

# Detection of Polyclonal Increases in Immunoglobulin G4 Subclass by Distinct Patterns on Capillary Serum Protein Electrophoresis

## Diagnostic Pitfalls and Clinical Observations in a Study of 303 Cases

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**Key Words:** IgG4; Serum protein electrophoresis; IgG4-related disease; Autoimmune

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### ABSTRACT

**Objectives:** Autoimmunity, hypersensitivity, and the recently recognized set of syndromes collectively termed immunoglobulin G4-related disease (IgG4-RD) may be associated with increased serum IgG4 levels. We reviewed our experience detecting increased IgG4 by distinct serum protein electrophoresis (SPEP) patterns.

**Methods:** We studied 303 capillary SPEP cases with dome-like anodal  $\gamma$  changes and increased measured serum IgG4.

**Results:** IgG4 ranged from 208 to 6,670 mg/dL (normal, <201 mg/dL). Seventeen of 91 cases evaluated by immunosubtraction appeared monotypic (16  $\kappa$ , 1  $\lambda$ ), but all five cases further analyzed by isoelectric focusing appeared polyclonal. Six cases with markedly increased IgG4 had presumptive evidence of IgG4-RD. Sixteen of 45 assessed patients had autoantibodies.

**Conclusions:** Increased polyclonal IgG4 has a characteristic SPEP pattern that may mimic monoclonal gammopathy, even on immunosubtraction.  $\kappa$  Pseudo-restriction might reflect the naturally high  $\kappa/\lambda$  ratio of the IgG4 subclass. Autoantibodies were common, and the greatest IgG4 increases had clinical features of IgG4-RD.

Upon completion of this activity you will be able to:

- describe the distinct pattern on capillary serum protein electrophoresis (SPEP) created by serum increase in IgG4 subclass.
- describe potential pitfalls in the evaluation of SPEP samples with increased serum IgG4, including the potential for pseudoclonal immunofixation or immunosubtraction results.
- correlate increases in serum IgG4 level with associated clinical conditions.
- describe aspects of the biochemistry of IgG4 that are distinct from other immunoglobulin subclasses.

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Immunoglobulin G4 subclass (IgG4) has been the subject of intensified focus in the medical literature, largely due to a growing awareness of IgG4-related disorders in recent years. Serum levels of IgG4 are increased in a variety of medical conditions, including allergic/hypersensitivity conditions, allergy desensitization therapy, microscopic vasculitis and other autoimmune processes, certain helminthic infections, and IgG4-related disease (IgG4-RD; also called IgG4-related sclerosing disease)—a collective term for a diverse set of systemic disorders that share in common focal or mass-like infiltrative plasmacytic or lymphoplasmacytic

sclerosing inflammatory lesions rich in IgG4-secreting plasma cells.<sup>1-3</sup> Certain idiopathic or autoimmune-mediated inflammatory conditions, including sclerosing pancreatitis, sclerosing sialadenitis (“Kuttner tumor”), sclerosing dacryoadenitis, Mikulicz disease, and some forms of interstitial lung disease and interstitial nephritis, are now proposed as belonging to the spectrum of IgG4-RD.<sup>3-9</sup> The focal lesions associated with IgG4-RD may mimic a broad spectrum of diseases, including lymphoma and other neoplasms.<sup>6,10-12</sup> Furthermore, patients diagnosed with IgG4-RD often respond quickly and profoundly to corticosteroid therapy, highlighting the importance of correct detection and classification of these disorders.<sup>13,14</sup> Most patients with active IgG4-RD have increased serum levels of IgG4, and those who do may have more disseminated or aggressive disease.<sup>15</sup>

IgG4 has chemical and immunologic properties distinct from other immunoglobulin subclasses. In contrast to other immunoglobulin subclasses, IgG4 molecules have relatively unstable disulfide bonds between heavy chains, often resulting in a single heavy chain/light chain combination (as opposed to the typical two-heavy chain/two-light chain immunoglobulin structure).<sup>16</sup> This may result in functionally monovalent “half-molecules,”<sup>17</sup> or in the ability of the heavy chains of molecules of different idiotype specificity to bind together, forming functionally bispecific antibodies.<sup>16,18</sup> IgG4 appears to lack the effector function of other IgG subtypes and does not have the ability to fix complement.<sup>19</sup> As a result, some have theorized that a major role of IgG4 may be in the tempering of other immune response mechanisms, including autoimmunity and immunoglobulin E (IgE)-associated hypersensitivity reactions.<sup>20</sup> The latter possibility is supported by the observation of increased serum IgG4 levels in patients undergoing allergy desensitization therapy. Based on this finding and other observations, such as an increase in serum IgG4 levels in experienced beekeepers,<sup>20</sup> it is thought that increases in IgG4 levels may be proportional in part to the duration of exposure to stimuli of autoimmunity or hypersensitivity.

