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Systematic overestimation of human serum albumin by capillary zone electrophoresis method due to monoclonal immunoglobulin interferences

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ABSTRACT

Background: The capillary zone electrophoresis method of albumin measurement is frequently used in monoclonal gammopathy patients but some studies suggest poor performances of the method in this population. The aim of this study was to analyse the impact of serum monoclonal immunoglobulins on human serum albumin determination by capillary zone electrophoresis method compared to other available methods.

Method: We prospectively measured albumin in 100 freshly collected non-frozen serum samples in a monoclonal gammopathy patients population, by using four different methods: the capillary zone electrophoresis method, the bromocresol purple dye method, the nephelometric method and the turbidimetric method. Differences in albumin values between the different methods were analysed with respect to serum monoclonal immunoglobulin concentration. These differences were further investigated by measuring albumin levels in human serum samples spiked with exogenous monoclonal immunoglobulins.

Results: Human serum albumin difference values between capillary zone electrophoresis compared to immunonephelometry method are significantly correlated with increasing monoclonal immunoglobulins concentrations: regression analyses revealed a correlation coefficient $r^2 = 0.60$ and a slope of 0.14 (0.12–0.17, 95% confidence interval). The capillary zone electrophoresis method overestimated serum albumin levels by up to 67% (12 g/L) when monoclonal immunoglobulin level was 63 g/L. The determination of albumin levels in human serum samples spiked with exogenous monoclonal immunoglobulins showed an overestimation of human serum albumin measurement by the capillary zone electrophoresis method proportional to the amount of monoclonal immunoglobulin added in the serum with a slope of 0.19 (0.18–0.20, 95% confidence interval).

Conclusion: Monoclonal immunoglobulins directly interfere with serum albumin measurement by the capillary zone electrophoresis method leading to a systematic overestimation of serum albumin concentrations proportional to the serum monoclonal immunoglobulin level.

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]. CZE is commonly used to perform serum protein electrophoresis (SPE) to detect and to monitor monoclonal gammopathies (MG) and to measure HSA concentrations in oncology and haematology patients [3]. 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.

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

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MGs are an heterogeneous group of hematological malignancies characterized by the clonal proliferation of plasma cells in the bone marrow and the production of monoclonal immunoglobulins (MI) [4]. A MG occurs in 1% of healthy individuals older than 50 years old and in 3% of those beyond 70 years old [5,6]. MG is a well-known interference source of many medical laboratory measurements that is frequently report in one-off cases of serum concentration misestimations [7–9]. Regarding HSA concentration determination, Quick et al. [10] observed substantial discrepancies, up to 10 g/L, between the CZE method and the BCP method in a MG patient population with a systematic overestimation by CZE method. Recently, Padelli et al. [11] reported that the CZE method tends to overestimated HSA concentrations by up to 25% (5 g/L) when the HSA levels are below 30 g/L compared to the IN, IT and BCP methods. These raise the question of the relevance of the CZE method for evaluating HSA in patients with MG requiring new data in this population.

The first aim of this work was to analyse the agreement between HSA measurement by the CZE method and HSA measurement by the IN, BCP and IT methods in a MG patient population. Then we studied the relationship between MI concentrations and HSA measurement results by CZE, IN, BCP and IT methods. Finally, we aimed to determine if monoclonal immunoglobulin is the causal agent of the results discrepancies.

2. Materials and methods

2.1. Patient samples

We prospectively tested HSA measurement of 100 successive freshly collected non-frozen patient serum samples having a monoclonal spike detected by serum protein electrophoresis (SPE). Haemolysed (haemoglobin > 100 mg/dL), icteric (bilirubin > 479 µmol/L), and lipemic (triglycerides > 10 mmol/L) specimens were excluded. Four different HSA measurement methods were performed: the CZE method, 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. The study was done with leftover samples, thus informed consent was not required in accordance with the French legislation.

