<Research Article>

Prediction of inflammatory response dynamics using albumin measurements by the modified BCP method

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Summary The present study examined changes in albumin levels during the exacerbation and remission of inflammation. The performance of each measurement method was initially assessed. The modified bromocresol purple (BCP) method, which is widely used in Japan, showed good results in terms of parallel precision and the stability of refrigerated samples. In comparisons with C-reactive protein (CRP) levels, a correlation was observed between albumin and CRP levels in all methods. In approximately 50% of cases, a decrease in albumin levels was noted prior to an increase in CRP levels, and was more pronounced with the modified BCP method than with the other methods. On the other hand, an increase in albumin levels before a decrease in CRP levels was not significant. These results suggests that during inflammation, blood albumin levels rapidly decrease due to increased vascular permeability, while during the remission of inflammation, they gradually increase as the liver synthesizes albumin. Therefore, a decrease in serum albumin levels may serve as a warning sign of inflammation, and was the most pronounced with the modified BCP method. Since the modified BCP method is widely used in Japan, but not internationally, the broader dissemination and standardization of this measurement method are necessary.

Key words: Modified bromocresol purple method, Bromocresol green method, Bromocresol purple method, C-Reactive protein

1. Introduction

The bromocresol green (BCG) method reported by Doumas et al.[1] in 1971 and the bromocresol purple (BCP) method described by Pinnell et al.[2] in 1978 are still used in routine laboratory testing to quantify serum albumin (ALB). One limitation of the BCG method is its low specificity for ALB because it also reacts with some acute-phase proteins, such as α-globulins[3]. Although the BCP method (the conventional BCP method) reacts specifically with ALB, its reactivity differs between reduced ALB (human mercaptoalbumin, HMA) and oxidized ALB (non-mercaptoalbumin, HNA), leading to falsely elevated values under conditions with increased HNA as well as in stored serum and control samples. The modified BCP method was developed to resolve the difference in reactivity between HNA and HMA in the conventional BCP method[4]. In Japan, the distribution of serum ALB measurement methods is currently 98.7% for the

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modified BCP method, 1.06% for the BCG method, and 0.16% for the conventional BCP method[5]. According to the 2023 CAP survey, the BCG method accounted for 67% and the BCP method for 32% internationally[6]. Although the survey did not specify whether the BCP method referred to the modified or conventional version, the majority of institutions outside Japan are using the conventional method.

The synthesis of ALB is impaired in inflammatory diseases, and the level of ALB decreases due to its leakage as a result of increased vascular permeability at the site of inflammation as well as its degradation at the site of inflammation and cell death[7]. At the onset of inflammation, ALB decreases almost simultaneously with elevations in acute-phase proteins, such as C-reactive protein (CRP), and also increased almost simultaneously with the reduction in CRP during remission [8,9]. However, the BCG method may yield falsely elevated values due to reactions with acute-phase proteins, and the conventional BCP method may also produce falsely elevated values due to an increased percentage of HMA.

Therefore, the present study examined changes in ALB levels measured by each method at the onset and remission of inflammation and investigated the usefulness of ALB for the diagnosis of inflammation.

2. Materials and Methods

Subjects

Serum samples were obtained from outpatients and inpatients at Takagi Hospital, Fukuoka, Japan. The present study was conducted with the approval of the Ethics Committees of our institution and Takagi Hospital (Approval No.: 23-Ifh-039). Liquid control serum as the quality control (C&C, FUJIFILM Wako Pure Chemical Corporation) was used in reproducibility testing.

The samples analyzed in the present study consisted of 1249 serum samples collected from patients at Takagi Hospital after ALB and CRP measurements. These samples were divided as follows: 478 for correlation studies, 22 for storage stability testing, and 749 for analyses related to inflammation. Inflammation-related studies included 142 patients who visited the emergency room and 607 surgical patients, including inpatients. Among them, cases in which ALB changes were observed through three or more blood collections comprised 6 cases from the emergency outpatient department and 98 surgical cases. Serum samples were collected on the day of blood sampling and were stored at −80°C for later analyses.

