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Albumin determined by bromocresol green leads to erroneous results in routine evaluation of patients with chronic kidney disease

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Abstract

Objectives: Measurement of plasma albumin is pivotal for clinical decision-making in patients with chronic kidney disease (CKD). Routinely used methods as bromocresol green (BCG) and bromocresol purple (BCP) can suffer from aselectivity, but the impact of aselectivity on the accuracy of plasma albumin results of CKD-patients is still unknown. Therefore, we evaluated the performance of BCG-, BCP- and JCTLM-endorsed immunological methods in patients with various stages of CKD.

Methods: We evaluated the performance of commonly used albumin methods in patients with CKD stages G1 through G5, the latter divided in two groups based on whether they received

hemodialysis treatment. In total, 163 patient plasma samples were measured at 14 laboratories, on six different BCG and BCP-platforms, and four different immunological platforms. The results were compared with an ERM-DA-470k-corrected nephelometric assay. The implications on outcome is evaluated by the proportion of patient results <38 g/L for the diagnosis of protein energy wasting.

Results: Albumin results determined with BCP- and immunological methods showed the best agreement with the target value (92.7 and 86.2 %, respectively vs. 66.7 % for BCG, namely due to overestimation). The relative agreement of each method with the target value was platform-dependent, with larger variability in agreement between platforms noted for BCG and immunological methods (3.2–4.6 and 2.6–5.3 %) as opposed to BCP (0.7–1.5 %). The stage of CKD had similar effects on the variability in agreement for the three method-groups (0.6–1.8 % vs. 0.7–1.5 % vs. 0.4–1.6 %). The differences between

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methods cause discrepancies in clinical decision-making, as structurally fewer patients were diagnosed with protein energy wasting upon using BCG-based albumin results.

Conclusions: Our study shows that BCP is fit for the intended use to measure plasma albumin levels in CKD patients from all stages, including patients on hemodialysis. In contrast, most BCG-based platforms falsely overestimate the plasma albumin concentration.

Keywords: analytical performance; analytical variation; chronic kidney disease; method comparison; plasma albumin

Introduction

Plasma albumin is an important biomarker for the diagnosis, treatment and prognosis in chronic kidney disease (CKD) patients (reviewed in 1). For instance, a lower plasma albumin concentration is associated with poor clinical outcomes in patients with all stages of CKD, including patients on dialysis and after kidney transplantation [2–6]. In dialysis patients, a decreasing albumin concentration is associated with poor nutritional status [7, 8]. In patients with nephrotic syndrome, prophylactic anticoagulation therapy is prescribed when plasma albumin is below 25 g/L [9]. Furthermore, a cut-off value of 38 g/L is used to diagnose protein energy wasting [10]. As the measurement of plasma albumin directly impacts clinical decision making, accurate evaluation of the plasma albumin concentration in all stages of CKD is pivotal.

In clinical practice, plasma albumin is commonly measured by the chromogenic methods of bromocresol green (BCG) and bromocresol purple (BCP) because of their automated 24/7 availability at relatively low cost. These methods however, both seem to suffer from aselectivity [1]. BCG-methods often overestimate plasma albumin due to alterations in plasma protein composition such as increased α 2-macroglobulin, haptoglobin, and immunoglobulins [11–16]. The magnitude of overestimation is variable as the mean bias differs between platforms in healthy persons and in patients with renal failure [16].

In contrast, BCP-methods outperform BCG-methods, both in healthy persons and in patients with nephrotic syndrome with normal renal function [1]. However, BCP-performance in haemodialysis (HD) patients is less conclusive; while some studies report a negative interference in patients on HD upon measuring plasma albumin with the BCP-methods [13, 17, 18], other studies report no effect [16, 19–24]. In this patient population, the presence of uremic toxins and/or albumin carbamylation can cause falsely decreased plasma albumin values [25]. The concentration of uremic toxins and the degree of protein carbamylation of plasma albumin is highly variable

during loss of kidney function and dependent on multiple factors [26].

For albumin, the primary reference measurement procedure according to the Joint Committee of Traceability in Laboratory Medicine (JCTLM) is the optimized immunonephelometric or -turbidimetric method [27]. Although immunological methods are highly specific, the suboptimal reference value transfer procedures in combination with the large uncertainty of the assigned value of European reference material ERM-DA-470k/IFCC result in considerable variation present among these methods [28]. Moreover, substantial lot-to-lot variation can be present, resulting in clinically significant bias in routinely used immunological systems [16, 29].

To the best of our knowledge, the performance of BCG-, BCP- and immunological methods in various stages of CKD has not been described before. In this study we investigated whether the performance of methods for measuring plasma albumin differs between patient populations at different stages of kidney function. Therefore, we have evaluated the performance of each method in individual samples of patients with stage G1–G5 CKD including HD, by comparing them to the JCTLM-endorsed reference method for plasma albumin. Furthermore, we have evaluated the impact of variation on outcome by using the clinical diagnosis of protein energy wasting.