2.2. Measurement of albumin, total protein and monoclonal immunoglobulin concentrations

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 MI concentration was calculated using the area under the spike from the absorbance baseline, after measurement of total proteins in the sample [12]. 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 [13]. 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) [14]. BCP binds to HSA *via* hydrophobic interactions, and this is followed by a colour change [15]. 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 [16]. 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) [17].

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. Preparation of the serum samples spiked with monoclonal immunoglobulin

Serum was obtained from a single healthy volunteer in 7 mL dry tubes centrifuged at 2700*g* for 10 min at 20 °C. Flixabi® infliximab powder (Samsung Bioepsis, South Korea), a biosimilar of Remicade®, a monoclonal immunoglobulin G1 (IgG1) anti-TNFα antibody, was used to obtain three MI concentration sera of 6 g/L, 12 g/L and 24 g/L and a control without MI. This experiment has been replicated five times.

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 3.5.1.

3. Results

Among the 100 patient serum samples having a monoclonal spike, two patients were excluded due to haemolysed serum. All 98 patients included in the study were hospitalized in a unit of the university hospital of Brest, France between April 1st, 2018 and May 15th, 2018. The population characteristic are presented in the Table 1. The patients analysed were 61 men and 37 women with a median age of 73.5 years (interquartile range (IQR): 63–79 years). Thirty-six patients had MGUS, 43 had myeloma, 8 had lymphoplasmacytic lymphoma and 11 had malignant lymphomas. Monoclonal components involved in MGs were 42 IgG Kappa, 25 IgG Lambda, 18 IgM Kappa, 4 IgM Lambda, 4 IgA Kappa, 4 IgA Lambda and one free light chains Kappa. The median serum concentration of MI was 7.6 g/L (IQR: 4.1–17.3 g/L). The median HSA concentrations were: 38.2 g/L (IQR: 33.4–41.6 g/L) by CZE method, 36.8 g/L (IQR: 32.5–40.3 g/L) by IN method, 36.8 g/L (IQR: 32.8–39.7 g/L) by IT method and 36.7 g/L (IQR: 32.9–40.5 g/L) by BCP method.

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 4.32% (95% limit of agreement: −11.89% and 20.52%). 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 $r^2 = 0.20$; slope of −0.52 (−0.73–-0.31, 95% confidence interval (CI)), which is significantly different from 1;

Table 1
Characteristics of patients.

	Study population (n = 98)
Demographics	
Age (years)	73.5 (63–79)
Gender, male	61 (62.2%)
Disease type	
MGUS	36 (36.7%)
Myeloma	43 (43.9%)
Lymphoplasmacytic lymphoma	8 (8.2%)
Other malignant lymphoma	11 (11.2%)
Laboratory results	
Serum MI concentration (g/L)	7.6 (4.1–17.3)
Ig classes	
IgG Kappa	42 (42.8%)
IgG Lambda	25 (25.5%)
IgM Kappa	18 (18.4%)
IgM Lambda	4 (4.1%)
IgA Kappa	4 (4.1%)
IgA Lambda	4 (4.1%)
FLC Kappa	1 (1%)
FLC Lambda	0
HSA concentration (g/L)	
CZE method	38.2 (33.4–41.6)
IN method	36.8 (32.5–40.3)
IT method	36.8 (32.8–39.7)
BCP method	36.7 (32.9–40.5)

Data presented as median (interquartile range) or number (percentage), where appropriate.
BCP: bromocresol purple; CZE: capillary zone electrophoresis; FLC: free light chain; HSA: Human serum albumin; Ig: immunoglobulin; IN: immunonephelometry; IT: immunoturbidimetry; MGUS: monoclonal gammopathy of undetermined significance; MI: monoclonal immunoglobulin.

