

γ /IgG Ratio: Role in Distinguishing Monoclonal Spikes From Fibrinogen

Maria Teresa Lee,¹ Patrizio Caturegli,¹ Richard L. Humphrey,¹ Richard E. Thompson,² and Barbara Detrick^{1*}

¹Immunology Laboratory, Department of Pathology, Johns Hopkins University, School of Medicine, Baltimore, Maryland

²Department of Biostatistics, Johns Hopkins Bloomberg, School of Public Health, Baltimore, Maryland

Serum protein electrophoresis (SPEP) is a standard screening method for detecting monoclonal gammopathies. Presence of fibrinogen, however, can mimic a true monoclonal spike and interfere with accurate monoclonal protein identification. We describe a novel approach for distinguishing fibrinogen spikes from true monoclonal spikes. We classified 600 individual patient samples into four groups: group 1, 58 samples with a fibrinogen spike; group 2, 127 samples with a spike due to a monoclonal gammopathy; group 3, 181 samples with previously established monoclonal gammopathies but resolved post-treatment; and group 4, 234 control samples without monoclonal gammopathies. The value of using a γ region

fraction/IgG ratio in distinguishing fibrinogen from true monoclonal spikes was assessed. The γ /IgG ratio in the fibrinogen group is significantly ($P < 0.0001$) higher than this ratio in the other three groups. A γ /IgG ratio cut-off value of 1.13 discriminates true monoclonal gammopathies from fibrinogen. Moreover, exclusion of elevated IgA or IgM cases improves the ratio's predictive power. The probability cut-off is 0.756, corresponding to a γ /IgG ratio of 1 (93% sensitivity, 91% specificity). Using the γ /IgG ratio improves the screening power of SPEP and offers a simple and reliable diagnostic tool for distinguishing fibrinogen spikes from true monoclonal spikes. *J. Clin. Lab. Anal.* 25:332–336, 2011.

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INTRODUCTION

Currently, in the clinical laboratory, a combination of methods is used to identify patients with a monoclonal gammopathy and other serum protein disorders (1–3). Serum protein electrophoresis (SPEP), serum immunofixation electrophoresis (SIFE), and nephelometric quantitation of immunoglobulins constitute a basic serum monoclonal gammopathy screen. On SPEP, a monoclonal gammopathy may be revealed by the presence of a homogeneous band in the γ or less often, in the β or α -2 region. Accurate quantitation of the monoclonal protein is critical for its identification, classification, and monitoring. On occasion, interfering substances may be present that generate misleading data, and thus cause special problems for interpretation. A number of proteins have been identified that create such difficulties, including lysozyme, C-reactive protein, hemoglobin–haptoglobin complex, transferrin, complement, and fibrinogen (4). Fibrinogen is a known

contaminant of poorly clotted serum samples. It can be detected as a discrete band that migrates at the junction between the β and γ fractions and is indistinguishable on SPEP from a monoclonal spike (1,2). In order to remove the fibrinogen interference, some laboratories induce a clot to remove fibrinogen or perform serum IFE with anti-fibrinogen antibody to discriminate between fibrinogen and a true monoclonal spike (1,5–11). These methods require further sample manipulation. In addition, they occasionally fail to effectively remove the fibrinogen and may, in some cases, create other artifacts (7,8). The aim of this study

*Correspondence to: Barbara Detrick, Johns Hopkins University, School of Medicine, Department of Pathology, Meyer B125A, 600 North Wolfe Street, Baltimore, MD 21287.

E-mail: bdetrick@jhmi.edu

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was to determine whether the γ /IgG ratio could assist in discriminating a fibrinogen-related spike from a true monoclonal spike on SPEP.

MATERIALS AND METHODS

Patient Groups and Design of the Study

The categorization of the patients into the four groups was selected by reviewing the Pathology database (from July to December 2005). The pathology database was queried for the following items: monoclonal gammopathy, SPEP, SIFE, monoclonal IgG spikes, monoclonal IgM spikes, monoclonal IgA spikes, monoclonal spike, and fibrinogen. Although the data were non-randomly selected, the statistician was masked to the data selection and the γ /IgG ratio was calculated only after the data were selected.

