Impact of Albumin Assays in the Diagnosis of Malnutrition in Hemodialysis Patients: A Cohort Study

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Objective: In hemodialysis (HD) patients, malnutrition should be diagnosed by several assessment tools including a plasma albumin concentration of less than 3.8 g/dL or 3.5 g/dL using bromocresol green or immunonephelometry (IN), respectively. However, albumin measurement is not yet standardized and two alternative methods are also commonly used in laboratories: bromocresol purple (BCP) and immunoturbidimetry (IT). This study aimed to revisit the hypoalbuminemia thresholds for BCP and IT, in HD patients.

Methods: Plasma albumin was measured by the four analytical methods during the monthly HD nutritional assessment of 103 prospectively included patients.

Results: Significant differences in albumin levels were observed in HD patients depending on the method used. Using BCP or IT with the cut-off at 3.5 g/dL (determined for the general population) we obtained 33% and 9.7% of false hypoalbuminemia in comparison to IN (mean bias of -0.4 g/dL and -0.065 g/dL, respectively). The best hypoalbuminemia threshold for BCP was 3.05 g/dL and 3.4 g/dL for IT. Twenty percent of HD patients were classified as malnourished when albumin was determined by IN. Similar rates were obtained using the new hypoalbuminemia cut-offs for BCP (18.5%) and IT (19.5%).

Conclusion: To avoid nutritional misclassification of HD patients, we should adjust hypoalbuminemia thresholds when BCP or IT methods are used in laboratories.

Keywords: Albumin; analytical method; cut-off; hemodialysis; malnutrition

Introduction

IN HEMODIALYSIS (HD) patients, hypoalbuminemia is the key biological parameter for the diagnosis of protein-energy wasting[1] or malnutrition,[2] in combination with clinical criteria. It is currently recommended to measure plasma albumin by either the bromocresol green (BCG) or immunonephelometry (IN) method. However, significant differences due to analytical interferences have been described between these two assays,[3–5] leading international guidelines to propose different decision thresholds for HD patients (albumin $<$ 3.8 g/dL with BCG or $<$ 3.5 g/dL with IN).[1,2] Standardization of albumin measurement is even more complex as two alternative methods (i.e., bromocresol purple [BCP] or immunoturbidimetry [IT]) are also widely implemented in laboratories. Decision thresholds for BCP and IT are already approved for the general population but not for HD patients. This raises a serious question because negative analytical interferences are majored in HD patients due to albumin carbamylation and uremia.[6] Discrepancies between analytical methods are known; however, the most recent guidelines for nutritional care in chronic kidney disease patients do not specify which one should be used.[7] In our study, we determined the analytical biases of the 4 methods for albumin measurement in 103 chronic HD patients and proposed new decision thresholds for hypoalbuminemia depending on the used method. This work highlights that the appropriate decision threshold for hypoalbuminemia can significantly improve the diagnosis of malnutrition in HD patients.

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Materials and Methods

Study Population and Outcome

This observational monocentric prospective study, including in-chronic and out-chronic HD patients, adheres to the Standard for reporting diagnostic accuracy studies[8] and follows the guidelines outlined in the Declaration of Helsinki and the Declaration of Istanbul, with a favorable opinion from the Research Ethics Committee. Participants aged more than 18 years (n = 103) were enrolled if they received HD for at least 3 months. As per the routine monthly nutritional assessment, blood was collected at the beginning of the middle week dialysis session. Demographic, clinical, and biological data were collected in dedicated health records DxCare (Table 1). As per French Haute Autorité de Santé guidelines,[2] the malnutrition status was defined as a body mass index (BMI) < 23 kg/m^2 (calculated using a dry weight at the end of the dialysis session) associated with hypoalbuminemia (defined as < 3.5 g/dL using IN, the reference assay).

Plasma Albumin Assays

The 4 albumin measurements were performed on the same day by the BCP and BCG methods (Abbott Diagnostics), the IT method (DiAgam), and the IN test (Siemens Healthineers).

Statistical Analysis

All analyses were performed using GraphPad Prism 5.0, as stated in the figures' legends. Differences were considered statistically significant if the double-sided *P* value was $< .05$.

Results

Baseline Characteristics of the Population

Patients, mostly with diabetic or vascular nephropathies, were included (n = 103) during their routine HD treatment. HD was used in the majority of cases (69.9%). Malnourished patients represented 20.4% of the population, with lower BMI (-6 kg/m^2), lower albumin levels (-0.3 g/dL), and similar inflammatory status (i.e., C-Reactive Protein level) compared to normal-nourished patients (Table 1).