Materials and methods

Patient inclusion

One hundred sixty-four participants were included from February 2020 until April 2021 representing various CKD-stages (G1, G2, G3a, G3b, G4, and G5; G5 consisted of patients on HD (G5-HD) and non-HD patients (G5)). A sample size of 30 participants per CKD-stage was selected based on a power-calculation (>0.8 – 0.95) aiming to detect a 1.4 % difference (i.e. the desirable bias based on biological variation) with an assumed within-method imprecision between 1.8 and 2.5 % (derived from external quality assurance (EQA) data of 2020). The medical ethical committee of the Radboudumc approved this study (File: 2019–5876). Informed consent was obtained from all participants. Lithium-heparin plasma was collected during routine phlebotomy at the Radboudumc. Demographic data and results for the biomarkers creatinine, estimated glomerular filtration rate (CKD-EPI, 2012 formula), urea, and urine protein to creatinine ratio (all determined on Cobas c702 analyzers from Roche Diagnostics, Basel, Switzerland) were derived from the electronic patient dossier (EPD). One patient with cryoglobulinemia was excluded, resulting in a final inclusion of 163 participants.

Patient sample handling and analysis

Blood samples were centrifuged for 10 min at 2000 g. Plasma was divided into 450 μ l aliquots, which were kept frozen at -80 °C until analysis. All patient samples underwent one freeze-thaw cycle [30].

Samples were distributed on dry-ice to 14 laboratories and analysed in single-fold on 16 unique platforms (21 platforms in total). Participating laboratories were selected based on their performance in the EQA programme of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML) in 2020 and the relevance of the platform (e.g. number of participants and/or emerging new platform) [31].

Aliquots were measured on six platforms using the BCP-method from four manufacturers: two Siemens platforms (Advia and Atellica, Siemens Healthineers, Marburg Erlangen, Germany), one Beckman Coulter DxC700 platform (Beckman Coulter, Krefeld, Germany), one Abbott C16000 platform (Abbott, Chicago, IL) and five Roche platforms (one Cobas c503 and four c702, Roche Diagnostics, Basel, Switzerland). Aliquots were measured on six platforms with BCG-methods from three manufacturers: two Abbott (Alinity and C8000), one Beckman Coulter AU 5800 and three Roche (one Cobas c501/c502, one Cobas c503 and two Cobas c702). A total of four platforms from two manufacturers were used for the immunological analysis: one nephelometer (Image 800, Beckman Coulter) and four turbidimeters (two Cobas c501/c502, one Cobas c503 and one Cobas c702, Roche). Internal quality control data was provided by the participants, with the long-term imprecision for each platform described in Supplemental Table 1.

Evaluation of method performance

The within-method performance in individual patients was evaluated by calculating the difference between the obtained value of each patient with the target value as determined by the reference procedure (described below). The range in obtained differences was calculated per method by subtracting the minimal obtained value from the maximum obtained value per patient.

To evaluate the effects of platforms and CKD-stage on the plasma albumin results, the between-CKD-stage variation and between-platform variation were computed. First, we calculated the mean relative deviation for each unique combination of CKD-stage, method, and platform. Thereafter, the between-CKD-stage variation was calculated by estimating the standard deviation of the obtained mean relative differences per method/platform. In contrast, the between-platform variation was calculated by estimating the standard deviation of the mean obtained relative differences per CKD-stage.

The analytical performance specifications were specified by using the total allowable error (TEa) based on the within- and between-subject biological variation [32]. We calculated the desirable total allowable error by summing the uncertainty of the target value (u_{ref} , described below) of 3.25% with the TEa of 3.4% as established by $1.65 \times 0.5 \times CV_i + 0.25 \times \sqrt{CV_i^2 + CV_g^2}$, resulting in a desirable TEa of 6.65% [33].

Albumin reference measurement procedure

For the determination of the target value of plasma albumin, the reference method “Optimized immunoturbidimetry/immunonephelometry” as listed by the JCTLM was used in combination with the ERM-DA-470k/IFCC as the JCTLM-listed reference materials [27, 34]. Target albumin values for all samples were determined using an Atellica Neph630 Nephelometer (Siemens Healthineers, Erlangen, Germany) calibrated with the Siemens calibrator according to the manufacturer’s instructions. To correct for any deviations in the metrological traceability, ERM-DA-470k/IFCC was measured in twelve-fold. Subsequently,

the relative recovery of ERM-DA-470k/IFCC was used to correct the patient albumin values. We evaluated this method by assessing that the ERM-DA-470k-calibrated-and-corrected results were proportionally related to the ERM-DA-470k-corrected results, with an $R^2 > 0.99$ for both control materials and patient samples.

We calculated u_{ref} by deducing the square root of the sum of squares of the uncertainty of the assigned value of ERM-DA-470k/IFCC (1.2 g/L) with our within-run-imprecision (0.14 g/L, derived from 12-fold repeated measurements of ERM-DA-470k/IFCC during our target-value determination run). The u_{ref} (i.e. the uncertainty applicable to the target results of patients) was 1.21 g/L, or 3.25%.

We have verified commutability of the original ERM-DA470 on the AtellicaNeph630 by using the IFCC-endorsed method [35, 36]. We verified that the found bias between the Behring BN II (Dade Behring, Deerfield, Illinois, USA), of which commutability has been established, and the Atellica Neph630 is equivalent for patient samples and ERM-DA470 when the uncertainty of the bias is taken into consideration (data not shown). The results of that study were assumed to be valid for the currently used ERM-DA470k/IFCC as both materials are produced “in a similar manner” [37].