Reagents

The modified BCP method used the L-type Wako ALB-BCP reagent (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan)[10,11]. The conventional BCP method employed an in-house prepared reagent [pH 5.6, 125 mmol/L citrate buffer, 0.15% surfactant (Triton X-100), and 0.225 mmol/L BCP][12]. The BCG method used the HA Test Wako Albumin II reagent (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan)[13]. Calibration samples were prepared with the TP/ALB Calibrator (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

Instruments

Measurements were performed using the BM6010 automated biochemical analyzer (JEOL Ltd., Tokyo, Japan). The parameters for each measurement method are shown in Table 1.

Relationships between changes in CRP and ALB levels during the exacerbation or remission of inflammation

The relationship between ALB and CRP was examined. Changes in ALB levels in relation to the exacerbation and remission of inflammation, defined based on fluctuations in CRP levels, were evaluated. CRP judgment criteria conformed to the international reference range established by the World Health Organization[14]. An increase ≥1.0 mg/dL from stable or decreasing CRP levels was defined as the exacerbation of inflammation, a decrease ≥1.0 mg/dL from stable or increasing levels was defined as

Table 1 Parameters for each measurement method.

	modified BCP	BCP	BCG
Sample volume (μL)	2.6	1.2	1.4
Reagent 1 volume (μL)	140	170	140
Reagent 2 volume (μL)	70	-	-
Dominant wavelength (nm)	596	596	658
Complementary wavelength (nm)	658	694	751

BCP, bromocresol purple method; BCG, bromocresol green method

the remission of inflammation, and a fluctuation within ±1 mg/dL of CRP was defined as no change. We compared the average change in ALB levels (ΔALB) immediately before both the exacerbation and remission of inflammation. We then compared average ΔALB measurements for each method between the times immediately before both the exacerbation and remission of inflammation, between the time immediately before exacerbation and the time with no change, and between the time with no change and immediately before remission.

Furthermore, to establish whether changes in ALB levels predict inflammation earlier than CRP, we investigated if ALB decreased or increased by ≥0.1 g/dL immediately before exacerbation or remission, respectively. Similarly, to clarify whether changes in ALB levels synchronized with the exacerbation/remission of inflammation, we investigated if ALB decreased or increased by ≥0.1 g/dL on the same day that CRP levels changed during the exacerbation or remission phase.

3. Results

Reproducibility

Within-run reproducibility was evaluated (n=20) using two concentrations of patient serum. In both cases, the coefficients of variation (CV) were ≤0.7% (Table 2).

Storage stability

Twenty-two randomly selected patient serum samples were stored at 5°C and assayed for 7 consecutive days. An example of temporal changes in measurement values is shown in Fig. 1. Average CV for intra-laboratory reproducibility (7 days) in 22 cases were 0.92% for the modified BCP method, 1.25% for the conventional BCP method, and 1.02% for the BCG method. Fig. 2 shows the average change in ALB levels from the initial measurement day in 22 cases. The modified BCP method showed an increase of 0.04 g/dL from days 1-6, the BCP method showed an increase of 0.09 g/dL from days 1-6, and the BCG method showed an initial decrease followed by an increase of 0.04 g/dL from day 3.