Bias in Albumin Concentrations

Albumin levels in HD patients were analyzed using BCP, BCG, and IT and compared to the gold-standard IN. As expected, albumin levels were significantly overestimated by BCG (+0.24 g/dL) and underestimated by BCP (-0.43 g/dL). By contrast, IT and IN were highly correlated with a mean difference of -0.07 g/dL (Fig. 1).

Table 1. Baseline Characteristics of Hemodialysis Patients

Variable	Hemodialysis Patients			*P* Value
	Global, n = 103	Nonmalnourished, n = 82 (79.6%)	Malnourished, n = 21 (20.4%)*	
Demographic				
Age (years), median (IQR)	68 (52-79)	68.5 (54-79)	64 (36.5-80.5)	.3464
Male gender, n (%)	61 (59.2)	51 (62.2)	10 (47.6)	.2252
Kidney disease etiology				
Diabetic nephropathy, n (%)	24 (22.8)	20 (23.8)	4 (19)	.6053
Vascular and hypertensive nephropathy, n (%)	32 (31.1)	26 (31.7)	6 (28.5)	.7817
Glomerular nephropathy, n (%)	20 (19.4)	14 (17.1)	6 (28.5)	.2346
Hereditary nephropathy, n (%)	5 (4.8)	3 (3.5)	2 (9.5)	.2645
Other, n (%)	9 (8.6)	8 (9.5)	1 (4.7)	.4696
Undetermined nephropathy, n (%)	13 (12.4)	11 (13.1)	2 (9.5)	.6319
Modality for acute renal replacement therapy				
Hemodialysis, n (%)	72 (69.9%)	59 (72%)	13 (62%)	.3705
Hemodiafiltration, n (%)	31 (30.1%)	23 (28%)	8 (38%)	.3705
Nutritional status				
Anthropometric measurements				
BMI (kg/m^2), mean (SD)*	25.1 (5)	26.3 (4.8)	20.3 (2.1)	$< .0001$
Significant weight loss†, n (%)	13 (12.6%)	10 (12.2%)	3 (14.3%)	.7969
Biological data				
Albumin (g/dL), mean (SD)*	3.31 (0.45)	3.38 (0.45)	3.02 (0.36)	.0006
CRP (mg/dL), mean (SD)	1.92 (2.91)	1.95 (3.04)	1.68 (2.18)	.9936

Continuous variables were expressed as median (25th, 75th percentile) or mean ± SD and discrete variables as absolute (relative) frequencies of patients. To compare the differences between malnourished and nonmalnourished HD patients, we used the Wilcoxon-Mann-Whitney U test for quantitative variables and the Chi-squared test for categorical variables. Age, gender, etiology of kidney disease, and therapy modality are independent of the nutritional status of HD patients.

*Malnutrition was defined as BMI < 23 kg/m^2 associated with albumin concentration < 3.5 g/dL measured by IN method (12).

†Unintentional weight loss $> 10\%$ of the normal body weight over 6 months.

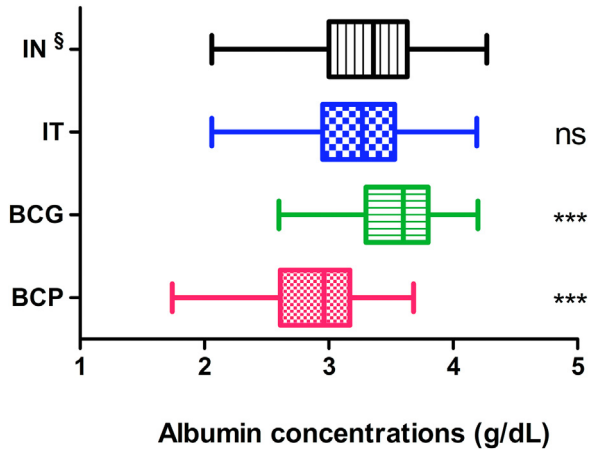

Figure 1. Box-and-whiskers plots representation of albumin concentrations in 103 hemodialyzed patients, measured by BCP (red box), BCG (green box), and IT (blue box) compared to IN method (black box). One-way ANOVA and Bonferroni's multiple comparison tests were used for comparing all 4 methods. BCG, bromocresol green; BCP, bromocresol purple; IN, immunonephelometry; IT, immunoturbidimetry; ns, nonsignificant; ***, $P < .001$; §, reference method (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Hypoalbuminemia Classification

Despite a good linear association between IN and BCP (Fig. 2A, $P = .96$), 33% of HD patients classified as normoalbuminemic with IN, were misclassified as hypoalbuminemic using BCP with a cut-off at 3.5 g/dL (Fig. 2A, hatched area). Less discrepancies were observed between IT and IN at the recommended threshold of 3.5 g/dL, with only 9.7% of false hypoalbuminemia (Fig. 2B, hatched area).