EQA assessment

The performance of the platforms used to measure plasma albumin in the Netherlands was assessed by using the EQA data of 2021 from the “Clinical Chemistry in Blood” and “Plasma Proteins surveys” from the SKML. Albumin results with a target value between 30 and 50 g/L as determined with the albumin reference procedure, were included in the analysis. In total, 23 platforms from four manufacturers were included in the data: four from Abbott (Alinity c, and Architect C4000, C8000 and C16000), nine from Beckman Coulter (AU 400, 480, 5800, 680, 700, DxC600, DxC700 AU, DxC800, and the Image), three from Roche (Cobas c501/502, c503 and c702), and seven from Siemens (ADVIA, Atellica CH, Dimension Vista, Advia XPT, AtellicaNeph630, Behring BN II and BN ProSpec). In addition, the preceding EQA sample relative to the measurements of the G1-patient cohort against the target value as determined by the ERM-DA-470k/IFCC-based albumin reference procedure.

Evaluating impact of albumin variation on clinical decision

The impact of the variability in obtained albumin results on clinical decision-making was evaluated using the cut-off value of 38 g/L for the diagnosis of protein energy wasting [10]. Per CKD stage, we established the proportion of patients below 38 g/L. However, the obtained target values are accompanied with uncertainty (i.e. 3.25%), which influences the calculated proportion of patients with results below 38 g/L. Therefore, we established the ‘target range’ of proportions per CKD stage by calculating the proportion of patient values below 38 g/L at the 2.5 and 97.5 percentile with an uncertainty of 3.25%. We compared these target ranges with the proportion of patient results below 38 g/L based on the laboratory results, and established the number of laboratories that obtained results outside the range.

Statistical analysis

The statistical analysis was performed in R (version 4.1.2), using ggplot2 (version 3.3.6) and tidyr (version 1.2.0) packages [38, 39]. The assessment

of overall significance in patient differences between groups was performed using Kruskal-Wallis rank sum (K-W-test) tests, whereas post-hoc groupwise comparisons were achieved using Wilcoxon-rank tests with Bonferroni correction (W-R-groupwise). The evaluation of significance in proportion of results outside the allowable performance between results with a target value of <40 or ≥ 40 g/L was evaluated by a chi-squared test, whereas for the evaluation of significance between variances, the F-test was used.

Results

Patient characteristics

Patient characteristics are shown in Table 1. As expected, patients with more severe CKD-stages were significantly older ($p < 0.0001$, K-W-test, Table 1). The male to female ratio was similar between the stages of CKD ($p = 0.139$, W-R-groupwise). The concentration of plasma albumin was higher in patients in early stages of CKD compared to patients with more advanced renal failure (G1 and G2 vs. G5-HD, $p < 0.05$, W-R-groupwise, Table 1). The median results of plasma urea and urine protein to creatinine ratio increased significantly with declining kidney function ($p < 0.005$, K-W-test, Table 1).

Albumin method performance in patients with CKD

First, the performance of BCG-, BCP- and immune-based albumin methods in individual CKD patient samples was

evaluated on 21 platforms in total ($n = 3040$ individual results, Figure 1A). Patient results ranged from -16.3 to $+18.9$ % of the target value, indicating substantial dispersion. Of the three method groups tested, plasma albumin results determined by BCP- and immunological methods were in best agreement with the target value (92.7 and 86.2 % of all individual results within allowable limits, respectively), compared to 66.7 % of individual results obtained by BCG-methods (Figure 1A). Specifically for BCG-methods, the proportion of results exceeding the allowable limits increased as the target albumin value decreased: 46.0 % of samples with a plasma albumin value below 40 g/L were reported outside allowable limits, vs. 22.6 % of results with albumin values above 40 g/L ($p < 0.0001$, chi-squared). The maximum difference obtained within one patient was lowest for BCP (5.4 g/L), whereas immunological methods and BCG had a similar maximum variation (8.3 and 8.2 g/L, Figure 1B).

Next, we assessed whether the within-method variation could be explained by CKD-stage or between-platform differences. Therefore, the albumin results were stratified per CKD-stage and per type of platform. For albumin concentrations determined by BCG, there was a significant variation between the platforms (Figure 2A). Whilst the median albumin levels determined by Roche Cobas c501/c502 platform and Abbot Alinity and C8000 were within allowable limits for all CKD stages, the median albumin value of one or more CKD-stages determined on the Beckman Coulter AU5800, Roche Cobas c503 and c702 platforms showed a positive bias outside allowable limits (Figure 2A). Moreover, the between-platform variation ranged from 3.2 to 4.6 %, exceeding the between-CKD variation which

Table 1: Patient characteristics stratified per CKD-stage. Significant differences are indicated with p-values. Data is presented as median [min–max].