Table 2 Within-run reproducibility (n=20)

		Modified BCP	BCP	BCG
Low	Mean (g/dL)	3.05	3.18	3.23
	SD (g/dL)	0.021	0.015	0.019
	CV (%)	0.69	0.47	0.59
High	Mean (g/dL)	4.51	4.88	4.97
	SD (g/dL)	0.015	0.018	0.027
	CV (%)	0.33	0.37	0.54

BCP, bromocresol purple method; BCG, bromocresol green method; SD, standard deviation; CV, coefficient of variation

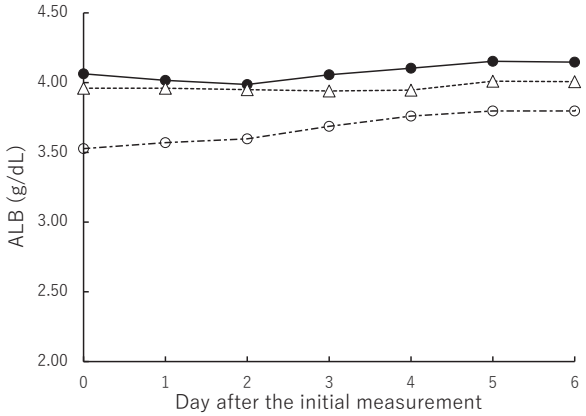

Fig. 1. Example of a stability test under refrigerated storage at 5°C. The x-axis shows the day after the initial measurement. The y-axis shows the variation in ALB values measured by the three methods. ●, modified BCP. ○, BCP. △, BCG.

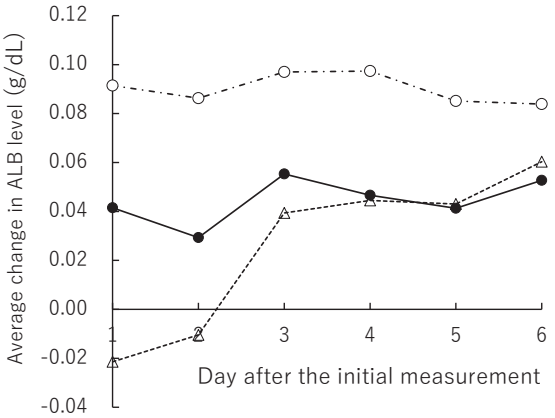

Fig. 2. The average change in albumin levels from the initial measurement day in 22 cases. The x-axis shows the day after the initial measurement. The y-axis shows the average change in albumin levels. ●, modified BCP. ○, BCP. △, BCG.

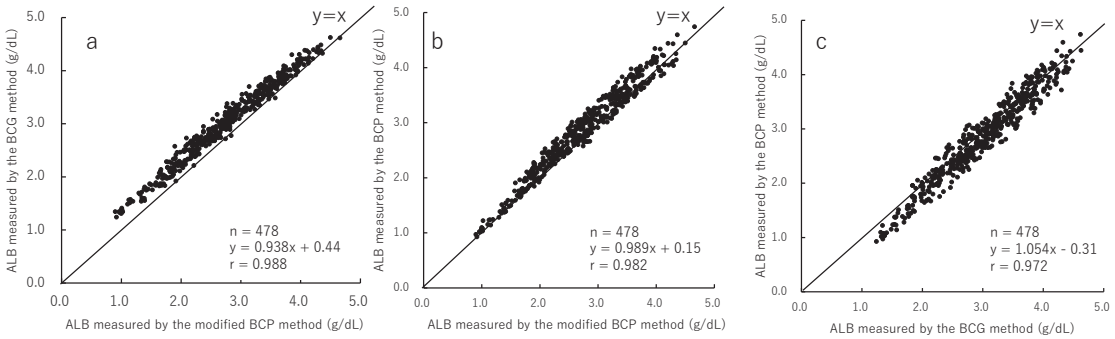

Fig. 3. Relationship between albumin measurement methods. Correlations between the modified BCP and BCP methods (a), modified BCP and BCG methods (b), and BCG and BCP methods (c).

