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Original Article

Albumin as an outcome measure in haemodialysis in patients: the effect of variation in assay method

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Abstract

Background. Serum/plasma albumin is an important predictor of future mortality/morbidity in haemodialysis (HD) patients and has been proposed as an important audit measure. Different methods of albumin assay give different results and the bias between methods may be greater in renal failure patients.

Methods. Albumin concentration in plasma was measured by three methods, two dye-binding methods (bromocresol green (BCG) and bromocresol purple (BCP)) and an immuno-turbidimetric (ITM) method, in 143 HD patients (group I) and 49 non-renal patients (group II). Comparisons were made between means, variation in differences across a range of albumin concentrations and on the percentage of patients within the normal range.

Results. In HD patients (group I), BCG overestimated plasma albumin compared with the other two methods. The difference could be as much as 10 g/l and was more marked in hypoalbuminaemic patients. The BCP method gave results closer to the ITM method, particularly in HD patients. These differences were less marked in group II patients but both methods overestimated albumin compared with the ITM method. Using the BCG local laboratory normal range, 84% of HD patients had plasma albumin concentrations within the normal range but this fell to 57% if the BCP results were used.

Conclusions. The method for determining albumin concentration has a marked effect on the results particularly in HD patients. BCG, the most commonly used method, gives higher results than other methods and correlates poorly with an immunological method. These differences make comparative audit between nephrology units difficult and have implications for other biochemical variables and other specialties.

Keywords: albumin; audit; haemodialysis; outcome measure

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Introduction

Serum/plasma albumin is an important predictor of future mortality/morbidity in patients with renal failure [1,2]. This predictive power is a reflection of the effect of inflammation (albumin is a negative acute phase reactant) and malnutrition on albumin concentration [3]. Hypoalbuminaemia is a marker for patients who are unwell for other reasons (e.g. malignancy, infection, severe vascular disease), who are malnourished or both. There are no data to prove that interventions directed to raising serum albumin could improve the outcome in these patients. A number of authorities have recommended the audit of albumin concentrations as an outcome measure of dialysis treatment. The Renal Association standards document [4] recommends that serum albumin should be 'within the normal range quoted by the local pathology laboratory' and this has been used as an important outcome measure in the first report of the UK Renal Registry [5]. The use of the local range recognizes that a number of different assay methods are used for measuring albumin and that these methods give different results [6]. We have noted that there are often major differences in albumin measurements between two laboratories routinely analysing samples from our haemodialysis (HD) patients. These laboratories use different assay methods but have similar normal ranges. However, we have found consistently that albumin concentrations, determined by the bromocresol purple (BCP) dye-binding method, are lower than the bromocresol green (BCG) dye-binding method. These differences have been reported in renal failure patients before [7–10]. In this study, we sought to characterize the magnitude of the difference in a larger population, compared with a 'gold standard' method for albumin assay based on an immunological method. We also assessed the effect of different assay methods on the use of albumin as an outcome/audit measure in HD patients.

Patients and methods

Samples were collected from 143 HD patients (group I) by collecting excess plasma from samples drawn pre-dialysis for

routine monthly biochemical testing. In the central laboratory at Leicester General Hospital, plasma samples are used for albumin assay and blood samples are collected in lithium heparin tubes. Plasma samples were also collected from 49 anonymous non-renal patients (group II) whose serum samples were analysed in the routine chemical pathology laboratory in the same week. These samples were selected to have serum creatinine <120 μmol/l and a range of albumin concentrations.

Plasma was analysed using three different methods for albumin measurement. The BCG dye binding assay was performed on fresh plasma using a dry chemical method (Vitros ALB slides). The quoted laboratory normal range is 35–55 g/l. The coefficient of variation (CV) was 2.3–3.9% within batch. The BCP assay was performed on a Beckman CX7 analyser. The laboratory normal range of 35–52 g/l was based on data from the manufacturers modified according to results from the regional Protein Reference Unit and from the results of 400 requests for liver function tests from primary care physicians. Within batch, CV was 2% and between batch CV ranged from 2.9 to 4.0% across a range of albumin concentrations. The immunoturbidimetric (ITM) albumin assay was performed on a Cobas Fara Analyser using an anti-human albumin antibody (Incstar Ltd, UK). The CV for high and low albumin concentrations were $<5\%$ within and between batches. Samples for both BCP and ITM method had been stored for up to 2 weeks at -20°C. The laboratories using the dye binding methods participate in the UK National External Quality Assurance Scheme for albumin measurement.

The results are shown as means, standard deviations (SD) and percentage within range for dye-binding methods. The bias and 95% limits of agreement were calculated for differences between dye-binding methods and the ITM method. The bias is the mean difference between the methods and the 95% limits of agreement are the range covered by the mean difference ± 2 SD of the difference [11]. The correlation plots of dye binding and the immunoturbidimetric results (taken as reference method) are shown although correlation coefficients were not calculated. To determine whether the differences between methods varied at different albumin concentrations, we have also plotted the difference between paired methods against the mean value of these two methods [12].