New Hypoalbuminemia Cut-Offs for BCP and IT

Using area under the receiver operating characteristic curves, we determined a new decision threshold for BCP to 3.05 g/dL (sensitivity 0.936, specificity 0.900, positive predictive value 0.936, negative predictive value 0.900, and accuracy 0.922; Fig. 3A, B). For IT, the optimal threshold was 3.4 g/dL (sensitivity 0.936, specificity 0.875, positive predictive value 0.922, negative predictive value 0.897, and accuracy 0.910; Fig. 3C, D).

Hypoalbuminemia Thresholds and Malnutrition

Malnutrition was defined as the combination of a BMI < 23 kg/m^2 and hypoalbuminemia. IN and IT at the cut-off of 3.5 g/dL displayed an identical population of 20.4% of malnourished patients (n = 21/103) (Fig. 4A, B). This proportion increased by 4.8% when hypoalbuminemia was determined by BCG at the cut-off of 3.8 g/dL (n = 26/103, Fig. 4C). Finally, the malnutrition rate was

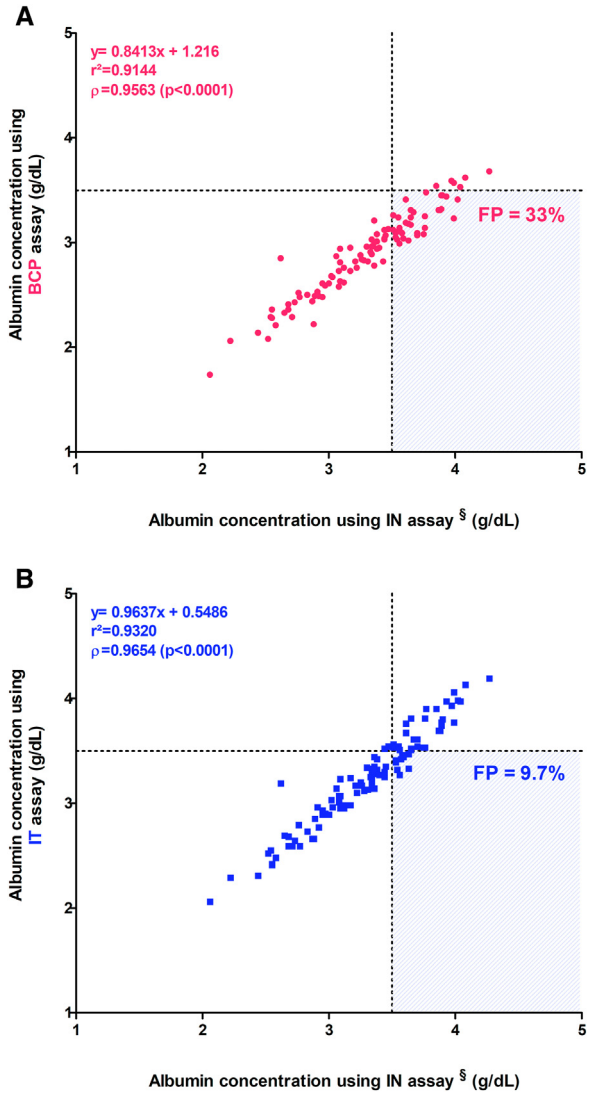

Figure 2. Pearson's correlation and linear regression analysis of the relationship between albumin levels in HDP by BCP and IN (A, red plots) and by IT and IN (B, blue plots). The dashed lines drawn at 3.5 g/dL represent the current recommended hypoalbuminemia threshold for IN, the reference method (at the intersection with the x-axis), and for BCP and IT (at the intersection with the y-axis, A and B, respectively). The hatched areas represent the false-positive hypoalbuminemia corresponding to albumin values classified as hypoalbuminemia as per BCP (A) or IT (B) threshold but considered as normal plasma albumin using IN thresholds. For each comparison, linear equation, R-squared and Pearson coefficient are mentioned. BCP, bromocresol purple; IN, immunonephelometry; IT, immunoturbidimetry; FP, false positive; §, reference method (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

