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Araştırma Makalesi / *Research Article*

Is Capillary Zone Electrophoresis a Reliable Alternative for BCG-Based Albumin Measurement

Kapiller Zon Elektroforezi, BCG Yöntemiyle Albümin Ölçümünde Güvenilir Bir Alternatif mi?

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ABSTRACT

Objective: This study aimed to evaluate the agreement between albumin measurements obtained via capillary zone electrophoresis (CZE) and bromocresol green (BCG) methods and to determine their interchangeability in clinical practice. Additionally, it explored how these results vary across different protein patterns.

Materials and Methods: Albumin concentrations in 373 patients were measured using the CZE and BCG methods. Subgroup analyses were conducted based on albumin, total protein, IgG, gamma band percentages, and M protein concentrations. Agreement was assessed using Bland-Altman analysis, while constant, proportional, and random errors were analyzed with Passing-Bablok regression. Clinical significance was determined by comparing total allowable error (TEa) with total analytical error (TAE).

Results: CZE method yielded albumin levels that were, on average, 0.28 g/dL lower than those measured by BCG. Greater bias (12.5%) was observed at low albumin levels. Regression analysis revealed constant, proportional, and random errors. Subgroup analyses indicated that TAE exceeded the TEa threshold.

Conclusion: Significant analytical errors exist between albumin concentrations measured by CZE and BCG, making these methods non-interchangeable. Detailed subgroup analyses revealed that this discrepancy consistently persisted across different protein patterns. These findings emphasize the importance of cautious use of the CZE method in clinical decision-making.

Keywords: Albumin, Capillary Electrophoreses, Bromcresol Green, Reproducibility of Results, Bias

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ÖZET

Amaç: Bu çalışmanın amacı, kapiller zon elektroforezi (CZE) yöntemi ile bromokrezol yeşili (BCG) yöntemine dayalı albümin ölçümlerinin uyumunu değerlendirmek ve bu yöntemlerin klinik uygulamalarda birbirinin yerine kullanılıp kullanılamayacağını belirlemektir. Çalışma ayrıca, albümin ölçüm sonuçlarının farklı protein paternlerine göre nasıl değişkenlik gösterdiğini incelemiştir.

Gereç ve Yöntem: CZE ve BCG yöntemleriyle albümin konsantrasyonları 373 hastada ölçüldü. Albümin ve total protein seviyeleri, IgG konsantrasyonları, gama bandı yüzdesi ve M protein konsantrasyonları analiz edilerek alt gruplar oluşturuldu. İki yöntem arasındaki uyum Bland-Altman analizi ile değerlendirildi. Sabit, oransal ve rastgele hatalar Passing-Bablok regresyonu ile analiz edildi. Klinik anlamlılık, toplam kabul edilebilir hata (TEa) ile toplam analitik hata (TAE) değerlerinin karşılaştırılmasıyla değerlendirildi.

Bulgular: CZE yöntemi, albümin düzeylerini BCG yöntemine kıyasla ortalama 0.28 g/dL daha düşük ölçtü. Düşük albümin seviyelerinde (%12.5) daha yüksek yanlılık saptandı. Sabit, orantılı ve rastgele hataların mevcut olduğu görüldü. Alt grup analizleri, TAE'nin TEa'yı aştığını ortaya koydu.

Sonuç: CZE ve BCG yöntemleri arasında belirgin analitik hatalar saptanmış ve bu yöntemlerin birbirinin yerine kullanımı önerilmemiştir. Farklı protein paternlerinde yapılan detaylı alt grup analizleri, bu uyumsuzluğun tutarlı bir şekilde devam ettiğini göstermiştir. Bulgular, CZE yönteminin klinik karar süreçlerinde dikkatle değerlendirilmesi gerektiğini açıkça vurgulamaktadır.

Anahtar kelimeler: Albümin, Kapiller Elektroforez, Bromkrezol Yeşili Sonuçların tekrarlanabilirliği Yanlılık

INTRODUCTION

Human serum albumin (HSA) measurement serves various clinical purposes, including the assessment of nutritional status, liver and kidney function, coronary heart disease, multiple myeloma, neurometabolic disorders, diabetes (1), and the prediction of corrected calcium levels (2). Additionally, low albumin levels have been associated with increased mortality, while appropriate treatment has been shown to improve patient outcomes (3). Traditional HSA detection methods primarily rely on dye-binding and immunoassay techniques, which are widely used in clinical diagnostics (4). Over time, advanced technologies such as fluorescent probe assays, nano-material-based detection, and biosensors have been developed. However, despite these advancements, comprehensive reviews covering these methods remain scarce (5).