		G1 (n=17)	G2 (n=28)	G3a (n=25)	G3b (n=31)	G4 (n=30)	G5 (n=18)	G5-HD (n=14)	p-Value (K-W-test)
Demographic	Age, years	32 [22–70]	55 [24–80]	65 [25–75]	64 [27–86]	67 [24–82]	73 [57–90]	73 [52–86]	<0.000001
	Female, %	50 %	32 %	48 %	55 %	43 %	17 %	57 %	0.1393 (NS)
	Plasma albumin, g/L	42 [31–50]	42 [38–48]	40 [32–47]	41 [31–46]	40 [28–45]	39 [33–43]	36 [31–44]	0.0001835
Laboratory parameters	Creatinine, $\mu\text{mol/l}$	75 [48–90]	94 [72–124]	121 [84–162]	151 [104–179]	242 [165–380]	458 [276–801]	654 [507–923]	–
	eGFR, CKD-EPI, 2012, mL/min/1.73 m ²	>90 [90->90]	72 [61–89]	51 [45–59]	37 [30–44]	22.5 [15–28]	10 [5–15]	NA	–
	Urea, mmol/L	5.0 [3.8–7.8]	7.0 [4.2–9.9]	7.4 [4.7–15]	11.4 [6.7–17.6]	17.6 [8.6–29.6]	25.3 [14.1–35.6]	21.4 [6.9–31.9]	–
	Protein-creatinine-ratio, g/10 mmol	0.15 [0.08–2.43]	0.21 [0.04–2.26]	0.18 [0.06–2.76]	0.38 [0.06–5.36]	0.38 [0.08–6.29]	1.26 [0.18–6.29]	NA	–

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate as determined with the CKD-EPI:2012 formula; NA, not available.

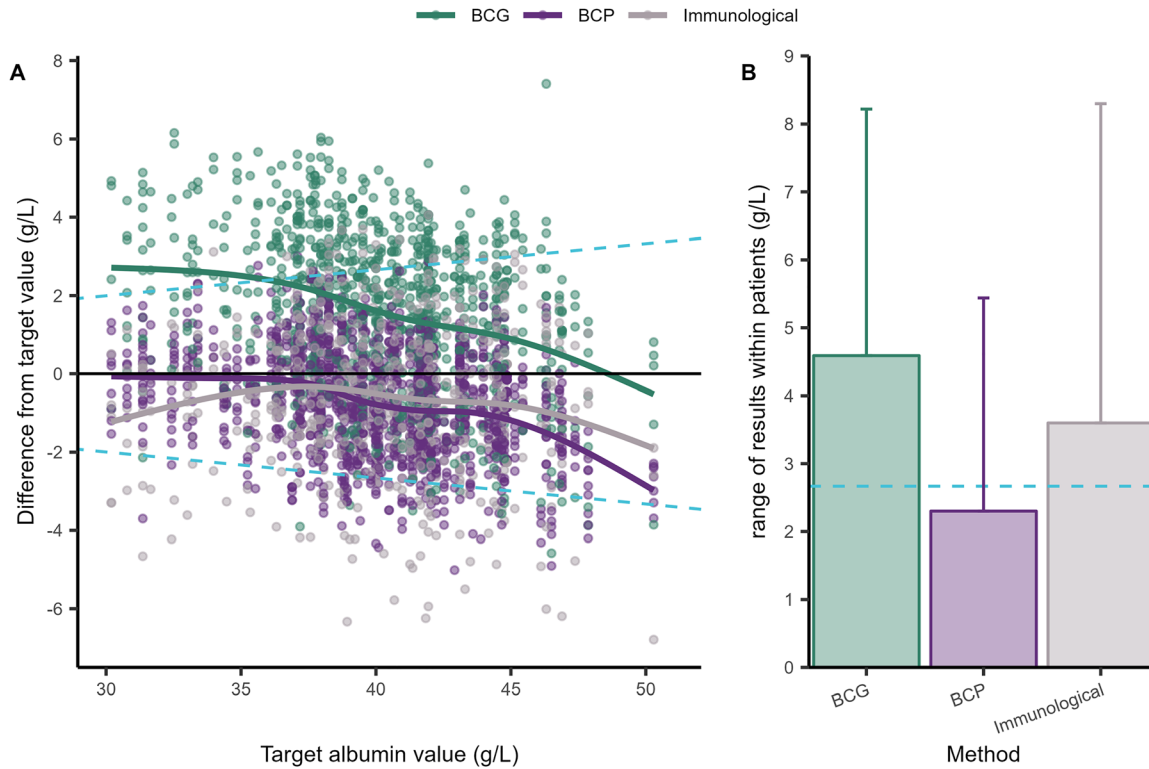


Figure 1: (A) Plasma albumin difference from the target value of individual CKD patients. Dashed lines indicate the desirable total allowable error of 6.65 % around the target value (---). The green, purple, and grey solid lines indicate the average deviation compared to the target value for BCG-, BCP-, and immunological methods, respectively. (B) Maximum between-platform difference obtained for each patient. The data is stratified for BCG, BCP and immunological methods (shown in green, purple, and grey, respectively). The dashed horizontal line indicates the TEa of 6.65 %. Error bars indicate the maximum. BCG: bromocresol green, BCP: bromocresol purple.

ranged from 0.6 to 1.8 % (Supplemental Figure 1A and B). This indicates that between-platform variation is the major contributor to the within-method variation for BCG in individual patients.

