Comparison of Bromcresol Green and Agarose Protein Electrophoresis for Quantitation of Serum Albumin in Multiple Myeloma

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Background: The International Staging System for multiple myeloma has increased the importance of accurate measurement of serum albumin. Two common albumin assays, bromcresol green (BCG) and agarose gel protein electrophoresis (PEL), frequently yield discordant results, creating confusion regarding which assay is superior for use in myeloma.

Methods: We measured albumin by BCG on a Roche Modular system, by PEL with a Helena SPIFE SPE Vis agarose gel, and by immunonephelometry performed on a Dade Behring BNII nephelometer. BCG and PEL were used to measure albumin in 5777 patient samples, and all 3 methods were used in an additional 252 samples. The clinical impact was assessed on 698 myeloma patient samples.

Results: For sera with zero/low monoclonal immunoglobulin protein (M)-spike (0 to <15 g/L), results for both BCG and PEL correlated well to nephelometry, although median PEL results were 8 g/L lower than corresponding BCG measurements. Correlation between PEL and nephelometry or BCG diminished with increasing M-spike, with PEL eventually overestimating albumin compared with both other assays. IgG and IgA M-spikes showed significantly different effects on albumin discordance. For 35% of myeloma patients, discrepancy between BCG and PEL had a potentially clinically significant effect on staging, but no difference in group survival was found.

Conclusions: Both BCG and PEL correlate well to nephelometry in sera with zero/low M-spike, whereas BCG compares well with nephelometry regardless of M-spike. Thus, albumin measurement can be performed reliably in myeloma patient sera by use of inexpensive, automated BCG assays.

Discordant results in the measurement of serum albumin concentrations have long been recognized with various assays, including agarose gel protein electrophoresis (PEL)4 bromcresol green (BCG), and bromcresol purple (BCP) (1, 2). This issue has recently increased in importance with development of the International Staging System (ISS) for multiple myeloma, which estimates prognosis according to serum β2-microglobulin (SB2M) and albumin concentrations (3). Stage I (longest median survival) is indicated by SB2M <3.5 mg/L and albumin ≥35 g/L and stage III (shortest survival) by SB2M ≥5.5 mg/L irrespective of albumin concentrations. Stage II (intermediate survival) is indicated by concentrations that differ from those characterizing stages I and III. Although clinical progression for myeloma patients is still measured by the size of the monoclonal immunoglobulin protein (M)-spike produced by the malignant cells, SB2M and albumin independently provide an initial measure of prognosis.

Three methods for measuring albumin are used in our clinical laboratories: automated analysis of BCG binding, PEL quantified by densitometry, and immunological quantification by nephelometry. Only PEL and BCG measurements of serum albumin are routinely used in clinical
practice. Immunonephelometric quantification is usually reserved for cerebrospinal fluid, which has lower albumin concentrations (4). Although nephelometry is arguably the most selective and therefore most likely to accurately quantify serum albumin, the relative expense and the requirement for additional analytical equipment often make nephelometry impractical.

To assess whether a particular albumin assay is preferable for use in multiple myeloma staging, we analyzed the relationship between PEL and BCG analyses of albumin in a large number (n = 5777) of patient samples both with and without a monoclonal immunoglobulin. In addition, we compared albumin concentrations from all 3 available methods (PEL, BCG, and nephelometry) in an independent group of patient samples (n = 252) over a range of M-spike values. Finally, we examined the effect of PEL and BCG albumin concentrations on staging and estimation of survival in a cohort of multiple myeloma patients to determine the clinical impact of the discrepancies in albumin measurement.