and an intercept of 22.83 g/L (15.10–30.57, 95% CI) (Figs. 1a and 2a). The agreement analysis between the CAPILLARYS 2 (CZE) and and DiAgam (IT) measurements (Figs. 1b and 2b) and between the CAPILLARYS 2 (CZE) Advia XPT (BCP) measurements (Figs. 1c and 2c) showed a mean bias of 3.53% (limit of agreement: −10.54% and 17.61%) and 4.09% (limit of agreement: −13.16% and 21.34%), respectively. Regression analyses revealed a correlation coefficient $r^2 = 0.12$, a slope of −0.35 (−0.55–-0.16, 95% CI) and an intercept of 16.24 g/L (9.08–23.40, 95% CI) for the comparison between CZE and IT and a correlation coefficient $r^2 = 0.33$, slope of −0.70 (−0.90–-0.49, 95% CI) and intercept of 29.08 g/L (21.71–36.45, 95% CI) for the CZE and BCP comparison. Compared to the IN, IT and BCP methods, the CZE method tends to overestimate HSA concentrations by up to 67% (12 g/L) when the HSA levels are below 30 g/L.

Regression analyses between HSA difference values between CZE, IN, IT and BCP methods and the serum MI amount are presented Fig. 3. HSA difference values between CZE and IN methods were significantly correlated with increasing MI concentrations, from 1 g/L to 63 g/L (Fig. 3a): regression analyses revealed a correlation coefficient $r^2 = 0.60$, a slope of 0.14 (0.12–0.17, 95% CI) and an intercept of −0.45 g/L (−0.86–-0.04, 95% CI). No significant correlation was observed between HSA difference values between IT and IN methods and MI concentrations (Fig. 3b) nor between IT and IN methods and MI concentrations (Fig. 3c).

The impact on HSA measurements of the addition of therapeutic MI in a serum is presented Fig. 4. HSA results by the CZE method increased significantly ($p < .001$) and proportionally to the amount of MI added in the serum. Regression analyses revealed a slope of 0.19 (0.18–0.20, 95% CI) (Fig. 4a). No impact of MI on HSA measurements was observed on IN method (Fig. 4b), IT method (Fig. 4c) and BCP method (Fig. 4d) with a slope of 0.01 (−0.01–0.03, 95% CI), 0.01 (−0.02–0.05, 95% CI) and 0.00 (−0.01–0.01, 95% CI) respectively.

4. Discussion

Measuring HSA concentrations provides a marker of nutritional status and is a prerequisite for staging diseases, particularly cancer and haematologic malignancies [18,19]. Low albumin levels are associated with high mortality risk [18–22], and an appropriate therapeutic use of albumin might improve the prognosis [22]. HSA concentrations are also used to estimate corrected calcium levels by the Payne formula, thus reducing misestimations of calcium concentrations [23]. Although CZE is frequently used to perform SPE in oncology and haematology patients, the performances of CZE method to measure HSA has been poorly assessed in the MG population [10].

Our study identified two causes that can lead to an overestimation of HSA measurement by the CZE method in MG patients. First, we observed that CZE method overestimates HSA measurement when HSA concentrations are below 30 g/L, as previously shown in a hospitalized population without MG [11]. Second, the differences between HSA measurements by the CZE method and by the IN method were correlated with increasing MI serum concentrations whereas no such effect was observed for the IT and the BCP methods relative to the IN method. The higher the concentration of monoclonal immunoglobulin in patient sera, the more the CZE method overestimated HSA compared to the IN method. These lead to an overestimation of HSA measurement by up to 67% (12 g/L): when MI amount was 63 g/L, HSA concentration result was 18 g/L by IN method and was 30 g/L by CZE method. This observation is consistent with the study conducted by Quick et al. [10] which observed substantial discrepancies in HSA determination in presence of MI between CZE and BCP methods. To our knowledge, this study is the first to provide evidence that MI is a causal agent of the overestimation of HSA by the CZE method in patients with MGs, thanks to spiked serum samples.