This query yielded 58 fibrinogen reports. In each of the 58 reports, a spike was observed on SPEP that mimicked a monoclonal gammopathy. However, after performing serum IFE, the spike was identified as fibrinogen. This set of patients constituted the first group of patient samples, group 1 ($N = 58$, monoclonal spike due to fibrinogen). We next identified three additional patient groups: a group of patient samples with a proven monoclonal gammopathy that had a discrete band on the SPEP and a band of restricted electrophoretic mobility on serum IFE, group 2 ($N = 127$, gammopathy with a monoclonal spike); a group of patient samples with a previously established monoclonal gammopathy but after patient treatment no monoclonal spike was observed on SPEP and SIFE, group 3 ($N = 181$, gammopathy without a current spike); and last, a group of control patient samples without a monoclonal gammopathy—that is, the serum sample was sent to the laboratory for routine gammopathy screening—and after SPEP evaluation no band was observed, group 4 ($N = 234$, control patients without a spike). The age and sex distribution of the 600 patient samples included in the four groups are identified in Table 1. The study was approved by the Johns Hopkins Medicine Institutional Review Board.

Laboratory Methods

SPEP was performed on agarose gels in alkaline buffer (Sebia Laboratories, Norcross, GA). The albumin, α -1, α -2, β , and γ fractions were digitalized and their percent protein concentration was multiplied by the separately determined total protein measurement to calculate the mg/dl in each fraction. Total protein concentration was determined by a colorimetric assay (Roche/Hitachi 917 modular P and D, Indianapolis, IN). Serum IgG, IgA, and IgM immunoglobulins were quantified by nephelometry on the IMAGE instrument (Beckman,

TABLE 1. Age and Sex Distribution of the 600 Subjects Included in the Study

Diagnostic category	Female	Male	Total
Group 1: Fibrinogen spike			
Sample size	38	20	58
Age: Mean (SD)	61 (16)	61 (14)	61 (15)
Group 2: Gammopathy with spike			
Sample size	53	74	127
Age: Mean (SD)	64 (13)	65 (12)	65 (12)
Group 3: Gammopathy without spike			
Sample size	111	80	181
Age: Mean (SD)	69 (13)	64 (14)	67 (13)
Group 4: Control patients			
Sample size	135	99	234
Age: Mean (SD)	62 (15)	63 (14)	62 (15)
Total subjects			
Sample size	327	273	600
Age: Mean (SD)	64 (15)	64 (13)	64 (14)

Fullerton, CA). SIFE was performed on the Paragon system (Beckman-Coulter, Fullerton CA). Anti-fibrinogen antibody (Binding Site, San Diego, CA) was used in SIFE assay to identify fibrinogen. All assays were performed according to the manufacturer's procedures.

Statistical Analysis

The outcome measure analyzed was the γ /IgG ratio. This was calculated by dividing the concentration of the γ globulins obtained from the SPEP (mg/dl) by the concentration of the IgG value obtained from nephelometry (mg/dl). Because the ratio was highly skewed, we compared differences in the mean ratio among the groups using nonparametric Kruskal–Wallis test. Univariate logistic regression was then used to assess how the binary outcome (presence or absence of a true monoclonal spike) related to the γ /IgG ratio. Multivariate logistic regression was performed to adjust for age and sex of the patients. The area under the receiver operating curve (ROC) was used to assess how well the logistic model discriminated among the four diagnostic groups. A model with no predictive power has an area of 0.5 or less, whereas a perfect model has an area of 1. Sensitivity, specificity, positive, and negative predictive values were also calculated following the regression analysis. All statistical analyses were performed using Stata software, release 10.0 (Stata Corporation, College Station, TX).