Capillary zone electrophoresis (CZE) is a widely used technique for detecting and monitoring monoclonal gammopathy. This method enables the electrophoretic separation of serum proteins, including albumin, by determining the percentages of protein fractions. It requires total serum protein concentration, measured by a different method, to convert these percentages into concentrations (6). Despite its widespread application, limited data exist regarding the agreement between HSA measurements obtained through CZE and other methods in medical laboratories (6,7).

This study aimed to evaluate the agreement between serum albumin concentrations obtained using capillary zone electrophoresis (CZE) and the bromocresol green (BCG) method, and to determine whether CZE can serve as a reliable alternative in clinical practice. Additionally, this study analyzed how discrepancies between these methods vary across different total protein concentrations and protein patterns, such as gamma band percentages, M protein concentrations, and IgG levels, through detailed subgroup analyses.

MATERIAL AND METHOD

Study Design: This cross-sectional, retrospective study included data from 373 patients aged 18–90 years, who had serum protein electrophoresis, total protein, albumin, and immunoglobulin results under the same barcode. Analyses were conducted in our laboratory over one year, from September 1, 2023, to September 1, 2024.

Patients with a hemolysis index >500, icterus index >20, lipemia index >550, CRP >5 mg/L, or biclonal gammopathy were excluded. Ethical approval was obtained from the local ethics committee (Decision number 22, dated November 28, 2023).

Categorization and Creation of Subgroups: Serum albumin concentrations measured by the bromocresol green (BCG) method and capillary zone electrophoresis (CZE) were first compared. Patients were then categorized into two groups based on albumin concentrations obtained using the direct measurement method:

- Albumin (g/dL): <3.5 and 3.5–5.2 (2 groups).

For a more detailed analysis, 11 additional subgroups were created based on laboratory reference ranges, categorized as follows:

- Total Protein (g/dL): <6.4 and 6.4–8.3 (2 groups)
- IgG (g/L): <7, 7–16, and >16 (3 groups)
- Gamma Band (%): <9.69, 9.69–18.9, and >18.9 (3 groups)
- M-Spike (g/dL): Absent, <0.1, and ≥0.1 (3 groups)

The M-spike was categorized using a threshold of 0.1 g/dL. This threshold ensures reliable quantification, as M-bands below this level, particularly in the presence of a polyclonal gammaglobulin background, are often reported as "trace" or "small band," reflecting their limited clinical significance. This categorization was selected to enhance measurement reliability and support clinical decision-making (8).

Capillary Zone Electrophoresis (CZE): The CZE analysis was performed using the Helena V8 Nexus instrument (Helena Biosciences, Gateshead, UK), equipped with eight fused silica capillary tubes (50 μm in diameter, 300 mm long). Migration occurred in a buffer with a pH of 9.9 under high voltage, while the temperature was maintained between 20 and 25 °C using a Peltier cooling system. The optical system included a deuterium lamp and a monochromator set to 214 nm. CZE determines the percentages of albumin, M protein, and other protein fractions converted to concentration equivalents using the total protein concentration.

Serum Total Protein, Albumin, and Immunoglobulin Measurements: Total protein, albumin, and immunoglobulin levels were analyzed on a Cobas c702 (Roche Diagnostics, Mannheim, Germany) autoanalyzer using the Biuret, bromocresol green (BCG), and immunoturbidimetric methods, respectively.

Statistical Analysis The comparison between the two methods was evaluated using a Bland-Altman plot to assess agreement and a Passing-Bablok regression analysis to determine the correlation. CUSUM test was used to validate the linearity of the Passing-Bablok regression analysis. According to interpretation guidelines, the methods are considered comparable within the studied concentration range if the 95% confidence interval (CI) of the intercept (a), representing the constant error, includes 0, and the CI of the slope (b), indicating proportional error, includes 1. A CI for the intercept excluding 0 indicates a constant error, while a CI for the slope excluding 1 suggests a proportional error (9).