Determining the serum total protein concentration by a separate assay is required for the quantification of albumin by CZE. The performance of the serum total protein measurement method used may affect the albumin quantification. In view of our current knowledge, we cannot discern the role of the CZE and the role of the serum total protein measurement in the overestimation of albumin by the CZE method. The Biuret method used in this study is the most common spectrophotometric method of measuring serum total protein concentration and it is the method of choice for samples with high protein concentrations (5–160 g/L) [24]. Not much interference is reported with the Biuret assay except for the rare cases of monoclonal protein interference [25] and bilirubin interference [26]. In our study, the exclusion of haemolysed, icteric and lipemic specimens allow us to rule out this interference as the cause of the disagreement in HSA measurements. Interestingly, Snozek et al. [27] reported that in a MG population HSA measurement by agarose gel protein electrophoresis (AGE) is impacted by MI concentration while BCG and IN method were not. There was a progression from underestimation of HSA at low MI level to an overestimation at high MI level. Total protein was measured on a Hitachi 912 automated analyser, by use of a Roche colorimetric Biuret assay. Further investigations might be useful to study the potential involvement of Biuret assay in the observed HSA measurement discrepancies in presence of serum MI.

Furthermore, HSA determination seems to be also affected by carbamylation [28], a non-enzymatic process resulting from urea decomposition, notably for the CZE method [29]. *In vitro* experiments have shown that albumin carbamylation is responsible for the shift in the albumin peak symmetry [30].

Discrepancies in HSA measurement observed in this study in a MG population could lead to an overestimation of the nutritional status, an inappropriate scoring of the disease and a delay in nutritional treatment [31]. In our point of view, the significant impact of the presence of MI on the HSA determination by CZE observed supports the need for HSA determination by a second method when a monoclonal peak is detected by SPE and, a fortiori, when MI level is high. To date, no gold standard