**Materials and Methods**

This study followed a protocol approved by the Mayo Institutional Review Board. Albumin results were retrieved retrospectively on 5777 consecutive patient sera analyzed from August 1, 2005, to August 1, 2006; all patients studied had given research authorization. The only inclusion criterion was that both BCG and PEL assays had been performed on each sample. Of 404 assays within a 1-year interval. Of the 5777 samples analyzed, 5373 were from patients with no quantifiable M-spike; M-spikes in the 404 remaining samples were 1 to 15 g/L, n = 118) and half had an M-spike of 0–55 g/L (n = 134). Of 166 samples containing an M-spike, 280 (69.3%) were IgG, 47 (11.6%) IgA, 59 (14.6%) IgM, 1 (0.2%) IgD, 4 (1.0%) light chain only, 11 (2.7%) biclonal with 2 different isotypes, and 2 (0.5%) did not have the isotype determined.

An additional 252 sera were collected prospectively and analyzed by all 3 albumin methods; consecutive samples were selected such that roughly half had little or no M-spike (0 to <15 g/L, n = 118) and half had an M-spike of 0 to 55 g/L (n = 134). Of 166 samples containing an M-spike, 280 (69.3%) were IgG, 47 (11.6%) IgA, 59 (14.6%) IgM, 1 (0.2%) IgD, 4 (1.0%) light chain only, 11 (2.7%) biclonal with 2 different isotypes, and 2 (0.5%) did not have the isotype determined.

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Data from a cohort of 1027 multiple myeloma patients were retrieved retrospectively; this patient population is well-defined and has been previously described (5). All serum measurements were made within 30 days of diagnosis; the 698 patients described herein were chosen for having had both PEL and BCG measurements of albumin performed on the initial serum sample. The type distribution in these samples was as follows: 359 (51.4%) IgG, 152 (21.8%) IgA, 2 (0.3%) IgM, 11 (1.6%) IgD, 128 (18.3%) light chain only, 36 (5.2%) nonsecretory, and 10 (1.4%) biclonal with 2 different isotypes. Sera whose M-spike isotype was not determined, sera with biclonal (2 isotypes) M-spikes, and sera from nonsecretory myeloma were excluded from the analysis of the influence of isotype on albumin discordance.

Statistical analyses were performed in SAS version 9.1 (SAS Institute) and Splus version 7.0 (Insightful) by the Mayo Clinic Division of Biostatistics. Recursive partitioning [r-part analysis, (6)] was used to determine optimal M-spike values for partitioning differences in PEL and BCG albumin measurements (i.e., points where the difference PEL – BCG changes as a function of M-spike), with M-spike and PEL – BCG as the independent and dependent variables, respectively. Standard linear and Deming regression analyses were used to assess the relationships among albumin assays. For the myeloma patient cohort, survival was defined as time from diagnosis of multiple myeloma to death or last follow-up. Estimates were generated by use of the method of Kaplan and Meier, and survival curves were compared by use of log-rank tests.

BCG measurement of albumin was performed with Roche reagents on a Roche P-modular automated analyzer according to the manufacturer’s procedure. Electrophoretic quantitations of both M-spike and albumin were performed on a Helena SPIFE 3000 electrophoresis unit by use of a SPIFE SPE Vis agarose gel, which was stained with Acid Blue to visualize proteins and scanned with a Helena Quick Scan 2000 densitometer. The relative amount of each protein fraction was determined by multiplying the relative amount by the total serum protein concentration. Total protein was measured on a Hitachi 912 automated analyzer, by use of a Roche colorimetric biuret assay.

Nephelometry was performed on a Dade Behring BNII immunonephelometer. Albumin was measured by use of Dade Behring antibodies specific to albumin; after antibody binding, light scatter at 840 nm was used to quantify the target protein. In addition, for IgG M-spikes >30 g/L, antiserum to the gamma chain was used to accurately quantify the monoclonal protein, because IgG M-spikes of >30 g/L may saturate the electrophoresis gel (7, 8).