Results

The mean concentrations and SD for plasma albumin in both groups measured by the three methods are shown in Table 1. A difference in the percentage of patients with albumin within the normal range were observed with only 57% of measurements in group I within the normal range for the BCP method compared with 87% for the BCG. In group II, 84% of subjects were within the normal range for both methods.

Table 1. Mean, SD and percentage within normal range for different assay methods

	Group I ($n=143$)			Group II ($n=49$)		
	BCG	BCP	ITM	BCG	BCP	ITM
Mean	38.4	34.9	33.7	38.1	38.4	34.8
SD	3.6	4.5	4.6	5.3	6.8	6.9
% in normal range	87	57	N/A	84	84	N/A

The BCG method demonstrated a positive bias for albumin compared with other methods. This affected all subsets but was most marked for HD patients and those with hypoalbuminaemia (<35 g/l) (Table 2). Albumin could be over-estimated by up to 10 g/l using BCG (assuming the ITM method as 'gold standard') and the error increased in patients with the lowest albumin concentrations (Fig. 1B). The BCP method gave results closer to the ITM method in group I patients even when albumin was <35 g/l (Table 2). The average difference between both dye-binding methods and ITM method was similar in group II patients. The difference between BCP and ITM methods did not show any trend over the range of albumin concentrations (Fig. 2B).

Discussion

Despite the importance of albumin as an outcome-measure in dialysis patients, comparatively little attention has been paid to the inherent difficulties in albumin measurements. The use of local normal ranges as suggested by the Renal Association [4] is designed to take account of different assay methods. The UK Renal Registry has considered this problem in detail in its first report [5].

The accurate measurement of serum albumin has exercised clinical biochemists for over 30 years. The development of automated dye binding methods utilizing BCG was hailed as a significant advance [13]. However, within 14 years, one of the workers who developed the method was so concerned about inaccuracies, due to non-specific binding to other plasma proteins, that he recommended Chemical Pathology departments should no longer use this method [14]. However, this continues to be the favoured method in the UK and other countries.

A number of studies have looked at this problem in patients with renal failure but not all have reached similar conclusions. Some studies of small numbers of HD and renal transplant patients in the 1980s reported that the BCP method gave falsely low values for albumin concentrations and postulated that a uraemic toxin interfered with BCP-albumin binding [7–9]. However, a larger study from the USA found a better correlation between the BCP method and an immunonephelometric method and highlighted that quality assurance programmes using albumin as an outcome measure needed to consider the implications of different methodologies [10]. Our results confirm and extend this work. BCG overestimates albumin and the biggest differences are in hypoalbuminaemic HD patients. In HD patients, BCP shows a much smaller difference compared with the reference method. In non-renal patients, both dye-binding methods show similar differences to reference method across a range of albumin concentrations.

Table 2. Comparison of bias between dye-binding and ITM methods

	Bromocresol purple		Bromocresol green	
	Bias	95% limits	Bias	95% limits
All ($n=192$)	1.8	−2.4; +6.0	4.4	−0.8; +9.6
Group I ($n=143$)	1.2	−2.6; +5.0	4.8	−0.2; 9.8
Group II ($n=49$)	3.6	+0.8; +6.4	3.2	−2.0; +8.4
Alb <35 g/l ($n=100$)	2.1	−1.5; +5.7	5.7	+1.3; +10.1
Alb >35 g/l ($n=92$)	1.5	−2.9; +5.9	2.9	−1.5; +7.3
Group I Alb <35 g/l ($n=81$)	1.7	−1.6; +5.0	5.8	+1.8; +9.9
Group II Alb <35 g/l ($n=19$)	3.7	+0.5; +7.0	5.0	−1.0; +11.0

Values are average differences and 95% limits of agreement (see text) comparing dye-binding methods to the ITM method. Division into high and low serum albumin is based on ITM assay result.