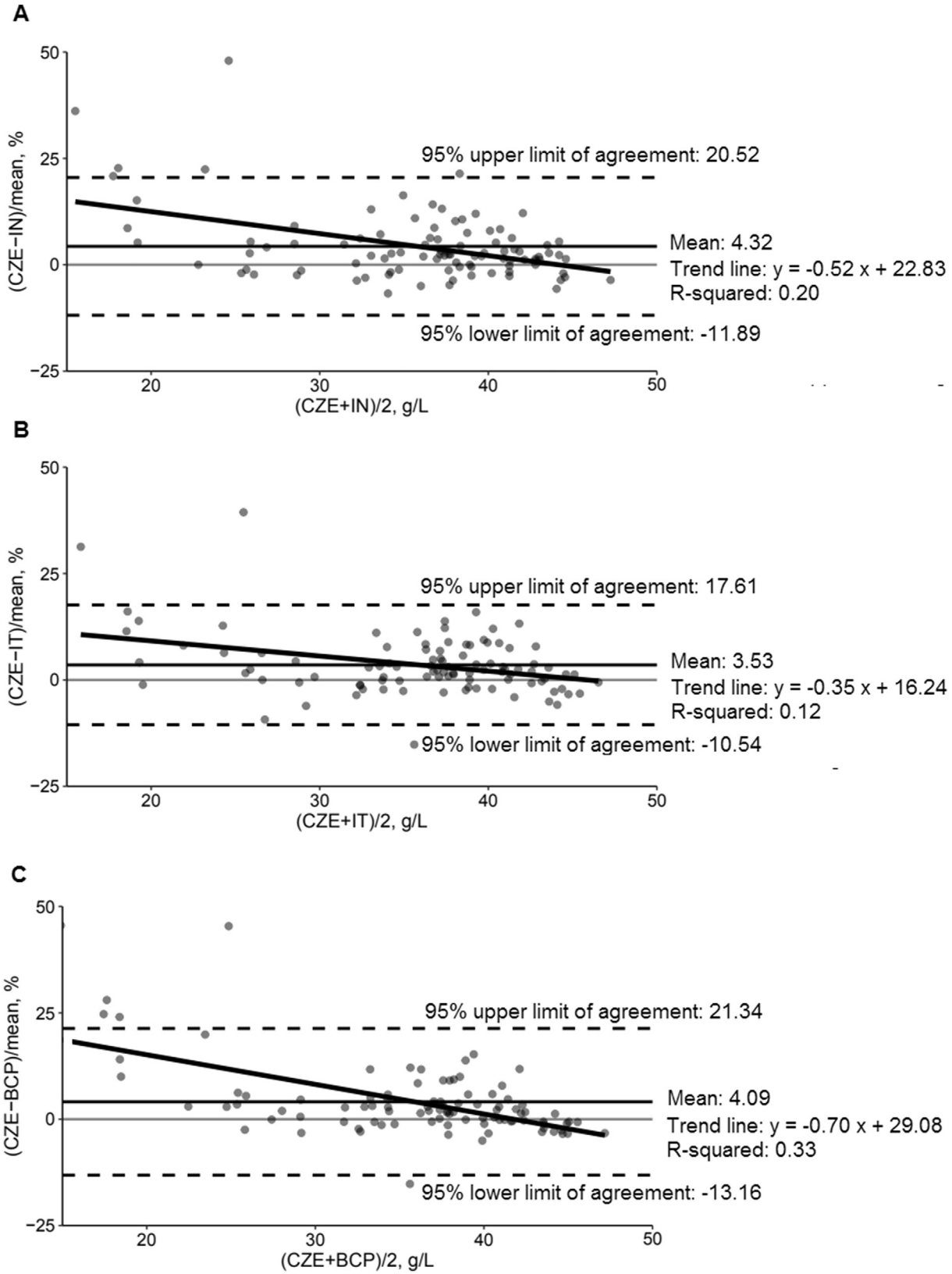

Fig. 1. Bland-Altman plots. Comparison of the CAPILLARYS 2 CZE method (A) *versus* the BN ProSpec IN method, (B) *versus* the DiAgam IT method, and (C) *versus* the Advia XPT BCP dye method.

The solid horizontal line represents the mean differences between the assays. The dashed lines represent the bias ± 1.96 standard deviation limits. The diagonal line was obtained by the least squares method. BCP, bromocresol purple; CZE, capillary zone electrophoresis; IN, nephelometry; IT, turbidimetry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