RESULTS

γ /IgG Ratio is Significantly Higher in the Presence of Fibrinogen

The analysis of the γ /IgG ratio in the four patient groups revealed that the fibrinogen group had a

significantly higher ratio (1.45 ± 0.44 , mean \pm SD) when compared with the other groups ($P < 0.0001$, Kruskal–Wallis test; Fig. 1A). The three other patient groups had a γ /IgG ratio closer to 1. For example, a ratio of 1.05 ± 0.54 was obtained in the group with a proven gammopathy, group 2 (Fig. 1B), 0.94 ± 1.28 was revealed in the group with a previously established monoclonal gammopathy currently without a spike, group 3 (Fig. 1C), and 0.92 ± 0.12 was obtained in the control patient group, group 4 (Fig. 1D). There was no significant difference in the γ /IgG ratio among these three groups.

The serum γ SPEP levels correlated, as expected, with the serum IgG levels (adjusted $r^2 = 0.906$, $P < 0.0001$), but this correlation improved when the 58 fibrinogen samples were excluded (adjusted $r^2 = 0.933$, $P < 0.0001$). In fact, this fibrinogen group (closed circles in Fig. 2A) tended to have a higher serum concentration in the γ region for a given IgG value when compared with the group identified with a monoclonal gammopathy and a spike (open circles). Linear regression analysis showed that the intercept and the slope in the fibrinogen group (the intercept = 406 mg/dl and slope = 0.97 mg/dl) were higher than the gammopathy group identified with a monoclonal spike (intercept = 237 mg/dl and slope = 0.83 mg/dl) ($P = 0.105$ and $P = 0.049$, respectively). There were no differences observed in the intercept or slope when the monoclonal gammopathies without a spike (Fig. 2B; closed circles) were compared with the control patient group (Fig. 2B; open circles).

A logistic regression model was used to determine if the presence or absence of a true monoclonal gammopathy can be predicted by the γ /IgG ratio. In fact, the ratio is highly predictive of this binary outcome; that is, the odds of a SPEP spike representing a true

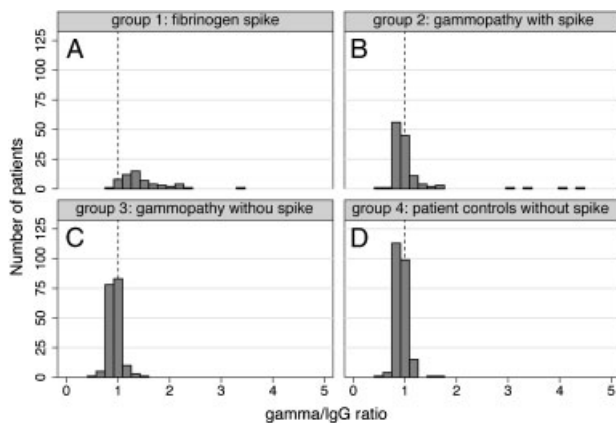


Fig. 1. Frequency distribution of the γ /IgG ratio in the four diagnostic groups. The dotted lines represent a ratio of 1 (i.e., no difference between SPEP γ region and IgG concentrations as determined by nephelometry).

monoclonal gammopathy (as opposed to fibrinogen) decreased 82% for every unit increase in the ratio (odds ratio: 0.18, 95% confidence limits: 0.076–0.44, $P < 0.0001$). The estimation of the goodness of the model by ROC curve revealed an area under the curve of 0.872 (Fig. 3A).

γ /IgG Cut-Off Value of 1.13 Discriminates a True Monoclonal Gammopathy From Fibrinogen

The logistic regression model was also used to identify the value of the γ /IgG ratio that distinguished a SPEP spike as a true monoclonal spike or a spike due to fibrinogen. Displaying sensitivity and specificity as a function of the probability cut-off, we found the probability of predicting the outcome that had the highest sensitivity and specificity was 0.707 (Fig. 3B; dotted line), which corresponded to a γ /IgG ratio of