Clinical significance was determined by evaluating whether the total analytical error (TAE) exceeded the total allowable error (TEa). TEa was calculated using intra-individual and inter-individual biological variations derived from the median albumin concentrations measured by the direct bromocresol green (BCG) method. TAE was calculated as the sum of constant error (a), proportional error (b−1), and random error (1.96×RSD [Residual Standard Deviation]). The factor 1.96 was used to account for the 95% confidence interval of the residual standard deviation, ensuring that random differences were evaluated within this statistical range. These analyses were

performed for all patients and across the 11 subgroups. Statistical analyses were conducted using MedCalc V22.023.

RESULTS

Albumin concentration data from the 373 patients included in the study are presented in Figures 1a and 1b. Figure 1a illustrates the Bland-Altman difference plot, comparing albumin concentrations obtained by the direct measurement method and capillary zone electrophoresis (CZE), with a mean bias of 0.28 g/dL (95% limits of agreement: -0.05 to 0.61). Figure 1b depicts the Passing-Bablok regression analysis, which assesses the correlation between the two methods using the same dataset. Both constant and proportional errors were observed in albumin measurements by CZE across the entire study group, with a correlation coefficient of $r^2 = 0.929$. The intercept of -0.6081 (95% CI: -0.7600 to -0.4554), which does not include zero, indicates a constant error, while the slope of 1.0774 (95% CI: 1.0417 to 1.1134), significantly deviating from 1, suggests a proportional error. Additionally, residual analysis revealed significant random error. As shown in the residuals plot (Figure 3), the residuals did not predominantly distribute within the expected range of ±1.96 × residual standard deviation (RSD: 0.1154; range:-0.2261 to 0.2261). Instead, the majority of the residuals were concentrated below zero, indicating a skewed distribution rather than randomness. This observation highlights the presence of substantial random error, further limiting the agreement between the two methods.

In addition, patients were grouped based on albumin concentrations obtained from the direct measurement method: those with concentrations <3.5 g/dL (n=27) and those with concentrations between 3.5–5.2 g/dL (n=344). Agreement analysis between direct measurement and CZE-derived albumin concentrations showed that for low albumin concentrations (<3.5 g/dL), the mean bias was 12.5% (95% limits of agreement: -11% to 36%), while for albumin concentrations within the reference range (3.5–5.2 g/dL), the mean bias decreased to 6.6% (95% limits of agreement: -1.7% to 14.9%). Only two patients had albumin concentrations >5.2 g/dL. These findings are illustrated in Figures 2a and 2b.

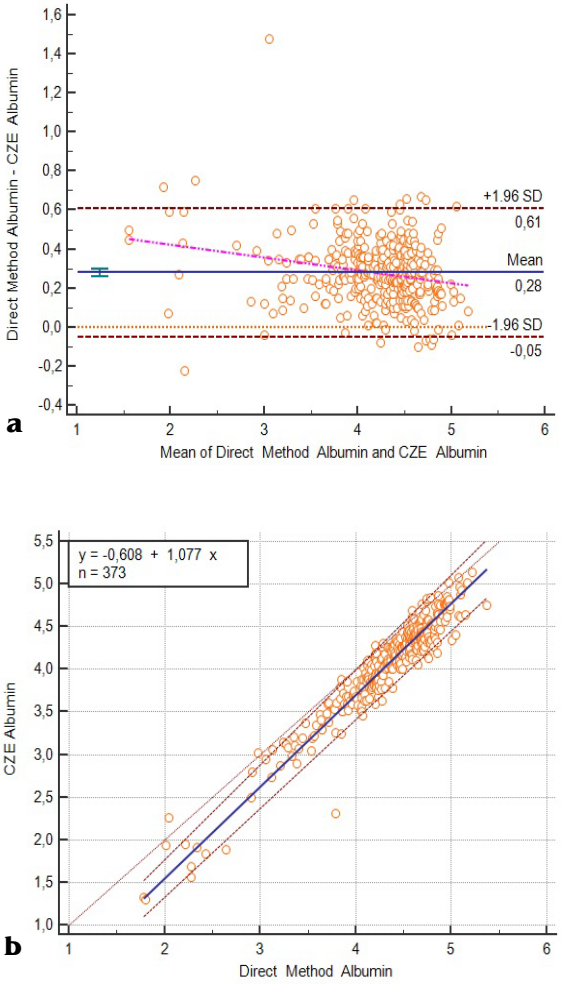

Figure 1. a. Bland-Altman plot comparing albumin concentrations obtained from the direct measurement method with those obtained from capillary zone electrophoresis (CZE) based on data from 373 patients. **b.** Passing-Bablok regression analysis comparing albumin concentrations obtained from the direct measurement method with those obtained from capillary zone electrophoresis (CZE) based on data from 373 patients.