IgG4 has a distinct pattern of migration on capillary electrophoresis, forming a narrow, smooth, bell-shaped increase in the anodal  $\gamma$  region.<sup>21,22</sup> In extreme cases, this pattern may mimic a monoclonal gammopathy and can, therefore, pose a diagnostic challenge to the interpreter of serum protein electrophoresis (SPEP) patterns.<sup>21,22</sup> In this study, we describe our experience in detecting serum increases in IgG4 by examination of SPEP patterns by capillary electrophoresis on a clinical service. Our goals were to illustrate the distinct electropherogram patterns associated with IgG4 increases, to highlight potential diagnostic pitfalls (including clonality assessment in cases mimicking

monoclonal gammopathy), and to report associated clinical observations where available.

## Materials and Methods

With approval from the institutional review board of the St Joseph Mercy Health System, Ann Arbor, MI, 303 cases evaluated by SPEP and showing characteristic smooth domed or bell-shaped anodal increases in the anodal  $\gamma$  region were retrieved from the files of Warde Medical Laboratory. It is the practice in this laboratory to further evaluate such cases by evaluating IgG4 levels by nephelometry, and this study was limited to cases in which an IgG4 increase was confirmed in that manner.

SPEP was performed using capillary electrophoresis (Capillarys; Sebia, Norcross, GA). Total  $\gamma$  region protein levels were measured by integrating the  $\gamma$  region of the capillary electropherogram (Phoresis software; Sebia) and multiplying this percentage by a serum total protein measurement as performed by a Coomassie blue method on an Olympus AU400 analyzer (Beckman-Coulter Diagnostics, Brea, CA). In all cases, serum IgG4 levels were measured on a Siemens BN II nephelometer (Siemens, Malvern, PA). In 91 of the cases, the observed SPEP pattern raised substantial concern for the presence of a monoclonal protein in the anodal  $\gamma$  region. For these 91 cases, further assessment for clonality was performed by an immunosubtraction method (Immunotyping; Sebia). To further clarify what appeared to be a clonal pattern of immunosubtraction/immunotyping, five cases were further analyzed for clonality by a higher resolution method, agarose isoelectrophoresis (isoelectric focusing) with immunofixation and visualization using peroxidase-conjugated anti-IgG antiserum (Hydragel 9; Sebia). Not all pseudoclonal cases were evaluated by isoelectric focusing once the pattern of apparent  $\kappa$  light chain restriction in patients with documented polyclonal IgG4 increases became apparent. In 16 cases, serum free light chain analysis was ordered by the clinical service and performed using anti- $\kappa$  and anti- $\lambda$  reagents (Freelite; The Binding Site, Birmingham, UK) on a Siemens BN II nephelometer (Siemens). In all applicable cases, total protein, serum IgG4 level, serum free light chain analysis, capillary electrophoresis, immunotyping, and isoelectric focusing were performed according to the manufacturer’s specifications.

Where available, online medical records were reviewed to evaluate for medical history of conditions or findings potentially associated with increased serum IgG4 levels. It must be emphasized that this study was conducted from a reference laboratory and that access to electronic health records was limited, inconsistent, and dependent on studies ordered by the respective clinical services. Radiologic and

anatomic pathology records were reviewed where available. Records were also reviewed for results of autoimmune serologic tests (antinuclear antibodies [ANAs], antibodies to specific extractable nuclear antigens, anti-neutrophil cytoplasmic antibodies [ANCA], etc), serum IgE levels, serum complement levels (total complement [CH50], C3, and C4), and absolute eosinophil count, where available.

## Results

Serum IgG4 levels as measured by nephelometry ranged from 208 to 6,670 mg/dL (SI units: 2.08-66.70 g/L) (median, 390 mg/dL; reference range, 3-201 mg/dL). In some cases, directly measured IgG4 level exceeded the total  $\gamma$ -region protein measurement, likely due to extension of IgG4 into the  $\beta$  region of the electropherogram in those cases and/or differences in the precision of measurement between nephelometry and software-driven integration of electropherogram regions. For cases in which IgG4 formed a distinct peak, the mean ratio of the x-axis migration channel of the IgG4 peak to the serum albumin peak was 2.71 (range, 2.60-2.82), resulting in a distinct, dome-shaped pattern in the early  $\gamma$  region (Figure 1). In 34 (11%) cases, the increase in IgG4 was accompanied by an increase in acute phase proteins (relative or absolute increase in both  $\alpha_1$  and  $\alpha_2$  regions, with or without a decreased transferrin peak). The median level of the total  $\gamma$  region (as measured by the integrated percentage of protein in the  $\gamma$  region on the electropherogram, multiplied by the measured total protein level) was 1,300 mg/dL (range, 500-5,700 mg/dL). One hundred fifteen (38%) cases had a total  $\gamma$  region above the upper reference range limit of 1,400 mg/dL.

