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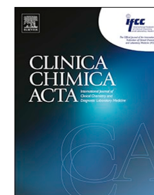

Is capillary zone electrophoresis a suitable method for estimating serum albumin: A comparison of four methods

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ABSTRACT

Background: The capillary zone electrophoresis method of albumin measurement is frequently used for oncologic and haematologic patients but few data exist about the agreement between the albumin measurements performed by capillary zone electrophoresis and other methods. The aim of this study was to analyse the agreement between human serum albumin measurements by capillary zone electrophoresis and by the nephelometry, bromocresol purple and turbidimetry methods.

Method: We prospectively measured 100 freshly collected non-frozen patient serum samples, by using four different methods: the capillary zone electrophoresis method performed with a CAPILLARYS 2 instrument, the bromocresol purple dye method performed with an Advia XPT analyser, the nephelometric method performed with a BN ProSpec analyser and the turbidimetric method with reagents from DiAgam and performed with the Advia XPT analyser.

Results: A bias towards higher values in the lower range of albumin concentrations was observed with capillary zone electrophoresis compared to immunonephelometry: correlation coefficient $r^2 = 0.925$; slope of 0.86 (0.82–0.89, 95% confidence interval), which is significantly different from 1; and an intercept of 4.94 g/L (3.67–6.16, 95% confidence interval). Similar results were observed when comparing capillary zone electrophoresis to the bromocresol purple and immunoturbidimetry methods. The capillary electrophoresis method overestimated low albumin levels by up to 25% (5 g/L).

Conclusion: Compared to the nephelometry, turbidimetry and bromocresol purple methods, the capillary zone electrophoresis method tends to overestimate human serum albumin concentrations for levels below 30 g/L. This discrepancy could lead to an overestimation of the nutritional status, an inappropriate scoring of the disease and a delay in nutritional treatment.

1. Introduction

Measurements of human serum albumin (HSA) concentrations are currently performed to explore nutritional status, hepatic function and nephritic syndrome [1]. At least five different HSA measurement procedures are used in medical testing laboratories; these methods include bromocresol green colorimetry (BCG), bromocresol purple colorimetry (BCP), immunological quantification by turbidimetry (IT) or nephelometry (IN) and capillary zone electrophoresis (CZE) [2]. Several studies have noted discordant results among the HSA concentrations measured using the BCG, BCP and IN methods [3,4]. The lack of agreement among HSA measurement methods may lead to inconsistent results between two institutions [4], which could result in inadequate patient care. Additionally, within the same laboratory, different HSA level measurement procedures can be performed according to clinical presentation, leading to a discrepancy in HSA results that could sow seeds of doubt among clinicians regarding laboratory results. In a recent study [4], Bachmann et al. compared the analytical performance of 24 commercially available HSA measurement products and showed significant differences that compromise the interpretation of HSA

Abbreviations: BCG, bromocresol green colorimetry; BCP, bromocresol purple colorimetry; CZE, capillary zone electrophoresis; HSA, human serum albumin; IN, immunological quantification by nephelometry; IT, immunological quantification by turbidimetry; QC, quality control; SPE, serum protein electrophoresis

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concentration results. The researchers included 9 BCG methods, 12 BCP methods, 2 IN methods and 1 IT method but no CZE method. However, CZE is commonly used to perform serum protein electrophoresis (SPE) to detect and to monitor a monoclonal gammopathy and to measure HSA concentrations in oncology and haematology patients [5]. The quantification of albumin by CZE relies on separating the proteins by electrophoresis, evaluating the percent of albumin in the protein fraction and determining the serum total protein concentration by a separate assay to convert the percentage to a concentration. CZE is sometimes used as a standard to estimate the performances of HSA measurement methods [6], but despite the current interest in this method [7–9] few data exist about the agreement between the HSA measurements performed in medical laboratories by CZE and other methods [10].

The aim of the study was to analyse the agreement between HSA measurement by the CZE method and HSA measurement by the IN, BCP and IT methods.

2. Materials and methods

2.1. Patient samples

We prospectively tested 100 freshly collected non-frozen patient serum samples that had HSA concentrations ranging from 18 to 50 g/L by using four different methods: CZE, the BCP dye method, the IT method and the IN method. A minimum volume of 7 mL of blood was collected in a dry tube and centrifuged at 2700*g* for 10 min at 20 °C. Haemolysed (haemoglobin > 100 mg/dL), icteric (bilirubin > 479 μmol/L), and lipemic (triglycerides > 10 mmol/L) specimens and samples with a monoclonal paraprotein detected by SPE were not included. The study was done with leftover samples, thus informed consents were not required in accordance with the French legislation.