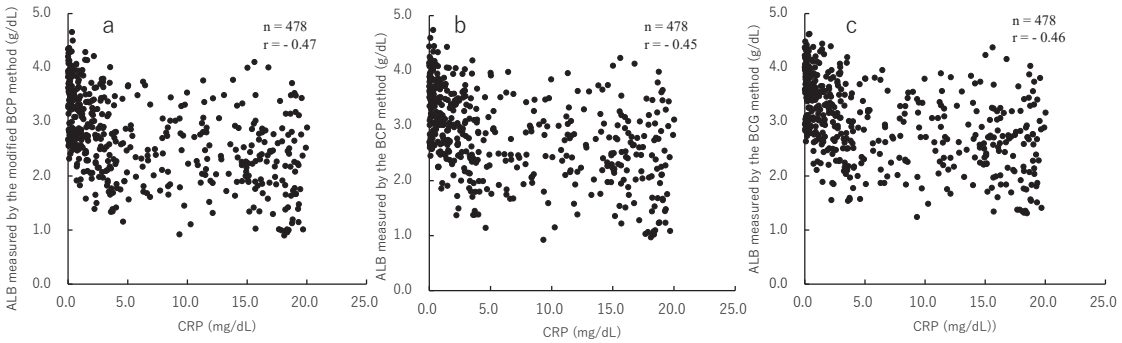

Fig. 4. Relationship between albumin and CRP. Correlations between CRP and the modified BCP method (a), CRP and the BCP method (b), CRP and the BCG method (c).

Relationships between methods

We analyzed 478 specimens to investigate the relationship between the three methods. These specimens were collected separately from the 749 specimens used specifically for the correlation test. An analysis of the 478 specimens to examine the relationship between the measurement methods yielded the results shown in Fig. 3. The correlation

coefficient was the highest between the modified BCP method and BCG method (r=0.988), followed by the modified BCP method and conventional BCP method (r=0.982) and the conventional BCP method and BCG method (r=0.972). In comparisons with the modified BCP method, the conventional BCP method had slightly higher values, while the BCG method showed a distinct constant positive systematic error.

Relationship between ALB and CRP

Fig. 4 shows the relationship between ALB and CRP levels for each method. Correlation coefficients were -0.47 for the modified BCP method, -0.45 for the conventional BCP method, and -0.46 for the BCG method, with the modified BCP method showing the strongest correlation.

Relationship between changes in CRP and ALB levels during the exacerbation or remission of inflammation

An example of temporal changes in measurement values is shown in Fig. 5. In 22 cases, 110 points (points at which the CRP value fluctuated by more than 1.0 mg/dL) were identified for the exacerbation or remission of inflammation.

In comparisons of ΔALB immediately before the exacerbation of inflammation and its remission, the modified BCP method showed a significantly larger decrease than the other methods. On the other hand, changes immediately before the remission of inflammation were not consistent (Table 3). In each method, ΔALB was compared between the periods immediately before both the exacerbation and remission of inflammation, between the period immediately before exacerbation and the time with no change, and between the time with no change and the period immediately before remission. The modified BCP method showed a significant difference between the periods immediately before both the exacerbation and remission of inflammation, and

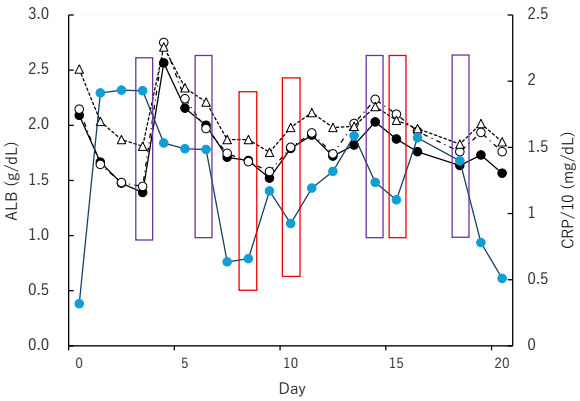

Fig. 5. Example of changes in CRP and ALB levels. For display CRP measurements on the same scale as albumin, they were divided by 10 and expressed as CRP/10. The red box indicates the day before the exacerbation of inflammation, while the purple box indicates the day before its remission. In this case, 24 points were identified where CRP fluctuated by ≥1.0 mg/dL. This graph shows temporal changes in ALB and CRP levels for a specific case. The red box indicates the ALB fluctuation on the day before the exacerbation of inflammation. The purple box indicates the ALB fluctuation on the day before the remission of inflammation. ●: Modified BCP, ○: BCP, △: BCG, ●: CRP/10.