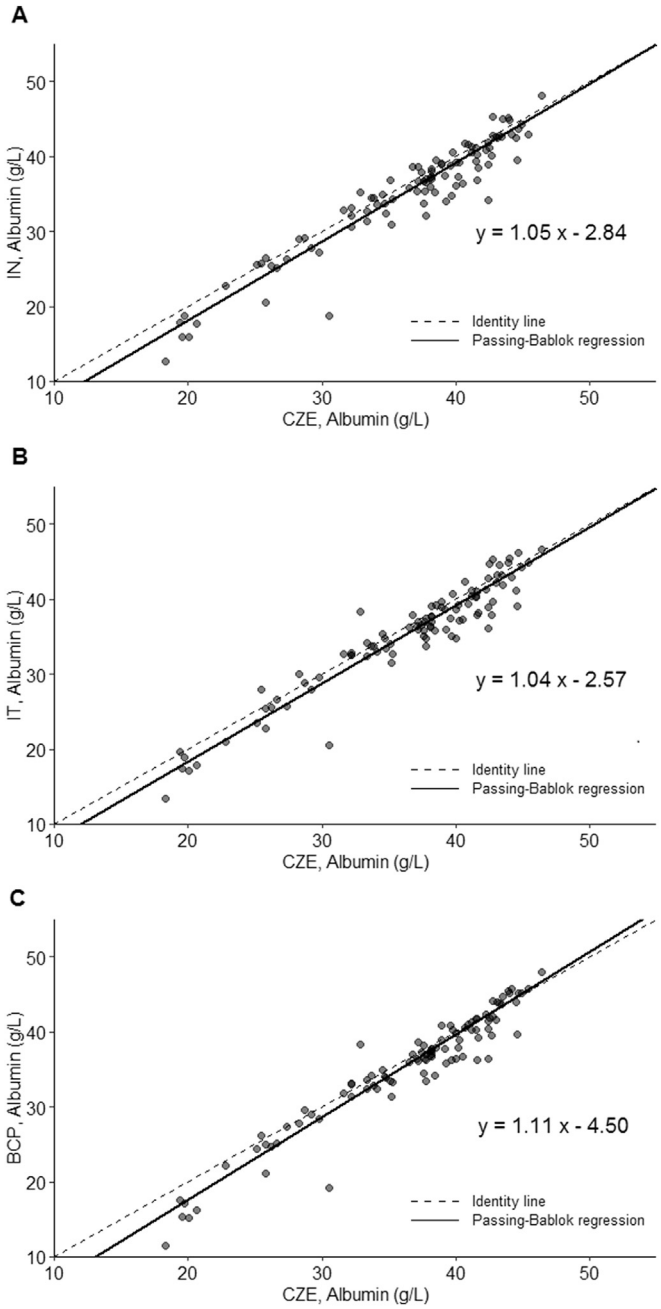

Fig. 2. Passing-Bablok regression analyses. Comparison of the CAPILLARYS 2 CZE method (A) *versus* the BN ProSpec IN method, (B) *versus* the DiAgam IT method, and (C) *versus* the Advia XPT BCP dye method.
The dashed lines represent the identity line. BCP, bromocresol purple; CZE, capillary zone electrophoresis; IN, nephelometry; IT, turbidimetry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