In contrast to BCG-based platforms, all BCP-based platforms produced median albumin results that were within the allowable limits for all CKD-stages (Figure 2B). For BCP-methods, the between-platform variation ranged from 0.7 to 1.5 %, which was significantly lower than BCG-methods ($p < 0.01$, F -test), whereas the between-CKD-stage variation was similar to BCG-methods (1.0–1.7 %, Supplemental Figure 1A and B). Taken together, the between-CKD-stage and between-platform variation contributed equally to the within-method variation obtained for BCP-methods.

For immunological methods, all three Roche platforms measured the median plasma albumin concentration within allowable limits (Figure 2C). In contrast, albumin was underestimated in patients when determined by the Beckman Coulter Immage platform, which was most pronounced in G5 patients on HD (Figure 2C, median deviation of -9.7 %). The between-platform variation of the

immunological assays tested (ranging from 2.6 to 5.3 %) was similar to BCG, most probably caused by the negative bias of the Beckman Coulter Immage. The CKD-stage dependent variation of immunological methods ranged from 0.4 to 1.6 % and was similar to the colorimetric methods (Supplemental Figure 1A and B).

Concludingly, the between-platform variation is larger for BCG and immunological methods compared to BCP-methods, while all methods showed a similar between-CKD stage variation. Thus, next to the method used, plasma albumin results are affected by the used platform, irrespective of the CKD-stage of the patient.

Albumin method performance by external quality assessment

In order to evaluate whether the between-platform deviation in the CKD cohort represents a general deviation for the included platforms, the performance of plasma albumin measurements on the vast majority of platforms used in the Netherlands was evaluated using SKML-EQA results.