2.2. Measurement of albumin/total protein

The CZE method was performed with a CAPILLARYS 2 instrument (Sebia, France) using 8 capillary tubes of fused silica, 25 μm diameter and 18 cm length. The migration occurred in a buffer of pH = 9.9 at a high electric potential difference of 7000 V, and the temperature was controlled to 35.5 °C by the Peltier device in the instrument. The optical system consisted of a deuterium lamp and a detection cell including a 200 nm UV filter. The total protein measurement was performed with an Advia XPT system (Siemens, Germany). The assay is based on the Weichselbaum method with a Biuret reagent. The peptide bonds of the protein interact with the cupric ions to form a purple complex measured at 545 nm as the endpoint of the reaction. CZE evaluates the percent of protein in the albumin fraction, and the total protein measurement enables conversion of the percentage to a concentration.

The BCP method was performed with an Advia XPT analyser (Siemens, Germany) *via* Biuret reagent with Gornall reagent. In alkaline environments, the peptide bonds of serum albumin form a blue-violet complex with the copper II ions of Gornall reagent; the colour intensity of the complex is proportional to the albumin concentration. After incubation at 37 °C for 10 min, the absorbance of the solution was measured at 545 nm.

IT was performed using a DiAgam (Belgium) reagent kit on the Advia XPT analyser. The turbidity induced by the antigen-antibody immune complex was measured at 340 nm and 700 nm. IN was performed with a BN ProSpec analyser (Siemens).

2.3. Quality control and calibrator

Quality controls (QC) were used for each HSA measurement method and tested before and after each series. A calibration was performed every month for each method excepted for CZE method. HSA measurement performances were evaluated every two months with an external quality assessment program comparing results to a peer group.

The CZE QC kits used were Sebia Normal Control Serum 30,037 and Sebia Hypergamma Control Serum 17,036. No calibrator was used for albumin measurement by CZE method. The BCP QC kits used were Biorad Liquid Assay Multiqual 45,771 and 45,772. The BCP calibrator kit was Siemens Enz3 8DD194. The IN QC kits used were Siemens N/T Protein Standard SL 084850F, 084751H and 084649 L. The BCP calibrator kit was Siemens N Protein Standard SL 083619A. The IT QC kits used were DiAgam Multiparametric Controls MP-COS-002 17H29, MPCON-002 17H24 and MPCOX-002 17H28. The IT calibrator kit was DiAgam Multiparametric Calibrator MPREK-000 17H28.

2.4. Evaluation of albumin carbamylation

Carbamylation of serum albumin was evaluated using the symmetry factor on the CZE instrument (CAPILLARYS 2). The symmetry factor has been defined as the distance from the centre line of the peak to the back slope, divided by the distance from the centre line of the peak to the front slope, with all measurements made at 10% of the maximum peak height [11]. Serum urea concentrations were assayed by a Siemens enzymatic and fluorometric method developed by Roch-Ramel using urease and glutamate dehydrogenase [12].

2.5. Statistical analysis

The agreement between the different methods was analysed by using a Bland-Altman plot (difference plot). The correlation between the different methods was assessed by Passing-Bablok regression analysis. All statistical analyses were performed using R Statistical Software version 3.4.3.

3. Results

All 100 patients included in the study were hospitalized in a unit of the university hospital of Brest, France (median age: 64 years, interquartile range: 47–72 years, 57 men and 43 women). The Bland-Altman difference plot of the CAPILLARYS 2 (CZE) and BN ProSpec (IN) HSA concentration measurements is presented in Fig. 1. The mean bias was 0.57% (95% limit of agreement: −13.58% and 14.72%). The correlation between the two methods was assessed by Passing-Bablok regression analysis (Fig. 2). A bias towards higher values in the lower range of albumin concentrations was observed with CZE: correlation coefficient $r^2 = 0.925$; slope of 0.86 (0.82–0.89, 95% CI), which is significantly different from 1; and an intercept of 4.94 g/L (3.67–6.16, 95% CI) (Figs. 1a and 2a). The agreement analysis between the CAPILLARYS 2 (CZE) and Advia XPT (BCP) measurements (Figs. 1b and 2b) and between the CAPILLARYS 2 (CZE) and DiAgam (IT) measurements (Figs. 1c and 2c) showed a mean bias of 2.35% (limit of agreement: −11.96% and 16.66%) and 3.75% (limit of agreement: −6.32% and 13.81%), respectively. Regression analyses revealed a correlation coefficient $r^2 = 0.929$, a slope of 0.96 (0.91–1.00, 95% CI) and an intercept of 1.99 g/L (0.18–3.73, 95% CI) for the comparison between CZE and BCP and a correlation coefficient $r^2 = 0.966$, slope of 0.95 (0.92–0.99, 95% CI) and intercept of 2.68 g/L (1.30–4.03, 95% CI) for the CZE and IT comparison. Compared to the IN, IT and BCP methods, the CZE method tends to overestimate HSA concentrations by up to 25% (5 g/L) when the HSA levels are below 30 g/L.

No correlations were observed between serum urea concentration and the difference between albumin measurements by the IN and CZE methods ($r^2 = 0.029$), nor between the symmetry factor and the difference between albumin measurements by the IN and CZE methods ($r^2 < 0.001$) (data not shown).

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