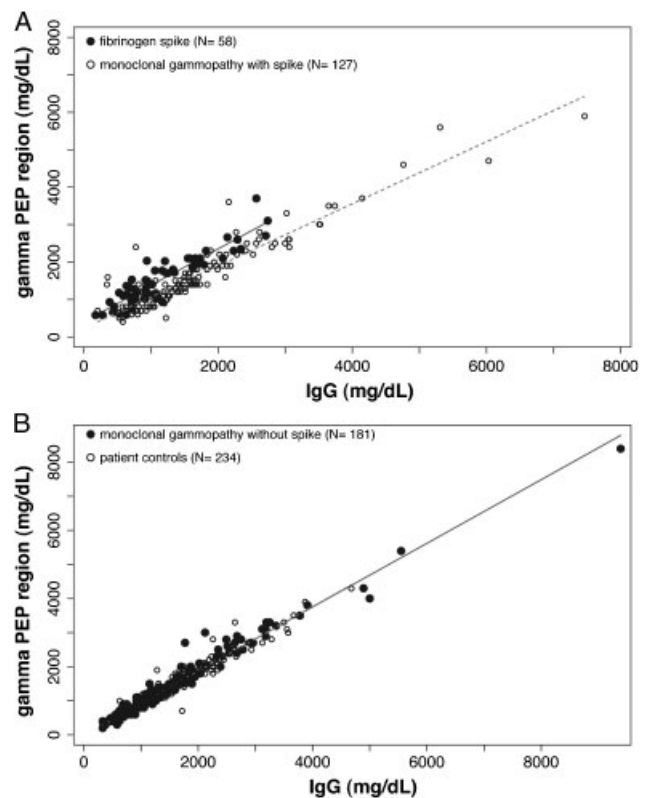


Fig. 2. Scatter plot identifies the relationship between the SPEP γ region (mg/dl) and IgG (mg/dl) levels. Panel A represents samples with a spike and panel B represents samples without a spike. The lines represent the fit from the linear regression analysis. In panel A, the closed circles and the solid line represent the fibrinogen group, group 1, whereas the open circles and the dotted line represent group 2, the monoclonal gammopathy samples with a spike. In panel B, the closed circles and solid line represent group 3, the monoclonal gammopathies without a spike, whereas the open circles represent group 4, the control patient group. The dotted regression line of the control patient group is not visible because it coincides with the solid line.

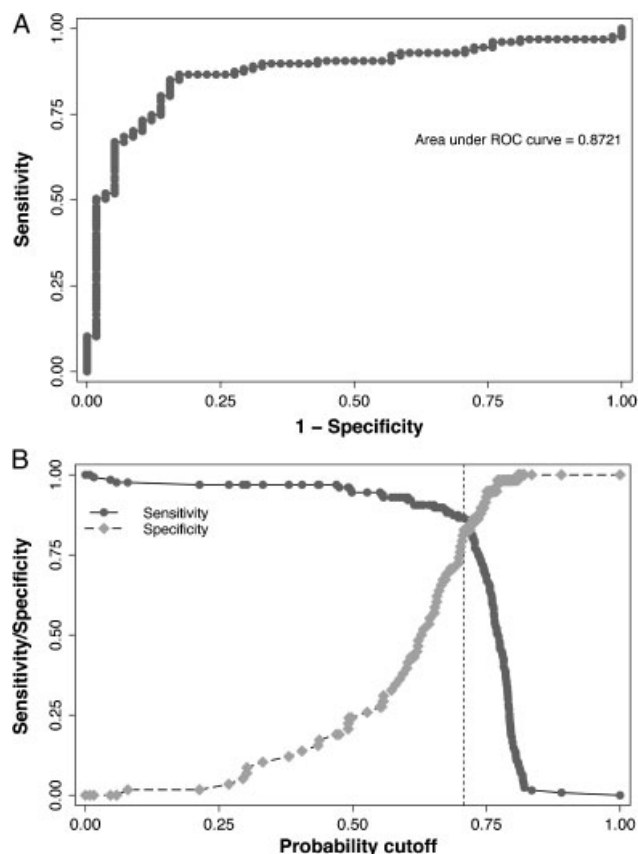


Fig. 3. ROC analysis. Panel A: ROC analysis performed after a logistic model regressing presence/absence of a spike over the γ /IgG ratio. Panel B: choice of the optimal cut-off value of the predicted outcome probability.