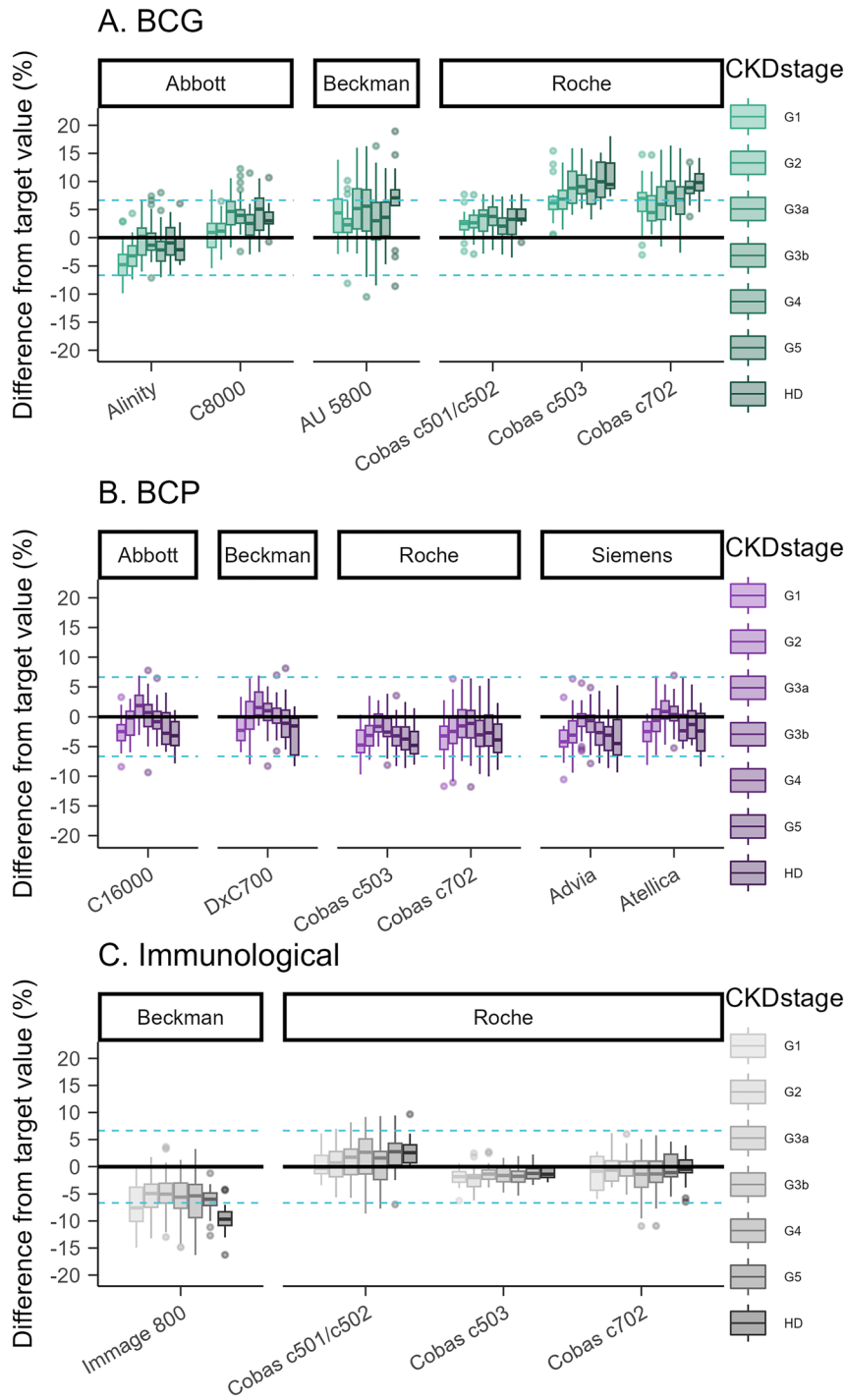


Figure 2: Albumin deviation per CKD-stage and analyzer-platform. Samples are depicted per CKD-stage, and stratified per method ((A) BCG, (B) BCP and (C) immunological methods). Horizontal lines indicate the desirable total allowable error (– – –). Error bars indicate 1.5 times the inter-quartile ratio. BCG: bromocresol green, BCP: bromocresol purple.

In 2021, 101 laboratories with 190 platforms participated in the EQA scheme for Clinical Chemistry in Blood, whereas 34 laboratories with 45 platforms participated in the EQA scheme for Plasma Proteins. The target value of the EQA samples was assigned using the target evaluation method for the patient samples. In these schemes, commutability is assessed yearly by comparing the variability of native patient samples (i.e. a spy sample) with the ‘nearest neighbour’ EQA sample.

The median albumin levels of EQA results obtained by BCG-, BCP-, and immunological platforms all were within allowable limits (Figure 3A–C). Additionally, the performance of the EQA-sample preceding the patient measurements coincides with the median obtained performance within patient samples (Figure 3A–C, squares vs. diamonds respectively). However, although the between-platform variation obtained by EQA results showed a similar trend as found upon assessing patient samples, the EQA-based between-platform

