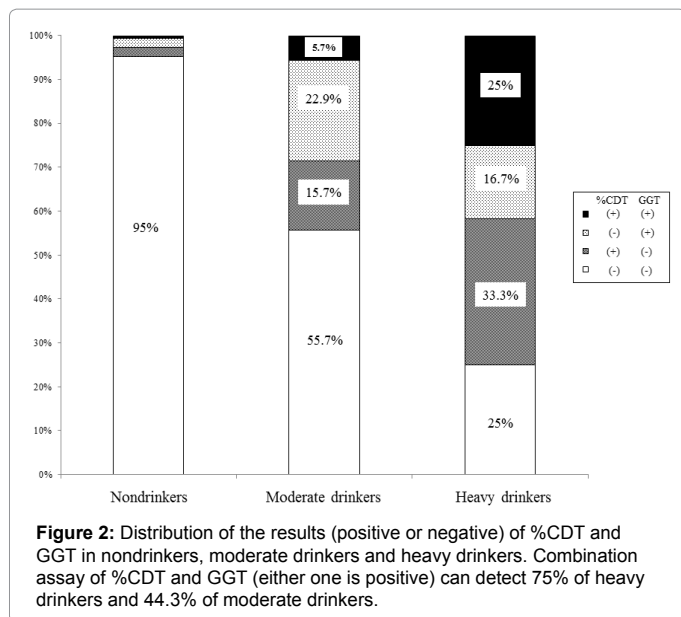
tentative reference interval for %CDT was 1.03-1.74 obtained by the non-parametric method based on data obtained from 160 nondrinkers.

The upper limit of the 95% confidence interval for %CDT was 1.74, which was considered as the cut-off level in the present study.

The reference intervals of CDT levels measured by N Latex CDT are different from those reported in Caucasians [10], indicating that the reference intervals for this direct immunoassay should be set considering their possible ethnic differences. It is important to know where the difference in the reference intervals comes from. We don't have straightforward answer for this question at the moment. But, we did some additional experiments on this matter. We determined CDT levels by capillary electrophoresis (Capillaryes 2 multicapillary CE system, Sebia, France) (data not shown). The reference intervals of

CDT obtained by the capillary electrophoresis were again lower than those for Caucasians, suggesting that the difference of the CDT levels between Japanese and Caucasians found in the present study is not necessarily specific to the N Latex CDT assay. The exact explanation for the difference in the reference intervals of CDT between Caucasians and Japanese remain to be studied. Since the reference intervals obtained in males and females were comparable (1.00-1.77 vs. 1.05-1.64), the values obtained in male nondrinkers and female nondrinkers were combined.

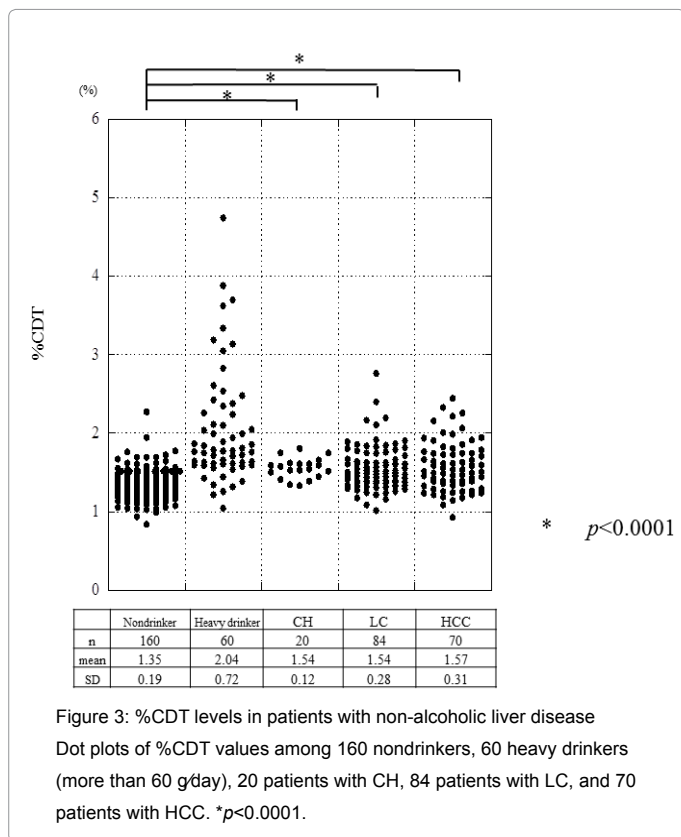
The most well-known marker of alcohol consumption is the serum enzyme GGT. In the present study, the upper limit of GGT was obtained exactly in the same way as for CDT; the 95% confidence interval for GGT obtained in the apparently healthy nondrinkers was



in all the heavy drinkers (Figures 1 and 2); 58% and 44%, respectively, in males and 58% and 17%, respectively, in females.

There was no significant correlation between the levels of %CDT and GGT in heavy drinkers as well known (data not shown); the combination assay of %CDT and GGT (that is, either %CDT or GGT-positive) resulted in 75% sensitivity and 95% specificity in detecting heavy drinkers among the general Japanese population. It is noteworthy that 57% of GGT non-responders could be detected by %CDT measurement. Thus, %CDT level measured by this assay is a sensitive marker of alcohol abuse complementary to GGT.