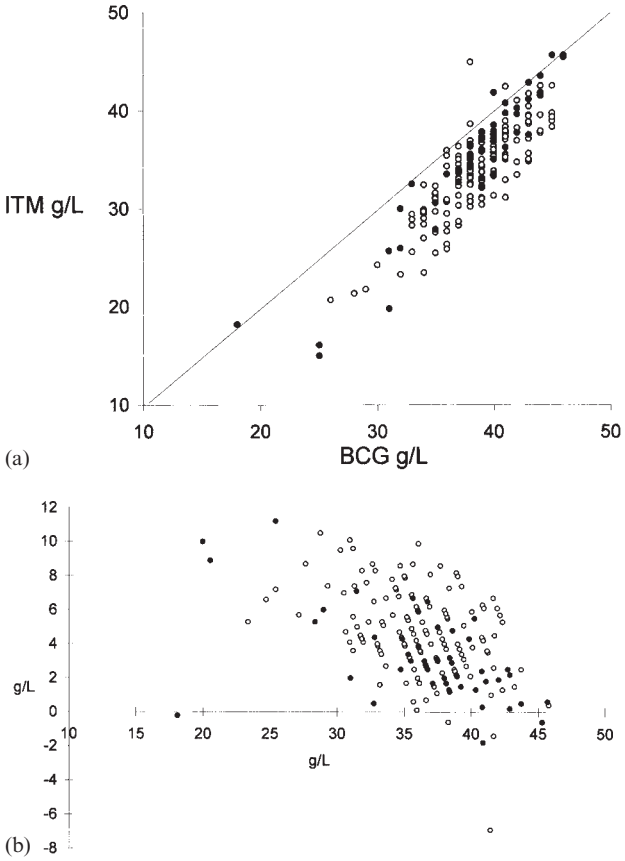

Fig. 1. Comparison of albumin concentrations measured by BCG and ITM methods. (**A**) Correlation plot and (**B**) plot of difference *versus* average value [12]. Group I patients represented by open symbols; group II by closed symbols.

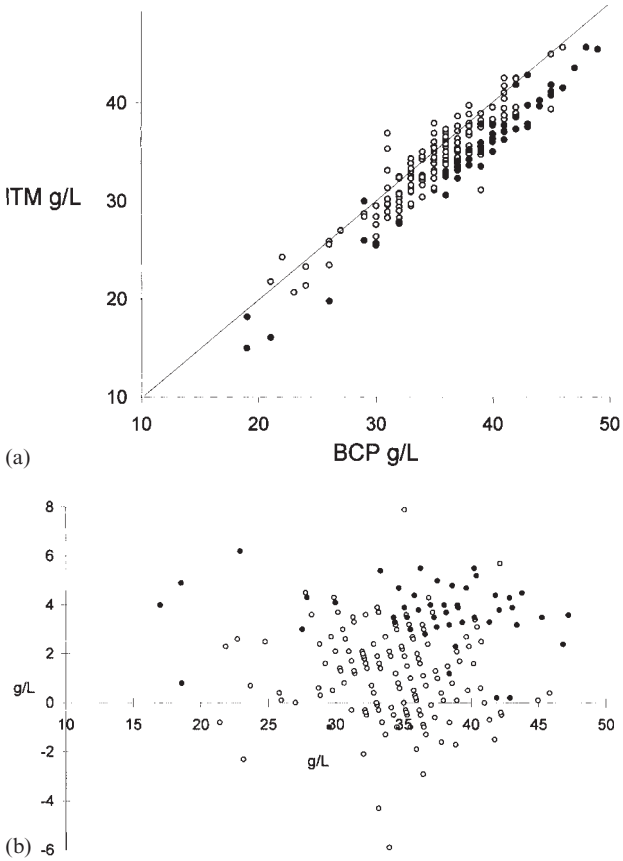

Fig. 2. Comparison of albumin concentrations measured by BCP and ITM methods. (**A**) Correlation plot (**B**) and plot of difference *versus* average value [12]. Group I patients represented by open symbols; group II by closed.

Therefore, the method of albumin assay has a major influence on results in HD patients and the normal ranges as defined in the normal population may not be applicable to renal failure patients. The use of the BCG method may lead to a systematic overestimation of albumin in dialysis patients, particularly in hypoalbuminaemic patients—the very group at risk and those where accurate measurement is most important. The difference in albumin concentrations using the two methods is not linear across the range of albumin concentrations; therefore, a correction factor can not be applied to a group of patients. This has important implications for day-to-day patient care and for comparative audit if different methodologies are compared.

In this study, the normal ranges for both dye-binding methods had the same lower limit (35 g/l). Many studies quote a lower range often down to 30 g/l for the BCP method. If a lower limit of 30 g/l were used, 92% of the HD patients would be above this value, similar to the 87% within normal range for the BCG

method. However, this does not account for the discrepancies between the two groups. For non-renal patients, similar percentages were within the quoted normal ranges for both methods. The explanation for this is not clear but it may be that non-specific binding to BCG by other proteins is increased in uraemic plasma while the binding of BCP is less affected.

These results have important implications. Nephrologists may be lulled into a false sense of security by the relatively good results afforded by BCG methods. Inaccurate albumin measurements will also lead to inaccurate calcium measurements due to the adjustments made for low serum albumin, precisely the group with the biggest error. This leads to underestimation of adjusted serum calcium with subsequent increases in vitamin D supplements and risks of metastatic calcification and calciphylaxis. Other medical specialties may be affected when albumin measurements may be an important part of assessment of liver disease, inflammatory bowel disease, systemic inflammation and nutritional status (e.g. intensive care medicine, gastroenterology, rheumatology).

Comparative audit using biochemical variables is difficult when results are returned by different laboratories using different methods, even within one centre. For albumin, it is not possible to apply a correction factor because of variation in difference between methods over a range of albumin values. This problem is particularly important where comparative audit of albumin as an intermediate outcome measure is performed between hospitals or departments using different methodologies. Standardization of albumin assay would solve this problem. Although an antibody-based method would be the ideal solution, the cost is likely to be prohibitive. However, standardization using the BCP method would be an acceptable alternative.

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