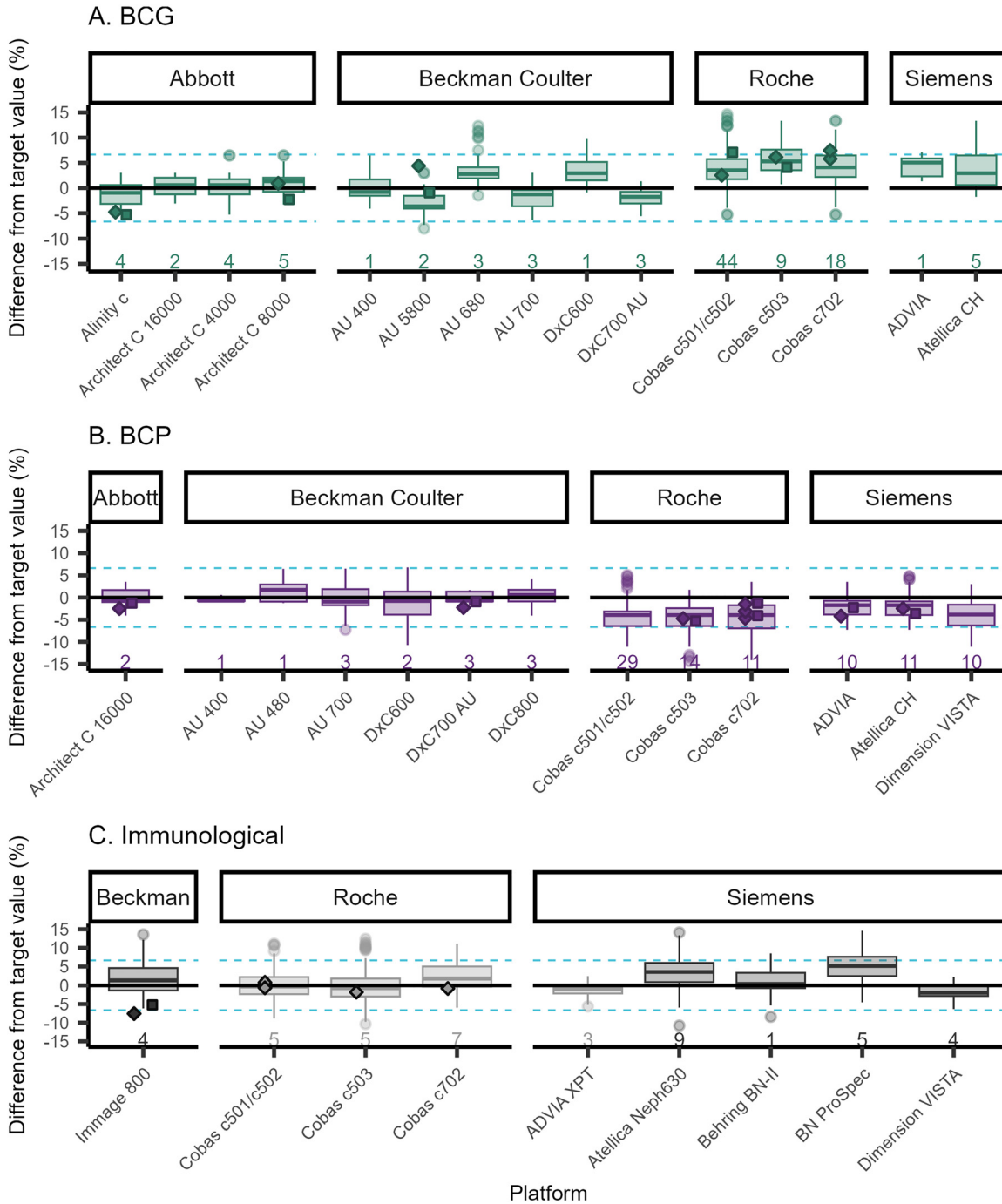


Figure 3: External quality assurance (EQA) performance of laboratories, stratified per method ((A) BCG, (B) BCP and (C) immunological methods). The median obtained patient deviation of patients from CKD-stage G1 from Figure 2 is presented as a diamond, with the result of the preceding EQA sample indicated as a square. The relative deviation from the target value was evaluated per analyzer platform. Horizontal lines indicate the desirable total allowable error (---). Numbers indicate the number of analyzers. Error bars indicate 1.5 times the inter-quartile ratio. EQA: external quality assurance, BCG: bromocresol green, BCP: bromocresol purple.

variation was larger than the CKD-sample-based between-platform variation for BCP- and immunological platforms, whereas BCG-platforms showed a lower between-platform variation for EQA as opposed to CKD-samples (Supplemental

Figure 2). When comparing EQA-based between-platform variation, a similar result to the patient study is observed: BCP-methods had a lower between-platform variation (ranging from 1.5 to 3.5 %) compared to BCG-methods (2.3–

3.4 %). However, both colorimetric methods outperformed the immunological methods, which showed substantial between-platform variation: nephelometry ranging from 2.3 to 8.2 % and turbidimetry ranging from 3.1 to 6.4 %. Taken together, we conclude that EQA-derived performance is optimistic in respect to the method-performance obtained with CKD patient samples but shows a similar trend.

Evaluating impact of albumin variation on clinical decision

To evaluate the clinical implication of the analytical variability, the proportion of patient results below 38 g/L as cut-off for diagnosing protein energy wasting was evaluated per CKD stage. As kidney function deteriorates, the proportion of patients diagnosed with protein energy wasting increases, and was highest in G5 patients on HD (56.5 %) (black bars in Figure 4). The number of laboratories that obtained proportions of patients below 38 g/L that are outside the ‘target range’ (as estimated using the 2.5 and 97.5 percentile using 3.25 % uncertainty) is indicated in Figure 4. At least 40 % of laboratories using BCG obtained substantially lower proportions of patients with protein energy wasting, in any CKD-stage, except for G2. In contrast, laboratories that use BCP would find substantially higher incidence of patients with protein energy wasting, namely in patients with CKD stages G1 and to a lesser extent in G5-HD patients. Moreover, utilizing immunological methods may also cause increased dietary referral rates, as 20 % of laboratories referred substantially

more patients in CKD stage G1, G3b, G5, and G5-HD. Taken together, we note that, depending on the platform, BCG-methods may structurally underestimate the proportion of patients that could benefit from dietary intervention across the entire CKD stage spectrum, whereas for BCP and immunological platforms, the effects are not as widespread.

Discussion

We describe the performance of BCG-, BCP-, and immunological methods for plasma albumin in individual patient samples at different stages of CKD. Of the three methods available in routine medical laboratories, BCP-platforms were most accurate in all stages of CKD, including HD patients. However, large between-platform variation of BCG-methods led to substantial and differential bias in patient results. Interestingly, immunological methods reported the majority of patient results within allowable limits despite the large between-platform variation present. Moreover, the between-CKD-stage variation for all three methods was low and similar to one another, indicating that the different methods are equivocally affected by CKD-stage specific factors. Thus, either BCP- or immunological methods can safely be used for the determination of plasma albumin in the follow up of patients with progression of kidney failure, including patients on HD.

The large between-platform variation for BCG-methods we describe is consistent with earlier reports of Bachmann et al., who showed a large variability in positive bias between the average results obtained by different BCG-platforms in HD pooled plasma and healthy persons [16]. Additionally, other reports in patients with kidney disease are inconsistent in the amount of positive bias found, with reported mean biases ranging from -1.0 to $+7.3$ g/L [1]. As the majority of these studies each compared only one BCG-platform to one of the JCTLM-listed immunological assays, the choice of either platform could be the main contributor to the variation in reported bias in BCG-methods [40].

Previous studies report a variable performance of BCP-methods in HD patients, which is caused by uremic toxins and/or high urea levels that interfere with the binding of BCP-methods to plasma albumin [11, 13, 16, 18–22, 25, 41, 42]. However, the average bias reported in HD-patients ranged from -6.1 to $+1.2$ g/L, which possibly reflects the between-platform variation by the used reference methods. Additionally, Bachmann et al. found a similar low between-platform variation for BCP-methods as our study [16]. Another explanation for the limited bias of BCP-methods in HD-patients compared to previous studies may be the introduction of high-flux membranes in hemodialysis treatment, which are superior to low-flux membrane in terms of uremic toxin filtering