**Results**

To determine the relationship between PEL and BCG measurements of serum albumin, we retrieved information on all patient samples that had been subjected to both assays within a 1-year interval. Of the 5777 samples analyzed, 5373 were from patients with no quantifiable M-spike; M-spikes in the 404 remaining samples were 1 to 15 g/L. PEL and BCG values for albumin were compared for all 5777 patients. Recursive partitioning analysis suggested that the relationship between PEL and BCG changed with increasing M-spikes, with cutpoints at 13.5, 24.5, and 40.5 g/L M-spikes. We therefore categorized the patient samples into 4 groups according to the concentration of M-spike (Table 1). The mean difference between BCG and PEL values changed progressively with increasing M-protein concentrations, from −8 to 4 g/L. This change in the relationship between BCG and PEL was statistically significant (P <0.0001) and is shown in Fig. 1A.

To assess the relative accuracy of the BCG and PEL albumin values, an additional 253 consecutive sera with M-spikes of 0 to 55 g/L were analyzed by BCG and PEL.
and immunonephelometry as an independent 3rd method. Notably, these 252 samples included 81 patients with albumin values ≥35 g/L as measured by nephelometry; 35 g/L is the lower reference limit for albumin in our laboratory, as well as the ISS cutoff between stages I and II. For patients with zero/low M-spikes (<15 g/L), both BCG and PEL demonstrated good correlation to nephelometry ($R^2 = 0.92$ and 0.91, respectively; $n = 118$), although PEL showed a negative bias relative to nephelometry. On inclusion of samples with larger M-spikes, however, PEL analysis showed poor correlation to nephelometry ($R = 0.67$); Deming regression showed the following:

$$\text{(PEL)} = 0.48 \times (\text{Nephelometry}) + 15.98.$$  

This poor correlation was largely attributable to a progression from underestimation of albumin at low M-spikes to overestimation at large M-spikes (Fig. 1B). In contrast, BCG analysis showed a stable relationship to nephelometry across all M-spike values (Fig. 1C). The overall correlation between BCG and nephelometry was good ($R = 0.96$); Deming regression showed the following:

$$\text{(BCG)} = 0.81 \times (\text{Nephelometry}) + 7.17.$$  

To address the impact of disagreement between PEL and BCG measurements of albumin in multiple myeloma, we analyzed data from a well-defined cohort of 1027 myeloma patients (5). Of these, 698 had PEL and BCG assays run on the same serum sample within 30 days of diagnosis; these results were used to assess whether the immunoglobulin isotype of the M-spike could affect the disparity between PEL and BCG results. To obtain sufficient numbers, we combined the 698 myeloma patient results with the M-spike–containing samples from the 5777- and 252-patient groups. There were too few samples containing only IgM, IgD, or light chain to obtain meaningful statistical analyses for the largest M-spike categories, but when these isotypes were compared with the rest of the data set in the smaller M-spike ranges, no significant differences were seen. In contrast, IgG-containing sera showed significantly greater discordance in albumin concentrations in the highest and lowest M-spike categories than did IgA-containing sera (Table 2).  

Albumin concentration of 35 g/L is an important cutoff for defining ISS stage. We therefore determined how many of the 698 myeloma patient samples had discordant PEL and BCG values across this cutoff, i.e., 1 albumin measurement ≥35 g/L and the other <35 g/L. 

### Table 1. Relationship between BCG and PEL measurements of serum albumin.

<table>
<thead>
<tr>
<th>M-spike (g/L)</th>
<th>n</th>
<th>Median difference, PEL – BCG, g/L (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero/low (0 to &lt;15)</td>
<td>5617</td>
<td>-8.0 (-29.0, 15.0)</td>
</tr>
<tr>
<td>Low-intermediate (15 to &lt;25)</td>
<td>79</td>
<td>-5.0 (-20.0, 8.0)</td>
</tr>
<tr>
<td>High-intermediate (25 to &lt;40)</td>
<td>52</td>
<td>-2.0 (-13.0, 17.0)</td>
</tr>
<tr>
<td>High (≥40)</td>
<td>29</td>
<td>4.0 (-11.0, 14.0)</td>
</tr>
<tr>
<td>Total</td>
<td>5777</td>
<td></td>
</tr>
</tbody>
</table>

* A value of 0 may represent the lack of a monoclonal protein or the presence of a monoclonal protein that is too small to quantify.