The agreement and correlation between albumin concentrations measured by the direct method and CZE were analyzed across all patients and within 11 subgroups.

The Passing - Bablok regression results demonstrated constant error and proportional error in specific subgroups, while the Bland-Altman plot revealed varying mean bias values across the groups (Table 1). Bold values in Table 1 from the Passing-Bablok regression analysis indicate subgroups where no constant error or proportional error was observed, reflecting better agreement between the two measurement methods.

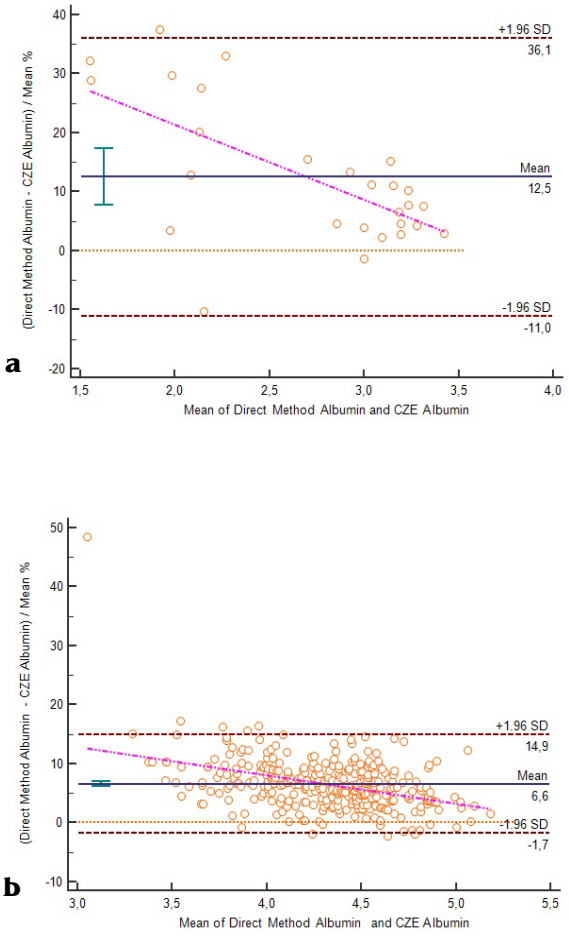

Figure 2. a. Bland-Altman plot comparing albumin concentrations obtained from the direct measurement method (BCG) with those obtained from capillary zone electrophoresis (CZE) for patients with albumin concentrations <3.5 g/dL (n=27). **b.** Bland-Altman plot comparing albumin concentrations obtained from the direct measurement method (BCG) with those obtained from capillary zone electrophoresis (CZE) for patients with 3.5-5.2 g/dL albumin concentrations (n= 346).

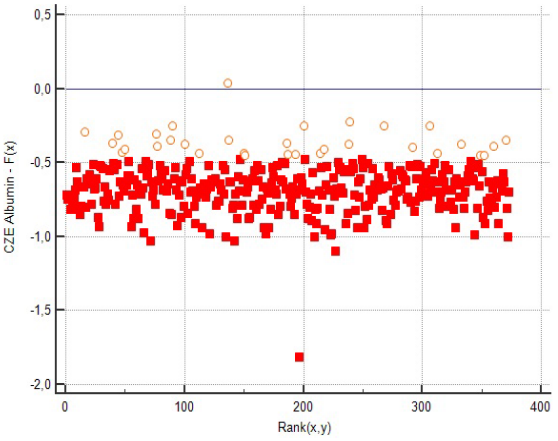

Figure 3. Residuals plot for albumin concentrations obtained using the CZE and BCG methods across the entire cohort (n=373).