30.1% (n = 31/103) when BCP was used with the recommended cut-off of hypoalbuminemia at 3.5 g/dL (Fig. 4D). By applying the new decision thresholds for IT and BCP (3.4 g/dL and 3.05 g/dL, respectively), the

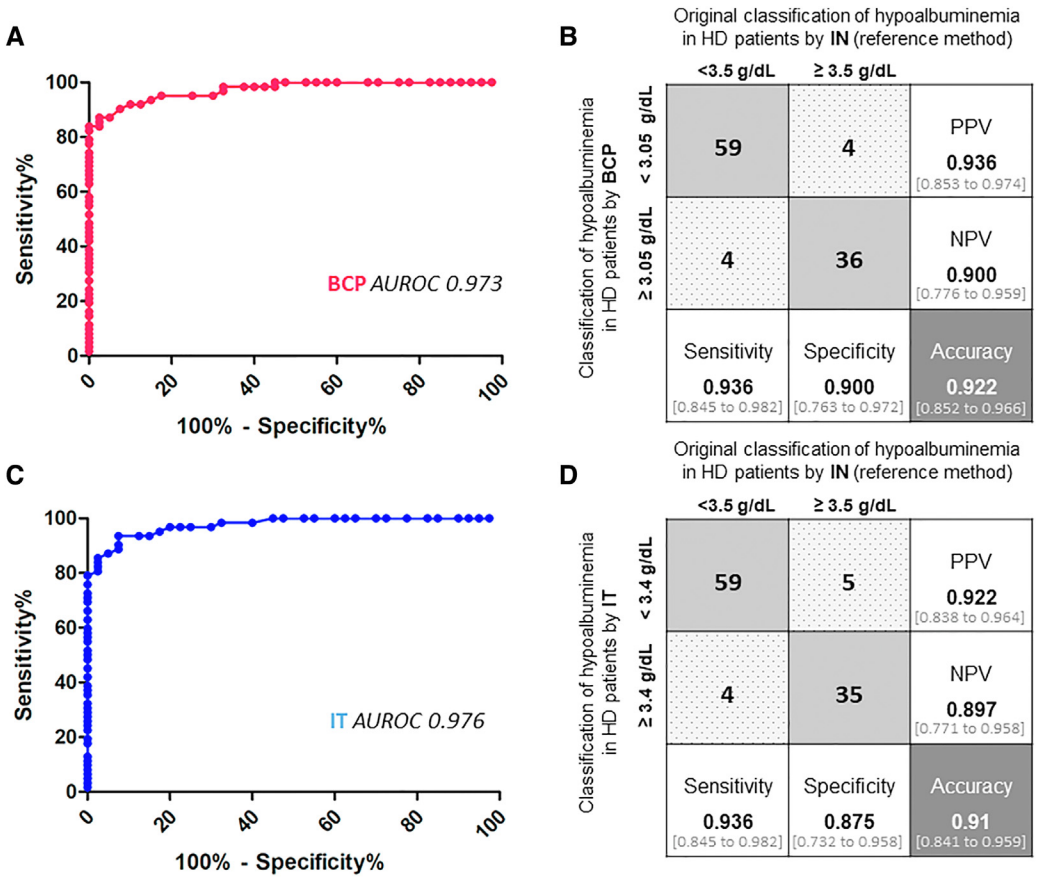

Figure 3. Receiver operating characteristic (ROC) curves and confusion matrix and performance for BCP (A and B) and IT (C and D) in determining hypoalbuminemia, considering IN as the reference method in hemodialyzed patients. Receiver operating characteristic (ROC) analyses were built and the best cut-off values of hypoalbuminemia were determined for each method by calculating the Youden index. The Areas Under Receiver Operating Characteristic curves (AUROC) are indicated on the graph legend. In the confusion matrix (B and D), gray squares correspond to true positive and negative values and spotted gray squares represent false positive and negative values. Predictions were calculated for a cut-off of hypoalbuminemia at 3.05 g/dL and 3.4 g/dL for BCP and IT, respectively. For each statistic, the 95% confidence intervals are indicated between brackets. BCP, Bromocresol Purple; IN, Immunonephelometry; IT, immunoturbidimetry; HD, hemodialysis; NPV, Negative Predictive Value; PPV, Positive Predictive Value (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

proportions of malnourished patients dropped to 19.5% (n = 20/103) for IT and 18.5% (n = 19/103) for BCP (Fig. 4E, F, respectively).