![Fig. 1. Variance in reported albumin concentrations by M-spike.](image)

Note that an M-spike of 0 may represent the lack of a monoclonal protein or the presence of a monoclonal protein that is too small to quantify. (A), difference (in g/L) between PEL and BCG albumin values for 5777 patient sera according to M-spike. Linear regression [(PEL – BCG) = 0.19 × (mean) – 7.9]; solid line and 95% confidence limits (dotted lines) are shown. (B), difference (in g/L) between electrophoresis (PEL) and nephelometry (neph) albumin values for 252 patient sera according to M-spike. (C), difference (in g/L) between BCG and nephelometry (neph) albumin values for 252 patient sera according to M-spike.
samples to assess the correlation of PEL or BCG values with patient outcome. For both assays, albumin concentration ≥35 g/L was predictive of longer survival than albumin <35 g/L (P < 0.001). Neither assay performed better than the other; Kaplan-Meier plots of survival in patients with albumin ≥35 g/L or <35 g/L are indistinguishable for PEL and BCG (Fig. 3). Similarly, the hazard ratios for albumin <35 g/L are identical at 1.3 (95% confidence limits 1.1, 1.6 and 1.1, 1.5 for BCG and PEL, respectively), indicating that PEL and BCG albumin concentrations are equally good predictors of patient survival in multiple myeloma.

Discussion

Measurement of serum albumin in multiple myeloma patients has increased in importance since the recent introduction of the ISS, which stratifies prognosis according to concentrations of albumin and Sβ2M (3). We used immunonephelometry as an independent 3rd method to clarify the discordance between BCG and PEL measurements of albumin, long recognized to have poor correlation (1, 2), and determined the impact of discrepant albumin concentrations on staging and survival of patients from a well-defined multiple myeloma cohort (5).

A retrospective look at 5777 serum samples analyzed by both BCG and PEL confirmed lack of agreement between the 2 assays and indicated that the relationship between them was unstable with increasing M-spikes. Although PEL underestimates albumin relative to BCG when M-spikes are low, this difference gradually decreases until, at M-spikes ≥40 g/L, the PEL albumin value is on average 4 g/L higher than the corresponding BCG value. The overall slope of this statistically significant change is 0.19, increasing from 0.07 for zero/low M-spikes to 0.21 in the high M-spike group.

In an additional 252 serum samples, the BCG assay showed good overall correlation to nephelometry (R = 0.96), with a consistent relationship across the full M-spike range. Interestingly, although PEL has previously been suggested to be a more accurate measurement of albumin (1, 2), we found the overall correlation of PEL to nephelometry to be low (R = 0.67) because PEL results

![Fig. 2. Discordance in albumin across the staging system cutoff.](image)

Albunin values from both assays were compared for 698 multiple myeloma patient sera. Samples with albumin results discordant across the 35 g/L cutoff set by the ISS were categorized by the assay that reported the higher result. Data are reported according to M-spike.

![Fig. 3. PEL and BCG albumin assays are equally good predictors of survival in multiple myeloma.](image)

Albunin values from PEL and BCG were compared with survival in 698 multiple myeloma patients. A cutoff of 35 g/L was used (P < 0.001, both assays); compared with the group with albumin ≥35 g/L, an albumin value <35 g/L showed a hazard ratio of 1.3 for both assays.

<table>
<thead>
<tr>
<th>M-spike, g/L</th>
<th>Median PEL − BCG, g/L (range)</th>
<th>Median PEL − BCG, g/L (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to &lt;15</td>
<td>218 −7.0 (−29.0, 6.0)</td>
<td>55 −6.0 (−14.0, 3.4)</td>
</tr>
<tr>
<td>15 to &lt;25</td>
<td>135 −5.0 (−20.0, 6.0)</td>
<td>50 −4.0 (−11.0, 8.0)</td>
</tr>
<tr>
<td>25 to &lt;40</td>
<td>212 −0.3 (−10.0, 17.0)</td>
<td>57 −1.0 (−13.0, 7.0)</td>
</tr>
<tr>
<td>≥40</td>
<td>190 5.0 (−4.3, 27.1)</td>
<td>62 3.0 (−13.0, 11.5)</td>
</tr>
</tbody>
</table>

α P = 0.019, IgG vs IgA.
β P < 0.0001, IgG vs IgA.