Table 3 Changes in albumin levels immediately before the exacerbation and remission of inflammation

g/dL

		modified BCP	BCP	BCG
Exacerbation group	mean	-0.26*	-0.11	-0.11
	SD	0.35	0.37	0.35
Remission group	mean	0.03	0.03	0.04
	SD	0.33	0.36	0.29

*A significant difference was observed between the modified BCP group and the other groups (the Student's t-test, P<0.05). BCP, bromocresol purple method; BCG, bromocresol green method; SD, standard deviation

also between the period immediately before exacerbation and the time with no change (Table 4). When examining the predictive ability of the exacerbation and remission of inflammation, the modified BCP method showed the highest prediction rates for the exacerbation and remission of inflammation, the highest probability of agreement with changes in CRP levels, and the highest average values of these parameters (Table 5).

4. Discussion

We herein examined the usefulness of changes in ALB measurement values for diagnosing the onset and remission of inflammation. Measurement methods for serum ALB currently include the modified BCP method, conventional BCP method, and BCG method. In Japan, the majority of facilities use the modified BCP method, whereas the conventional BCP and BCG methods are more commonly employed in Western countries. Therefore, we also compared the performance of these methods.

The verification of parallel precision showed that all methods achieved good results with CV ≤0.7%. Regarding the stability of measurement values when serum was stored under refrigeration, the results obtained were generally acceptable, with CV ≤1.4% (Table 2). However, in the conventional BCP method, values generally increased by approximately 0.1 g/dL from the following day onwards (Fig. 1-2). This was attributed to the higher reactivity of the conventional BCP method to HNA. In

Table 4 Changes in CRP and albumin levels

	modified BCP		BCP		BCG	
	F value	P value	F value	P value	F value	P value
Remission group vs. Exacerbation group *	0.80	<0.01	0.77	0.09	0.18	<0.05
No change group vs. Exacerbation group**	0.035	<0.01	0.065	0.12	0.048	0.13
No change group vs. Remission group*	0.077	0.41	0.15	0.49	0.83	0.24

Exacerbation group, CRP levels increase by ≥1.0 mg/dL; No change group, CRP levels within ±1 mg/dL; Remission group, CRP levels decrease by ≥1.0 mg/dL
*the Student's t-test; **Welch's t-test

Table 5 Prediction rates of exacerbation and remission

	modified BCP			BCP			BCG		
	Positive	Invariant	Inverse	Positive	Invariant	Inverse	Positive	Invariant	Inverse
The prediction rate of exacerbation (%)	50	12	38	50	12	38	45	15	40
The prediction rate of remission (%)	47	11	42	37	10	53	38	16	46
The concordance rate with CRP (%)	56	12	32	48	12	40	52	11	37

Positive, The percentage of cases where ALB decreases immediately before exacerbation, or the percentage where ALB increases immediately before remission; Invariant, The percentage of cases where ALB does not change immediately before exacerbation or remission; Inverse, The probability that ALB increases immediately before exacerbation, or the probability that ALB decreases immediately before remission. Exacerbation prediction rate, The percentage of cases where ALB decreases immediately before exacerbation; Remission prediction rate: The percentage of cases where ALB increases immediately before remission; Concordance rate with CRP, The percentage of cases where ALB decreases simultaneously with an increase in CRP, or the percentage where ALB increases simultaneously with a decrease in CRP

healthy individuals, the percentage of HMA is higher, whereas in various disease states in which ALB decreases, the percentage of HMA decreases and that of HNA increases. The increase in measurement values over time with the conventional BCP method was attributed to the oxidation of NMA in samples during refrigerated storage, resulting in its conversion to HNA.