Discussion

Plasma albumin monitoring is essential for nutritional assessment and therapeutic decisions in HD patients. Our work highlights the high complexity in interpreting albumin measurements as analytical assays are not currently standardized. BCG overestimates albumin,[6,9] whereas IT and especially BCP measure lower albumin levels than the reference IN, as previously reported in the general population[4] or HD patients.[6] Immunoassays are currently listed as the reference methods for albumin determination.[10] Furthermore, HAS' latest guidelines for the diagnosis and severity stratification of malnutrition in the general population recommend albumin measure by IT or IN but no longer by BCG which overestimates the concentration of albumin due to its interaction with alpha-1 and alpha-2 globulins.[5,11,12] However, analytical interferences with immunoassays are also described. Albumin carbamylation, an unavoidable consequence of excess urea in chronic kidney disease, negatively interferes with IT.[13] This may explain why the best hypoalbuminemia threshold with IT in the HD population was not 3.5 g/dL, but 3.4 g/dL. The albumin binding sites for BCP contain a lysine moiety susceptible to carbamylation, which may prevent BCP binding. In HD patients, this albumin modification lowered concentrations up to -0.6 g/dL,[14] in agreement with our observations. To circumvent difficulties of interpretation when albumin is determined by BCP in HD patients, we propose here to set the critical cut-off to 3.05 g/dL. Based on an analysis of 24,778 albumin concentrations determined by either BCP or BCG, Coley and Grant suggested lowering the reference range of 3.5-5.0 g/dL to

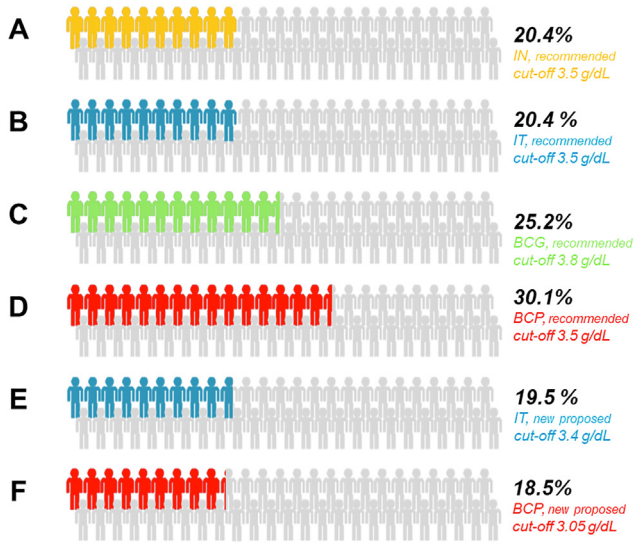

Figure 4. Schematic representation of malnourished patients in HD cohort (%) when albumin is measured by IN (yellow icons, A), BCG (green icons, B), and BCP (red icons, C) with the current recommended cut-offs for hypoalbuminemia (3.8 g/dL for BCG, 3.5 g/dL for IN and BCP). The same representation is applied when hypoalbuminemia is determined by IT and BCP, using the new proposed cut-offs: 3.4 g/dL for IT (blue icons, D) and 3.05 g/dL for BCP (red icons, E). BCG, Bromocresol Green; BCP, Bromocresol Purple; IN, Immunonephelometry; IT, immunoturbidimetry (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

3.1-4.5 g/dL. This adjustment resulted in comparable levels of hypoalbuminemia.[15] Interestingly, the lower limit proposed in this study is very close to the decision threshold we found for BCP. Using the new threshold at 3.05 g/dL, the rate of malnutrition is significantly reduced and comparable to that observed with IN. Concerning IT, since lowering the threshold by 0.1 g/dL does not improve the clinical classification of malnourished HD patients, we propose to maintain it at 3.5 g/dL. Although our results are crucial in routine nutritional HD management, there are some limitations. First, our study could be confirmed in an external and larger cohort of HD patients. Then, since albumin may be impacted by non-nutritional factors,[16] plasma albumin should be used in combination with other measures to assign malnutrition in patients with end-stage kidney disease.

Practical Application

This study aims to sensitize nephrologists and medical biologists to the analytical differences between colorimetric and immunological methods of albumin determination. The classification of malnourished patients should be improved by applying the appropriate hypoalbuminemia thresholds, adapted to the analytical method. This work could also be used as a reference for the establishment of guidelines for the nutritional management of HD patients.

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