1.13. This cut-off value of 1.13 classified correctly 158 of the 185 (85%) patients with a spike. In particular, 110 of the 127 patients with a true monoclonal gammopathy (86.6% sensitivity) and 48 of 58 patients with a spike owing to fibrinogen (82.7% specificity) were classified correctly. Among the 120 subjects with a ratio less than or equal to 1.13, 110 had a monoclonal gammopathy, resulting in a positive predictive value of 91.7%. Among the 65 patients with a γ /IgG ratio greater than 1.13, 48 were positive for a spike due to fibrinogen; this resulted in a negative predictive value of 73.8%. Age and sex had no significant influence, and therefore were not included in the final logistic regression model.

Exclusion of Elevated IgA or IgM Improves Predictive Power

The γ /IgG ratio is based on IgG, because IgG is the most abundant immunoglobulin isotype and the one most commonly involved in monoclonal gammopathies. Clearly, an IgA or an IgM monoclonal gammopathy would increase the γ /IgG ratio independently of

fibrinogen. We, therefore, assessed whether the already good predictive power (under the curve area of 0.87) of the γ /IgG ratio could be improved by excluding those patients with IgA >453 mg/dl ($N=38$) or IgM >304 mg/dl ($N=17$) or with elevation in both IgA and IgM ($N=2$). Repeating the logistic regression model in the remaining 128 patients with a SPEP spike (that is, 44 patient samples with fibrinogen and 84 patient samples with a monoclonal gammopathy), the model became almost perfect, with an area under the curve of 0.96. The probability cut-off of this model was 0.756, corresponding to a γ /IgG ratio of 1, a sensitivity of 93%, and a specificity of 91%.

DISCUSSION

For nearly 50 years, protein analysis by electrophoresis has played an important role in the assessment of certain body fluids, such as serum, urine, cerebrospinal, and synovial fluid.

In fact, high-resolution agarose gel electrophoresis is a recommended screening method for the identification of monoclonal proteins. During SPEP evaluation, the presence of a spike conveys the likelihood of a monoclonal gammopathy. Fibrinogen, a known contaminant of poorly clotted blood samples, can often interfere with the accurate identification of a true monoclonal spike. Laboratories have handled this difficulty in a variety of ways, such as the addition of ethanol or thrombin to the serum sample or evaluation by serum IFE with anti-fibrinogen antibody. However, to date no standard method has been identified.

In this study, we described a simple laboratory approach to distinguish a fibrinogen spike from a monoclonal spike. We formulated a hypothesis based on the following: IgG is the most abundant immunoglobulin in the serum, IgG gammopathy is the most common gammopathy, and IgG is most often located in the γ region. Moreover, there is an almost linear relationship between the γ region concentration and IgG level. Using this relationship, a γ /IgG ratio can be constructed and we suggest that utilization of this γ /IgG ratio will assist in discriminating a fibrinogen-related spike from a true monoclonal spike. We, therefore, attempted to explore this relationship with a variety of samples analyzed on SPEP. We calculated a simple γ region/IgG ratio in the samples from groups 2, 3, and 4 (samples containing a true monoclonal spike, samples previously containing with monoclonal gammopathy but presently without a monoclonal spike, and control patient samples). All samples had, as expected, an almost linear relationship with a γ /IgG ratio close to 1. In contrast, when we evaluated the samples in group 1, in those that contained a fibrinogen spike the γ /IgG

ratio was significantly greater than 1. This trend was more evident in samples that contained normal levels of IgA and IgM. We, thus, suggest that when a spike is present in the γ region, a γ /IgG ratio should be calculated. If the ratio is ≥ 1 , then the spike is most likely a nonmonoclonal spike, probably caused by the presence of fibrinogen.

In summary, the approach described herein is a rapid, alternative, and nonmanipulative method which offers preliminary information that a SPEP spike may be due to fibrinogen. Additional standard confirmatory testing (i.e., serum IFE with anti-fibrinogen antibody) should follow. Perhaps, this new approach will reduce the burden of additional clinical testing, such as bone radiographs and bone marrow aspirations and biopsies. The inclusion of the γ /IgG ratio will improve the screening power of the SPEP and offer the laboratory a simple and reliable diagnostic tool for distinguishing a fibrinogen-related spike from a true monoclonal spike.

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