In the 91 cases for which immunotyping was performed, there was a monotypic pattern of apparent light chain restriction in 12 (13%) cases (11  $\kappa$  and one  $\lambda$ ) (Figure 2). The remaining 79 cases showed polyclonal or oligoclonal immunotyping patterns. In five cases, further evaluation by isoelectric focusing confirmed the polyclonal nature of the IgG4 increase, despite the apparently monotypic light chain expression pattern (Figure 3). Of note, the one case that showed apparent  $\lambda$  light chain restriction also was demonstrated to be polyclonal by isoelectric focusing (Figure 4).

Serum free light chain measurements were available in 16 cases (5.3% of total cases) (Table 1). Serum free  $\kappa$  light chain was elevated above the upper limit of the reference range (1.94 mg/dL) in 14 cases and was within the reference range in two cases. Serum free  $\lambda$  light chain was elevated above the upper limit of the reference range (2.63 mg/dL) in 11 cases and was within the reference range in five cases. The serum free  $\kappa$  to  $\lambda$  ratio was increased ( $>1.65$ ) in two cases and was normal in 14 cases. The two cases with

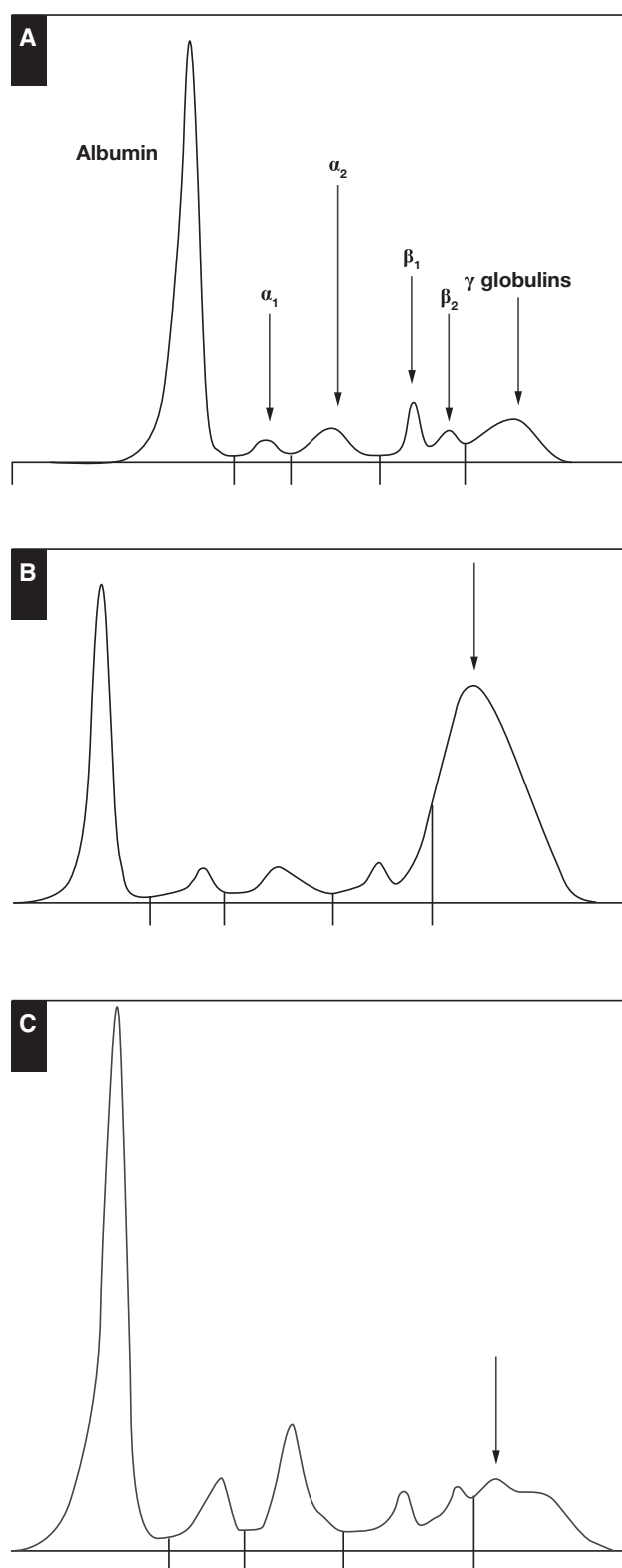


**Figure 1** Capillary electropherograms showing a normal pattern (A, with normal regions labeled), markedly elevated serum immunoglobulin G4 (IgG4) level (B, 6,670 mg/dL), and a more modest IgG4 increase (C, 257 mg/dL). The cases with increased IgG4 each show a characteristic dome-shaped peak (arrows) in the anodal  $\gamma$  region.