Table 2 compares TEa and TAE for albumin concentrations obtained through direct measurement and CZE across the entire group and subgroups. The TAE was compared with the TEa, and these analyses were conducted separately for all patients as well as for each of the 11 subgroups created. TEa was calculated based on the Desirable Biological Variation Database specifications on Westgard's website, updated in 2014. The desirable TEa (%) was calculated as $<1.65(0.50 \times CVw) + 0.25 \sqrt{(CV^2w + CV^2g)}$, where CVw represents within-subject biological variation, CVg represents between-subject biological variation, and TEa denotes the desirable specification for allowable total error. Accordingly, albumin's desirable TEa was established at 4.07% (10-12). Total allowable errors for each group were calculated in g/dL using the median albumin concentrations obtained from the entire group and each of the subgroups. These values were then compared with the TAE obtained from the Passing-Bablok regression analysis as described in the statistical analysis section. As shown in Table 2, the TAE values in the overall group and the subgroups were significantly higher than the calculated TEa values.

Table 1. Passing-Bablok Regression and Bland-Altman Plot Analysis of Albumin Concentrations: Direct Measurement Method vs. CZE Across All Patients and Subgroups.
Tablo 1. Albümin Konsantrasyonlarının Passing-Bablok Regresyon ve Bland-Altman Grafiği Analizi: Tüm Hastalar ve Alt Gruplar İçin Doğrudan Ölçüm Yöntemi ile CZE'nin Karşılaştırılması.

Groups		Passing-Bablok Regression Analysis				Bland-Altman Plot Analysis	
	n	Regression Equation	Intercept, 95% CI	Slope, 95% CI	Cusum Test	Mean Bias (g/dL)	p
All group	373	y = -0,612225 + 1,078331 x	-0,7647 to -0,4600	1,0427 to 1,1145	(P=0,49)	0,28	<0,0001
Albumin (<3.5)	27	y = -0,903180 + 1,210693 x	-1,5154 to -0,3413	1,0265 to 1,4079	(P=1,00)	0,29	<0,0001
Albumin (3.5-5.2)	346	y = -0,748478 + 1,108696 x	-0,9565 to -0,5389	1,0606 to 1,1558	(P=0,61)	0,28	<0,0001
TP (<6.4)	83	y = -0,423067 + 1,025410 x	-0,6901 to -0,1463	**0,9550 to 1,0933**	(P=0,40)	0,33	<0,0001
TP (6.4-8.3)	290	y = -0,627514 + 1,083834 x	-0,8568 to -0,4057	1,0327 to 1,1354	(P=0,33)	0,26	<0,0001
IgG (<7)	76	y = -0,492885 + 1,041147 x	-0,7509 to -0,2217	**0,9795 to 1,1030**	(P=0,71)	0,33	<0,0001
IgG (7-16)	251	y = -0,735088 + 1,106536 x	-0,9606 to -0,5113	1,0542 to 1,1573	(P=0,50)	0,28	<0,0001
IgG (>16)	46	y = -0,409529 + 1,043742 x	**-0,7516 to 0,04566**	**0,9442 to 1,1249**	(P=0,90)	0,20	<0,0001
Gamma (<9,69)	84	y = -0,594329 + 1,065281 x	-0,8916 to -0,2873	**0,9941 to 1,1340**	(P=0,92)	0,33	<0,0001
Gamma (9,69-18,9)	225	y = -0,754002 + 1,112379 x	-1,0155 to -0,5348	1,0621 to 1,1684	(P=0,53)	0,27	<0,0001
Gamma (>18,9)	64	y = -0,491639 + 1,063763 x	-0,8515 to -0,1050	**0,9752 to 1,1540**	(P=0,81)	0,23	<0,0001
M-Spike (Absent)	276	y = -0,665087 + 1,093554 x	-0,8309 to -0,4956	1,0542 to 1,1309	(P=0,30)	0,27	<0,0001
M-Spike (<0,1)	10	y = -1,248418 + 1,211516 x	**-3,4031 to 0,2171**	**0,8812 to 1,7061**	(P=0,77)	0,31	<0,0001
M-Spike (≥ 0,1)	87	y = -0,379982 + 1,016580 x	**-0,7836 to 0,01915**	**0,9261 to 1,1087**	(P=0,43)	0,32	<0,0001

CZE: Capillary zone electrophoresis, TP: Total protein, Gamma: The percentage of the gamma region in CZE, M-spike: M protein detected by CZE

Table 2. Comparison of Total Allowable Error (TEa) and Total Analytical Error (TAE) with its Components for Albumin Concentrations: Direct Measurement vs CZE
Tablo 2. Albümin Konsantrasyonları İçin Toplam İzin Verilebilir Hata (TEa) ve Toplam Analitik Hata (TAE) Komponentlerinin Karşılaştırılması: Doğrudan Ölçüm Yöntemi ve CZE.

