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Evaluation of automated serum des-gamma-carboxyprothrombin (DCP) assays for detecting hepatocellular carcinoma

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ABSTRACT

Objectives: We evaluated two new autoanalyzers, μTAS and Lumipulse for des-γ-carboxyprothrombin (DCP) assay.

Material and methods: Analytical performance was evaluated, and the upper reference limit of the 97.5th percentile for DCP was re-established using sera from 140 healthy individuals. DCP levels were determined by the two autoanalyzers and EIA in a total of 239 sera from HCC patients (n = 120) and those without HCC (n = 119).

Results: Total imprecision of the two automated assays was <5% CV. Analytical measurement ranges (AMRs) were verified to be linear. The new reference limits were 29.5 mAU/mL for μTAS and 35.0 mAU/mL for Lumipulse. There were proportional and constant biases between the results from the autoanalyzers and those from EIA.

Conclusion: The two newly developed DCP assays showed high analytical performance, but re-establishment of reference limits would be necessary. The new analyzers could be useful for clinical laboratories because of convenience of operation and wide AMRs.

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Introduction

Des-γ-carboxy prothrombin (DCP) is an abnormal prothrombin induced by vitamin K absence or antagonist (PIVKA-II) which lacks the capability of reciprocal action with other coagulation factors [1]. It is structurally distinguishable from normal prothrombin based on the differences in composed amino acid residues in the N-terminal Gla domain [2]. These Gla residues are originally formed from glutamic acid (Glu) residues in the precursor of prothrombin and are synthesized by the action of γ-glutamyl carboxylase along with vitamin K-dependent enzymatic reactions [3]. However, if the enzymatic reaction is not sufficient, in conditions such as vitamin K deficiency or after administration of vitamin K antagonists, DCP, which has remaining Glu residues due to decreased γ-carboxylation, is expressed and released into the blood [4]. In addition, DCP is elevated in patients suffering severe obstructive jaundice [5].

Measuring serum DCP has been identified as an effective serologic tool in early diagnosis of hepatocellular carcinoma (HCC), because DCP in circulation is primarily produced by HCC tissues and is elevated in proportion to tumor size [6]. Moreover, it may provide more information on biological status of HCC patients due to the correlation of DCP levels with several conditions such as intrahepatic metastasis and portal vein thrombosis [7,8]. After the first description by Liebman et al. in 1984 [9], the clinical usefulness and significance of DCP in patients with HCC has been consistently reported [10–12]. Although serum DCP in patients with HCC may not always originate from cancer tissues [13], DCP levels generally indicate the presence of HCC and are helpful in making patient prognosis [14–16]. Moreover, levels of serum DCP were not elevated in most cases of chronic liver diseases such as cirrhosis and hepatitis because of the highly specific characteristics of DCP [17]. In a previous study, DCP together with alpha-fetoprotein (AFP) and *lens culinaris* agglutitin-reactive fraction of AFP (AFP-L3) could enhance the diagnostic accuracy for detecting of HCC [18].

With increasing concern for DCP in HCC, various methods to determine DCP levels in the blood have been developed and a competitive radioimmunoassay was used in earlier eras [9]. Since then, an enzyme immunoassay (EIA) using an anti-DCP monoclonal antibody has been designed to quantify levels of DCP, and is now widely used in clinical laboratories. In addition, electrochemiluminescence immunoassay (ECLIA), which generally has higher analytical sensitivity than EIA and may have potential for detecting small HCC, has also been used to measure DCP levels [19,20]. Recently, technically advanced autoanalyzers such as liquid-phase binding assay (LBA) and chemiluminescence enzyme immunoassay (CLEIA) have been introduced [21,22].

In this study, we aimed to evaluate the newly developed autoanalyzers for DCP assays and to compare these assays with conventional EIA. We also re-established upper reference limits for the two

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automated DCP assays and estimated the diagnostic performance of these reference limits as cut-off values for discriminating HCC from other benign liver diseases.

Materials and methods

Principle of the DCP assays

Serum DCP concentration was measured by three assays including the commercially available EIA (Haicatch PIVKA-II, Eisai Co., Ltd., Tokyo, Japan) [9,23,24], a micro-total analysis system (μTAS) Wako i30 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and Lumipulse G1200 (Lumipulse) (Fujirebio inc., Tokyo, Japan) according to the manufacturers' instructions.

The EIA utilizes the solid-phase (micro-cup) sandwich method using an anti-human DCP mouse monoclonal antibody (Ab), which is pre-coated on the inner surface of the micro-cup. Serum DCP is captured and reacts with enzyme-labeled anti-human prothrombin Ab. After adding the substrate solution, the enzyme reaction generates products with color from substrates. The DCP level of the sample was determined from the calibration curve generated with the concentrations of calibrators and their optical densities.