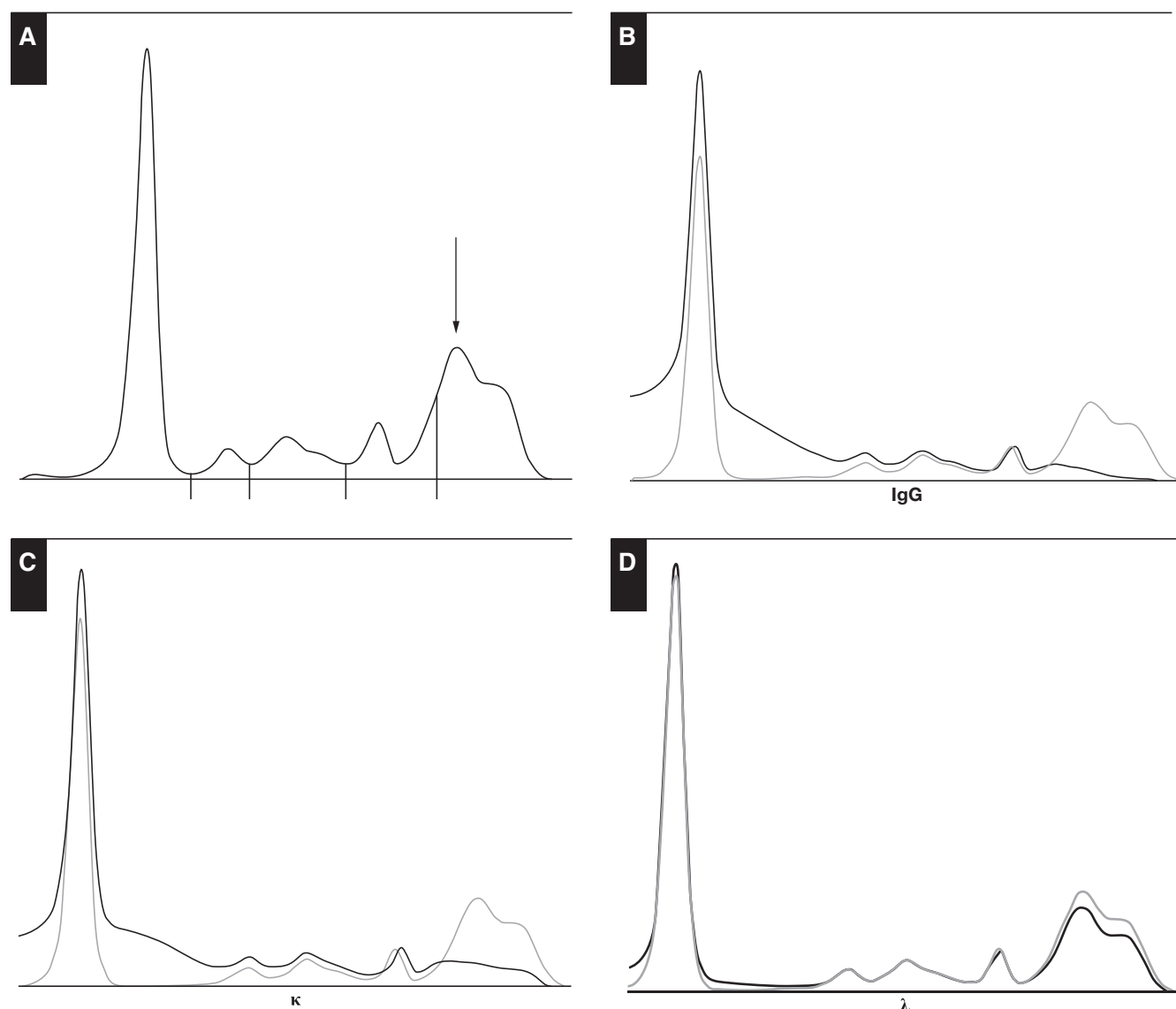


**Figure 2** A, Capillary electrophoresis pattern from a patient with a serum immunoglobulin G4 (IgG4) level of 2,500 mg/dL (reference range, 3-201 mg/dL). Arrow shows the polyclonal IgG4 peak in the anodal  $\gamma$  region, mimicking a monoclonal restriction. **B-D**, Immunotyping patterns for IgG (**B**),  $\kappa$  light chain (**C**), and  $\lambda$  light chain (**D**), highlighting the apparent  $\kappa$  restriction (pseudoclonality) of the IgG4 peak.

increased free serum  $\kappa$  to  $\lambda$  ratio had serum IgG4 levels of 1,640 mg/dL and 215 mg/dL, respectively.

Some level of clinical correlation was available in 112 of the 309 cases, although the retrospective nature of the study precluded absolute consistency in the nature of the medical information available, and conclusive clinicopathologic inferences could not be made. In six cases, the medical record contained presumptive clinical, radiographic, or biopsy evidence of possible IgG4-RD, as summarized in **Table 2**. In each of these cases, the total IgG4 level was at least 1,150 mg/dL (median, 1,605 mg/dL; range, 1,150-6,670 mg/dL) and represented the highest 10% of measured serum IgG4 in the study cohort, as well as six

of the nine highest measured IgG4 levels in cases for which clinical data were available. Specific mention of IgG4-RD was made in the medical record of only one case, specifically the case of the patient with the highest serum IgG4 level in the study (case 265, Table 1). This patient had a lymph node biopsy specimen with a prominent infiltrate of