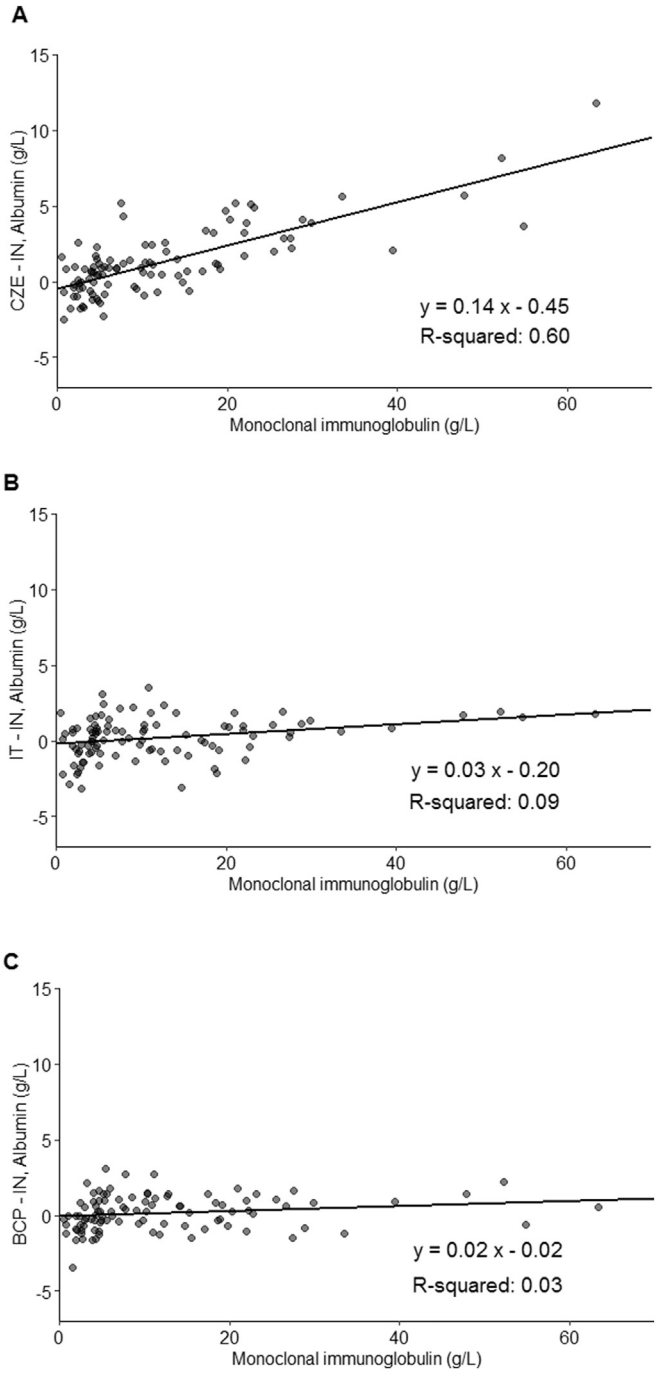

Fig. 3. Correlations between serum monoclonal immunoglobulin levels and differences in human serum albumin results between (A) the CAPILLARYS 2 CZE method and the BN ProSpec IN method (B) the DiAgam IT method and the BN ProSpec IN method, and (C) the Advia XPT BCP dye method and the BN ProSpec IN method.
The regression line was obtained by Passing-Bablok regression analysis. BCP, bromocresol purple; CZE, capillary zone electrophoresis; IN, nephelometry; IT, turbidimetry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

is available for measuring HSA [24], but it is common to consider the immunological methods superior to the dye-based albumin methods, especially for patients with low albumin values [32–34]. IN method is usually considered the reference method for albumin measurements [10,29,32,33,35]. The main advantage of IN method is the absence of cross-reactivity with non-albumin proteins [36]. IN method is usually not the first-line method in medical testing laboratories because it is technically demanding, time consuming, poorly automated and expensive. IT method has the advantage of being more automated than IN but has significant higher reagent costs than dye-binding assays. BCP dye method assays are cheaper alternatives to the immunological methods and are usually described as more specific to albumin than BCG because BCP reagents do not react with α-globulins [37]. In healthy subjects, the BCP method has been reported to closely agree with the IN method in albumin measurements [29,38,39].