μTAS has been developed as a specific autoanalyzer for HCC. It adopts a hybrid method based on the liquid-phase binding assay [22] of electrokinetic analyte transport assay (EATA) and capillary zone electrophoresis (CZE) as previously described by Kagebayashi et al. in 2009. Reagents in this system uses a Fab′ Ab produced from mouse monoclonal immunoglobulin G specific to DCP, negatively charged DNA and dye. After loading serum sample and reagents, DNA-Fab′-DCP-dye complexes are formed and moved to the electrophoresis zone by EATA. Finally, the complexes are isolated by CZE and measured in the detection area.

Lumipulse is designed to perform automated CLEIA using Lumipulse G reagents, conducting various processes including dispensation, agitation and photometric measurement. The CLEIA system carries out quantification of analytes using ferrite particle coated with antigen (Ag) or Ab conjugated with alkaline phosphatase, and chemical luminescent substrate. The luminescence, which is generated by CLEIA, is measured by a photometric detector.

Precision performance evaluation

The imprecision of the two autoanalyzers was evaluated according to the Clinical and Laboratory Standards Institute (CLSI) document EP5-A2. Two quality control (QC) materials with high and low DCP levels were assayed in duplicate with two runs per day over a 10-day evaluation period; repeatability, between-run, between-day and total imprecision for two levels of QC materials were calculated.

Validation of analytical measurement range (AMR)

The AMRs suggested by the manufacturers of the μTAS and the Lumipulse are 5 to 50,000 mAU/mL and 5 to 75,000 mAU/mL, respectively. Patients' sera with low and high DCP levels were selected to validate the AMRs. The linearity of the two autoanalyzers were analyzed using mixtures of samples with high and low DCP levels at the following ratios; 100:0, 75:25, 50:50, 25:75, and 0:100. These samples were assayed in duplicate, and the expected and measured concentrations were compared using linear regression analysis.

Evaluation of dilution recovery and carryover rates

To estimate dilution recovery rates, two sera with DCP over 2000 mAU/mL by EIA were selected from samples of patients with HCC and pooled. The two autoanalyzers were assessed with dilution of the pooled sample with high DCP level by ×2, ×4, ×6, ×8, and ×10 with a sample diluent. The pooled and diluted sera were measured in duplicate. Percent recovery of each dilution was calculated by comparing the measured and expected DCP concentrations. Additionally, the pooled serum with high DCP level (H) was employed to evaluate the carryover rates of the two autoanalyzers. The DCP level of 10 mAU/mL (L) was used as the low level sample for the carryover test. The H sample was consecutively assayed four times with the two analyzers, and the L sample was also assayed four times. The carryover rate (%) was not considered to be significant in a case below 1%, and the following equation was applied for the calculation of carryover rate [25]:

$$\text{Carryover}\ (\%) = [L1-(L3+L4)/2]/[(H2+H3)/2-(L3+L4)/2]\times 100$$

Study subjects

A total of 379 serum samples from patients with HCC (n = 120), those with HCC-free liver diseases (n = 119) and apparently healthy individuals (n = 140) were collected at Severance Hospital from January to April 2011. All the samples from the patients with warfarin administration during recent several days were excluded. All diagnoses of HCC were histologically confirmed by an ultrasonography-guided biopsy of the liver lesion. Patients with HCC-free liver diseases were selected as a control group, and no samples in the control group were collected from the patients suffering obstructive jaundice, in which the DCP can be elevated. The 140 healthy individuals were consisted of ages 30 to 39 (20 males and 20 females), 40 to 49 (20 males and 20 females), and 50 to 59 (30 males and 30 females).

Reference range establishments and diagnostic performances

The 97.5th percentile upper reference limits of DCP for the two autoanalyzers was estimated for the 140 healthy individuals according to the CLSI document C28-A2. The calculated upper limits were applied as cutoff values to estimate the sensitivity and specificity for differentiation of HCC from benign liver diseases.

Comparison of DCP levels between the three methods

The DCP levels measured by the two autoanalyzers were compared with those measured by conventional EIA. The measured DCP levels were also compared between the two autoanalyzers. Slope, intercept, and bias between the results by the three methods were calculated.

Statistical analysis

The Analyse-it Method Evaluation Edition version 2.22 software (Analyse-it Software Lit, City West Business Park, Leeds, UK) was used for all statistical analyses. Continuous variables between the study groups were compared using Mann–Whitney *U* test, and categorical variables were assessed by Chi-square test. To determine agreement on DCP levels between the three methods, Bland–Altman plotting of the paired differences was performed. The DCP levels of the three assays were also compared using Passing–Bablok regression analysis.

Results

Precision performance of the automated DCP assays

As shown in Table 1, the mean DCP levels of the two QC materials for μTAS were 2193.0 and 106.4 mAU/mL, and those for Lumipulse were 170.9 and 37.3 mAU/mL. For these levels, imprecision between runs and between days along with repeatability of the two assays

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