Table 2. Influence of M-spike isotype on albumin assay disagreement.
showed underestimation of albumin at low M-spikes, shifting to overestimation of albumin at high M-spikes. The latter effect is likely attributable to saturation of Acid Blue binding by large IgG M-spikes, leading to assignment of a falsely high percentage of the total protein to albumin (7,8). For this reason our laboratory recommends quantification of large (>30 g/L) IgG M-spikes by nephelometry; our in-house PEL assay validation studies of sera with large M-spikes suggest that dilution of the samples does not provide adequate linearity for accurate measurement of serum proteins (J.A.K., unpublished data). Saturation of dense IgG bands is not observed on capillary zone electrophoresis (7).

Analysis of the influence of M-spike isotype on albumin assay disagreement supports the concept that dye saturation by large IgG M-spikes affects the accuracy of PEL measurements. Although the number of samples was insufficient for analysis of other isotypes (e.g., IgM, light chain only) across the full range of M-spike values, comparison of IgG and IgA showed significantly larger albumin discrepancies in IgG-containing sera for both the lowest and highest M-spike categories. Interestingly, albumin measurements in 698 myeloma patients suggested that almost half (46.5%) of all IgG-containing sera in the highest M-spike category have PEL and BCG albumin values that are discordant across the 35 g/L ISS cutoff.

Of 1027 previously described multiple myeloma patients, 698 had both PEL and BCG measurements of albumin performed on the same serum sample within 30 days of diagnosis. About one third of these samples had discrepant albumin results that crossed the 35 g/L ISS cutoff, indicating that a substantial fraction of patients may be staged differentially by the 2 albumin assays, depending on $S_{\beta 2}$M concentrations. Despite these differences, PEL and BCG performed equally well as predictors of patient survival, likely because the effect of albumin on outcome is most strongly dictated by patients at the high and low extremes of the variable, and because patients with intermediate albumin concentrations (i.e., close to the 35 g/L cutoff) do not greatly impact the prognostic value of the test. This finding illustrates a point raised by the ISS, that albumin alone has limited prognostic value. This finding illustrates a point raised by the 35 g/L cutoff, indicating that a substantial fraction of patients likely because the effect of albumin on survival, likely because the effect of albumin on outcome is most strongly dictated by patients at the high and low extremes of the variable, and because patients with intermediate albumin concentrations (i.e., close to the 35 g/L cutoff) do not greatly impact the prognostic value of the test. This finding illustrates a point raised by the ISS, that albumin alone has limited prognostic value.

For the majority of the general population, M-spikes will be absent, or present only in very low concentrations (9); notably, both BCG and PEL demonstrated good correlation to nephelometry for M-spikes from 0 to <15 g/L. However, values from the 2 assays are often discrepant even in the absence of an M-spike, because in normal samples BCG typically overestimates albumin relative to PEL. Measurements from the same patient should therefore be performed by the same assay or interpreted with this relationship in mind. Although this study did not address the utility of BCP in sera containing M-spikes, it is likely that BCP assays will behave similarly to BCG for predicting prognosis in multiple myeloma. BCG and BCP tend to show acceptable linear correlation, with BCG albumin typically 3-5 g/L higher than the corresponding BCP results (10).

Staging of multiple myeloma requires accurate determination of albumin concentration in the presence of both small and large M-spikes; as many as one third of patient samples show discrepancies between BCG and PEL that may affect ISS staging. To our knowledge, this study is the first to address the utility of different albumin assays in the presence of large M-spikes. The data presented here suggest that an automated assay with BCG methodology provides consistent measurement of albumin regardless of the size of the M-spike; the PEL assay is less reliable with large M-spikes and may report artifactually increased albumin concentrations in these patients.

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References