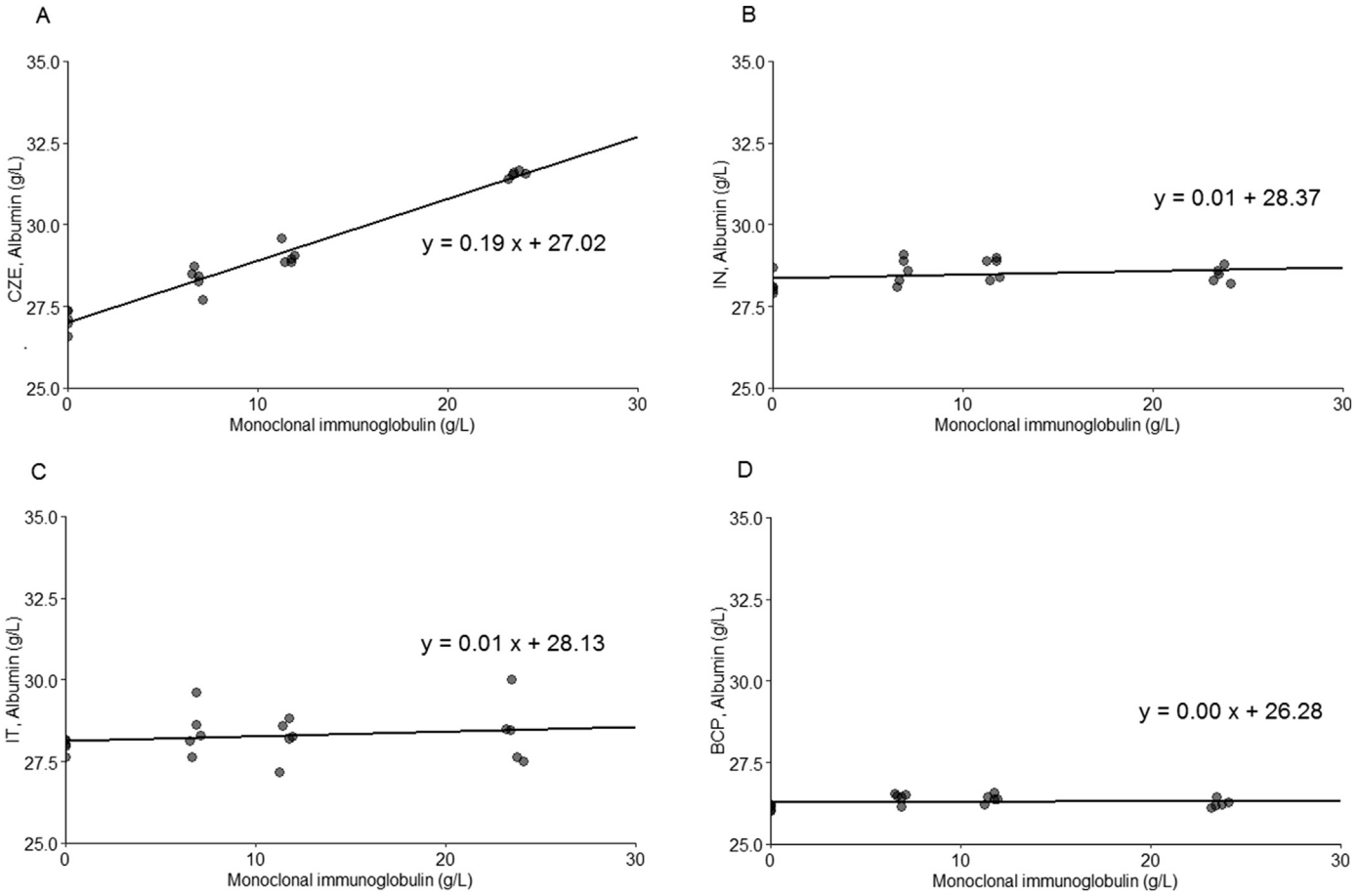

Fig. 4. Impact of the addition of therapeutic monoclonal immunoglobulin in a healthy volunteer serum on albumin results by (A) the CAPILLARYS 2 CZE method, (B) the BN ProSpec IN method, (C) the DiAgam IT method, and (D) the Advia XPT BCP dye method.
The regression line was obtained by Passing-Bablok regression analysis. BCP, bromocresol purple; CZE, capillary zone electrophoresis; IN, nephelometry; IT, turbidimetry. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5. Conclusion

Evaluation of HSA concentrations is crucial particularly for MG patients for whom albumin measurement is commonly performed by CZE during SPE. We highlight that MI directly interfere with HSA measurement by CZE method leading to a systematic overestimation of HSA concentrations proportional to serum MI amount. In addition, we showed that CZE method overestimates HSA in low albumin values in a MG population as previously observed in a hospitalized non MG population. The overestimation of HSA measurement by CZE method reached 67% (12 g/L) in the study population. Further studies are warranted to determine whether the MI interferes with the total protein assay or with capillary electrophoresis system.

The observed discrepancies could lead to an overestimation of the nutritional status, an inappropriate scoring of disease and a delay in nutritional treatment. In our point of view, the significant impact of the presence of monoclonal immunoglobulins on the HSA determination by the capillary zone electrophoresis method supports the need of a second method determination when a monoclonal spike is detected by SPE and, a fortiori, when MI level is high.

Author contributions

All authors confirmed that they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revision of the article for intellectual content; and (c) final approval of the published article.

Authors' disclosures or potential conflicts of interest

The authors have declared that no competing interest exists.

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