Although the N Latex CDT assay was applied to the Japanese



61 U/l. It is well-known that a combination of CDT and GGT, namely GGT-CDT, can have higher sensitivity than single markers alone in detecting excessive drinkers [16]. Although MCV is often included in the current standards of biomarkers for excessive drinking, sensitivity of MCV in detection of habitual drinkers is lower than those of GGT as demonstrated in several reports [17,18]. In the present study, the positive rates of %CDT and GGT in habitual heavy drinkers consuming more than 60 g of alcohol per day were 58.3% and 41.7%, respectively

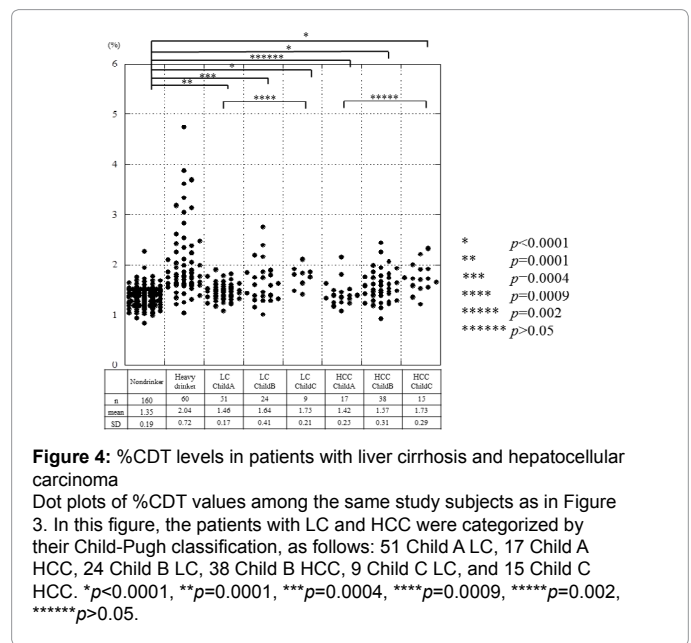


Figure 4: %CDT levels in patients with liver cirrhosis and hepatocellular carcinoma

Dot plots of %CDT values among the same study subjects as in Figure 3. In this figure, the patients with LC and HCC were categorized by their Child-Pugh classification, as follows: 51 Child A LC, 17 Child A HCC, 24 Child B LC, 38 Child B HCC, 9 Child C LC, and 15 Child C HCC. * $p < 0.0001$, ** $p = 0.0001$, *** $p = 0.0004$, **** $p = 0.0009$, ***** $p = 0.002$, ***** $p > 0.05$.

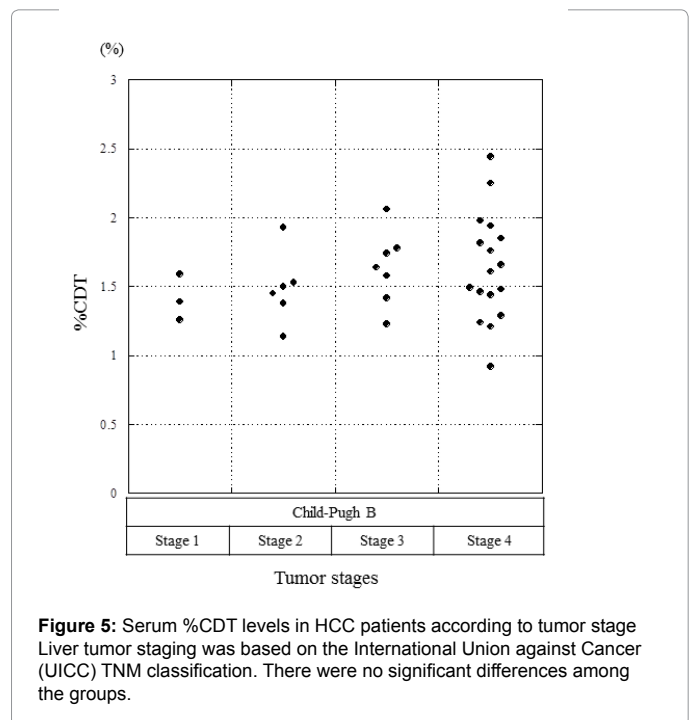


Figure 5: Serum %CDT levels in HCC patients according to tumor stage. Liver tumor staging was based on the International Union against Cancer (UICC) TNM classification. There were no significant differences among the groups.

population for the first time in the present study to evaluate the diagnostic performance and establish the reference values, diagnostic performances of this assay in other Asian populations remain to be tested. Healthy subjects as a reference population was defined as non-drinkers with normal serum transaminase levels and also negative for both HBsAg and anti-HCV. Since imaging studies such as ultrasound are not conducted on a routine basis, subjects with mild fatty liver but without elevation of serum transaminase levels are not necessarily excluded.

One of the concerns when CDT measurements are used in liver unit is whether or not the CDT values may change in patients with non-alcoholic liver disease. Indeed, it has been reported that CDT levels are higher in patients with non-alcoholic chronic liver disease, limiting its use as a screening marker for alcoholic liver disease [12,19,20]. It is possible, however, that false positive rates in non-alcoholic liver disease are dependent on the methods used to determine CDT. Therefore, we tested the performances of this new immunoassay in patients with non-alcoholic liver disease.

We found that serum %CDT was not altered in patients with chronic viral hepatitis, or Child A liver cirrhosis, but was increased in Child B or C liver cirrhosis compared with serum %CDT in nondrinkers. Chrostek et al. also reported that %CDT values determined by the N Latex CDT are increased in non-alcoholic liver disease [21], but the extent of increase was smaller than that reported in the present study.

The %CDT levels did not increase in direct relation to the stage of HCC, in contrast to results reported previously [12]. It is possible that this discrepancy resulted from the differences in methods; the RIA used in the previous report reacts with trisialo-transferrin, which does not react with the N-Latex CDT test.

In summary, the results of this study indicate that the serum CDT level as determined by N Latex CDT is useful as a combination assay with GGT in detecting excessive alcohol consumption at medical check-up settings in Japan. However, caution should be taken to interpret the results in clinical settings because patients with advanced non-alcoholic liver disease have high values irrespective of drinking history.

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