In the correlation analysis of the three methods, the highest value was observed between the modified BCP method and BCG method (r=0.988). The modified BCP method correlated well with the BCG method, which strongly reacts with ALB, suggesting that the modified BCP method reacts uniformly regardless of the ALB state[15]. In comparisons with the modified BCP method, the BCG method showed a constant positive systematic error, which was considered to result from falsely elevated values due to reactions with acute-phase proteins. This change was evident in the low ALB concentration range, which is consistent with our previous findings[16]. In contrast, the correlation between the modified BCP method and conventional BCP method was r=0.982, showing slightly greater variability, with the conventional BCP method yielding higher results. This may have been due to the stronger reactivity of the conventional BCP method towards HNA in samples. The correlation between the conventional BCP method and BCG method was the lowest (r=0.972), with the BCG method giving slightly higher values. This was considered to arise from differences in the ALB state and reactivity with acute-phase proteins between the two methods (Fig. 3).

During inflammation, ALB levels decrease. The present results showed a correlation between CRP and ALB values (Fig. 4). Furthermore, the concordance rate with CRP also showed an inverse trend toward CRP in 56% of cases(Table 5). In relation to CRP, the finding that the modified BCP method showed the highest correlation compared with the other methods was considered to suggest that the modified BCP method has higher accuracy. The reason for the decrease in albumin levels during the onset or exacerbation of inflammation was attributed to several factors, including ALB leakage caused by increased vascular permeability at the inflammation site, the local degradation of ALB itself, and decreased ALB synthesis in the liver because the liver produces acute-phase proteins[17]. Therefore, a decrease in ALB levels during inflammation may precede an increase in the level of CRP, an acute-phase reactant protein. However, few studies have examined changes in ALB levels before the exacerbation or remission of inflammation.

A combination of factors induce a decrease in the plasma concentration of ALB, including a poor nutritional status, decreased liver synthesis capacity, extravasation due to increased vascular permeability, renal excretion, and dilution from intravenous fluids. Therefore, difficulties are associated with assessing the exacerbation of inflammation based solely on ALB levels. In fact, albumin levels were also found to be elevated in 38% of cases before the rise in CRP. However, a novel result of the present study is that a decrease in ALB preceded CRP in 50% of cases of the exacerbation of inflammation (Table 5). This result suggests that a decrease in albumin may be a warning sign of the onset of inflammation, and is considered to be clinically significant.

Furthermore, this change was more pronounced with the modified BCP method than with the other methods (Tables 3-5), which may be because the modified BCP method accurately measures serum ALB levels, while the conventional BCP method exhibits different reactivities to HMA and HNA, and the BCG method shows non-specific reactions with acute-phase proteins. On the other hand, an increase in ΔALB immediately before the remission of inflammation was not clearly observed, which may be attributed to ALB synthesis in the liver not recovering quickly.

The limitations of this study include its focus solely on patients undergoing surgical treatment, the number of analyzed cases, and its single-center nature. Analysis of a broader range of cases is therefore necessary. Furthermore, there is potential to improve inflammation prediction accuracy by combining ALB with other laboratory parameters, which represents a future challenge.

Although the majority of serum ALB

measurements in Japan are performed using the modified BCP method, it has not been widely adopted in Western countries. This may be due to the limited range of reagents available for automated biochemical analyzers overseas, which restricts free reagent selection. The broader international adoption of the modified BCP method is expected to increase the global accuracy of ALB measurements.

5. Conclusion

The modified BCP method exhibited superior accuracy and the highest sample storage stability. A decrease in ALB levels measured by the modified BCP method may be a sign of impending inflammation, so caution is advised. The broader international dissemination of the modified BCP method is expected to improve measurement accuracy worldwide in the future.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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