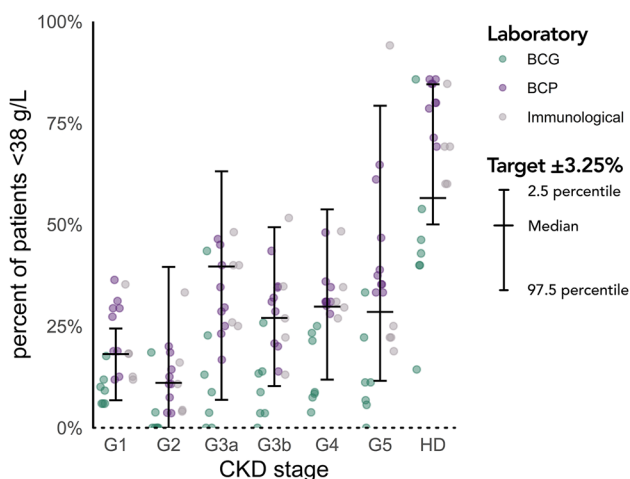


Figure 4: Clinical implications of using assays on proportions of patients referred for dietary intervention in regards to protein energy wasting, per CKD stage. The vertical error bars indicate the median and 2.5 and 97.5 percentile of obtainable proportions of patients using the uncertainty of $\pm 3.25\%$. In green, purple and grey the proportion of patients <38 g/L is indicated per laboratory. BCG: bromocresol green, BCP: bromocresol purple.

capacity, and thereby reduce the source of negative bias in BCP-methods [43]. Taken together, our study shows that currently employed BCP-methods do not suffer from clinically significant bias, and can be safely used for routine patient diagnostics for the follow up of patients with every CKD-stage, including patients on HD.

Our study showed a considerable variation between immunological methods, as patient results deviated between -16.3 and $+9.7\%$ from the target value. Similarly, the EQA-based performance confirmed this large variation between immunological platforms, exceeding the between-platform variation of both BCG and BCP. Between-reagent lot variation is a recognized pitfall of immunological methods, possibly caused by a combination of a large uncertainty of reference material ERM-DA-470k/IFCC and differences in the value assignment process between manufacturers and/or platforms. A particularly negative bias in patient- and SKML-EQA results was observed in the participating Beckman Coulter Immage. As EQA results of Immage users are generally within allowable limits, we note that the observed bias is due to temporary bias of this laboratory and does not reflect the Immage users in general (Figure 3C). Nevertheless, temporarily negative bias may strongly hamper adequate follow up of patients over time and stresses the need for commutable EQA materials at clinically relevant concentrations. These results clearly show the relationship between a momentary deviation and the effect on patient samples, and remind us that even though the JCTLM endorses nephelometry and/or turbidimetry-based immunological methods as the reference measurement procedure for the detection of plasma albumin, the quality of immunological methods should be closely monitored [27].

Next, we would like to stress that, despite the fact that the differences in performance have been known for over 50 years, BCG and BCP are still both used in routine practice today. In fact, survey analysis has shown that 76% laboratories (respectively 78.4 and 73.7% of BCG- and BCP-laboratories) have adopted identical lower reference intervals of 35 g/L (data not shown). The consequences of adopting identical clinical decision-limits when assays are clearly deviant from one another can negatively affect patient care. For example, our data shows that half of the patients requiring dietary intervention due to protein energy wasting may be missed when using BCG to determine albumin. For BCP, laboratories obtained plasma albumin values that would lead to more patients with dietary intervention in CKD stages G1 and G5 on HD. However, the diagnosis of protein energy wasting is namely relevant in patients with more severe loss of kidney function. Furthermore, the ongoing process of healthcare decentralization and the incentive to enable exchange of laboratory data may impede clinical decision-making if data from analytically

different systems are used interchangeably. Taken together, we stress that a decisive discontinuation of methods that are not fit for purpose is not only preferred, but also that the continuation of current practice is a disservice for nephrology patient care.

Although it could be seen as a limitation of our study that we did not explicitly confirm the commutability of ERM-DA470k/IFCC on the AtellicaNeph630 platform from Siemens, we believe that the established commutability of the original ERM-DA470 between the Behring BN II and the AtellicaNeph630 justifies the assumption of commutability of ERM-DA470k/IFCC, as both materials were produced in similar fashion [37]. In the unlikely event that ERM-DA470k/IFCC would be non-commutable, our conclusions regarding the between-method variability and the impact on protein energy wasting are still valid as these calculations rely on direct method comparison and therefore do not depend on commutability of the calibrator.

To our knowledge, this is the first study that analysed individual patient samples at different stages of CKD against JCTLM-listed reference methods and materials to establish the performance of BCG-, BCP-, and immunological methods. Firstly, we note that substantial variation between immunological methods occurs, and endorse efforts to further optimize standardisation of human plasma albumin measurements by immunological methods. As our results confirm earlier illustrations of positive bias and showcase differential bias between BCG-platforms in patients with various stages of CKD, we endorse the discontinuation of BCG-methods in its current form for use in nephrology care, and recommend reconsideration of decision limits derived with BCG-methods. In contrast, we found no evidence of clinically relevant bias in any of the BCP-methods, indicating that BCP-based methods are fit for use in patients regardless of kidney function, including patients on hemodialysis.

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