Groups	n	Median g/dL	TEa (g/dL)	CE	RE	PE	TAE
All group	373	4.46	0.18	0.61	0.22	0.07	**0.90**
Albumin (<3.5)	27	3.06	0.13	0.90	0.29	0.21	**1.40**
Albumin (3.5-5.2)	346	4.49	0.18	0.75	0.22	0.11	**1.08**
TP (<6.4)	83	4.05	0.16	0.42	0.24	0.03	**0.69**
TP (6.4-8.3)	290	4.56	0.19	0.63	0.22	0.08	**0.93**
IgG (<7)	76	4.41	0.18	0.49	0.21	0.04	**0.74**
IgG (7-16)	251	4.49	0.18	0.74	0.24	0.11	**1.09**
IgG (>16)	46	4.34	0.18	0.41	0.18	0.04	**0.63**
Gamma (<9.69)	84	4.46	0.18	0.59	0.21	0.07	**0.87**
Gamma (9.69-18.9)	225	4.53	0.18	0.75	0.23	0.11	**1.09**
Gamma (>18.9)	64	4.19	0.17	0.49	0.22	0.06	**0.77**
M-Spike (Absent)	276	4.47	0.18	0.67	0.21	0.09	**0.97**
M-Spike (<0.1)	10	4.45	0.18	1.25	0.16	0.21	**1.62**
M-Spike (≥ 0.1)	87	4.27	0.17	0.38	0.28	0.02	**0.68**

CE: Constant Error (Intercept in Passing-Bablok regression). **CZE**: Capillary Zone Electrophoresis (A method for serum protein separation). **Gamma**: Gamma Region Percentage in CZE. **M-spike:** M Protein detected by CZE. **PE:** Proportional Error (Deviation of slope "b" from 1). **RE:** Random Error (1.96 × Residual Standard Deviation). **TAE:** Total Analytical Error (Sum of CE, PE, and RE). **TEa:** Total Error Allowable (Calculated based on biological variations; set at 4.07%, determined using the median albumin values for the overall cohort and subgroups). **TP:** Total Protein

DISCUSSION

Numerous analytical methods are available for determining human serum albumin (HSA) concentration, ranging from traditional dye-binding and immunological methods to advanced techniques such as fluorescent probes, nanoparticles, biosensors, chromatographic techniques, and electrophoresis-based methods. Despite these advancements, dye-binding and immunological approaches remain the most commonly used in routine clinical practice due to their simplicity and cost-effectiveness. However, the clinical implementation of newer techniques, such as fluorescent probes and biosensors, requires further validation and optimization (5).

In our study of 373 patients, we observed that albumin concentrations measured by the CZE method were consistently lower compared to the direct BCG method. This underestimation was particularly pronounced at low albumin levels (<3.5 g/dL), with a discrepancy of 12.5%, while within the reference range (3.5–5.2 g/dL), the bias decreased to 6.6%.

Statistically significant constant, proportional, and random errors were observed in albumin measurements obtained via the CZE and BCG methods across the entire cohort and all 11 subgroups. Constant and proportional errors were demonstrated by the intercept and slope of the Passing-Bablok regression analysis, while residual analysis revealed significant random error. Most residuals did not lie within the expected range of ±1.96 times the residual standard deviation (RSD: 0.1154; interval: -0.2261 to 0.2261) and were predominantly distributed below zero. Furthermore, the TAE values in all groups exceeded the predefined TEa limits,

emphasizing that albumin concentrations measured by the CZE method may have clinical implications and potentially affect patient management.

In certain subgroups, the confidence intervals (CIs) of the intercept and slope were wider, suggesting potential challenges in interpreting the agreement between the methods. Notably, in cases where the CI of the intercept includes 0 and the CI of the slope includes 1, the methods might appear to be in agreement. However, as highlighted by Mayer et al. (13), this perceived agreement could be an artifact of small sample sizes, which tend to produce larger CIs. This methodological limitation is further supported by Ludbrook (14), who emphasized that method comparison studies with small sample sizes are inherently biased toward concluding agreement between laboratory methods. For robust Passing-Bablok regression analysis, a sample size of at least 50 is recommended, as stated by Passing and Bablok (15). Therefore, the apparent agreement observed in certain subgroups with limited sample sizes should be interpreted cautiously, as it may not reflect true methodological compatibility.

Previous studies have demonstrated that dye-binding methods based on bromocresol green (BCG) interact not only with albumin but also with other acute-phase proteins, such as α-globulins, particularly in samples with low albumin concentrations (<2.5–3.5 g/dL) (16). This interaction leads to a positive bias in albumin measurement, with discrepancies of up to 1.0 g/dL compared to bromocresol purple (BCP) or nephelometric (IN) methods (17). The positive bias observed with BCG is primarily attributed to its reactivity with proteins such as α2-macroglobulins and haptoglobin (18). Consequently, BCG-based assays may yield inconsistent results and should be interpreted cautiously, particularly in clinical scenarios such as nephrotic syndrome, where α2-macroglobulin levels are significantly elevated (19). Understanding these limitations is essential for ensuring the accurate application of the BCG method in our laboratory and for the reliable interpretation of clinical results.

Our study demonstrated that the CZE method tends to underestimate albumin levels compared to the BCG method, particularly in subgroups with and without M-spikes. However, Padelli et al. (7) reported a systematic overestimation of albumin levels by the CZE method in monoclonal gammopathy patients. This discrepancy may be due to methodological differences and the varying impact of monoclonal immunoglobulins on albumin measurements. Padelli et al. also observed a proportional overestimation correlated with increasing monoclonal immunoglobulin concentrations, which highlights the variability in CZE performance across different clinical scenarios.

The determination of albumin levels using the CZE method heavily relies on the accuracy of serum total protein measurements, which are performed as a separate test (6,7). In our laboratory, serum total protein concentrations are measured using the Biuret method, which, according to the kit insert, is prone to interference from conditions such as hemolysis, lipemia, icterus, and high dextran concentrations. However, as patients with values exceeding these thresholds were excluded from our study, we do not believe that these interferences affected the results. Additionally, rare cases of gammopathies, especially those involving IgM-type paraproteins (e.g., Waldenström's macroglobulinemia), can lead to unreliable total protein results measured by the Biuret method (20).

Previous studies have reported a lack of agreement between the CZE and BCG methods. Our study is among the first to systematically investigate how this disagreement varies across different protein patterns, including gamma band percentages, M protein concentrations, and IgG levels. These findings emphasize the

limitations of substituting one method for the other and underscore the impact of protein patterns on method agreement in clinical applications. Additionally, our results suggest that discrepancies observed in subgroups with absent and varying levels of M-spikes cannot be solely attributed to interference from Biuret-based total protein measurements. This indicates that other factors, beyond total protein interference, contribute to the observed lack of agreement between the methods.

One of the limitations of this study is the limited number of patients with low albumin concentrations (<3.5 g/dL). Although the study included 373 patients, only 27 patients fell within this range, which may limit the generalizability of the findings for low albumin levels. Additionally, the observed residuals demonstrated a skewed distribution, predominantly below zero, indicating significant random error that could influence the agreement between the methods. These findings suggest that further studies with larger sample sizes, particularly in the low albumin concentration range, are necessary to validate the results and explore the impact of random error more comprehensively. Moreover, the influence of other potential factors, such as variations in total protein measurements and their methodological interferences, should be considered in future research.

CONCLUSION

This study demonstrated that albumin concentrations measured using the CZE and BCG methods are not interchangeable due to statistically and clinically significant constant, proportional, and random errors. Careful selection of the albumin measurement method is crucial, particularly in clinical scenarios such as kidney and liver diseases or nutritional assessments, where accurate albumin values are critical for patient management and therapeutic decision-making.

Moreover, while previous studies have reported a lack of agreement between these methods, our study uniquely investigated how this disagreement varies across different protein patterns, such as gamma band percentages, M protein concentrations, and IgG levels, through detailed subgroup analyses. These findings underscore the limitations of the CZE method and emphasize the need for careful consideration when interpreting its results in clinical practice.

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Authors' disclosures or potential conflicts of interest

The authors have stated that there are no conflicts of interest to disclose.

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