IgG4-positive plasma cells and a note in the diagnostic report that, despite the lack of prominent fibrosis, this pattern “raised the possibility of” IgG4-RD. The remaining five cases in Table 2 did not have specific mention of IgG4-RD in the records available to us, although this does not rule out that IgG4-RD may have been considered in those cases.

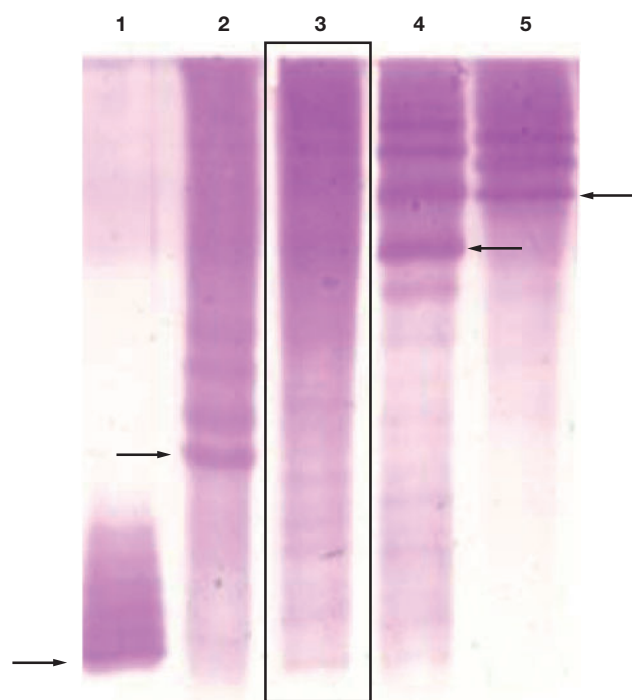


**Figure 3** Isoelectric focusing of a case that had demonstrated a pseudoclonal pattern of apparent  $\kappa$  restriction due to polyclonal immunoglobulin G4 (IgG4) increase. The polyclonal pattern of the index case (lane 3) is contrasted by monoclonal patterns in lanes 1, 2, 4, and 5. Arrows show the monoclonal “front” of the banding characteristic of monoclonal IgG proteins on isoelectric focusing.

In another five cases, the medical record alluded to some form of lymphadenopathy. In 28 cases, the medical history included renal insufficiency. In four cases, there was documentation of some form of allergic disorder, including increased serum IgE level (one case), allergy desensitization therapy with increased serum IgE level (one case), or less formal reference to allergic conditions (“allergic rhinitis,” “seasonal allergy”) (two cases). Unfortunately, only two patients in the study had serum IgE levels available in the electronic medical record. Some form of autoimmune serologic study was ordered by the clinical service in 45 of the 112 cases with available clinical data (44 included ANA testing). In 16 (36%) of these cases, there was documentation of at least one positive autoantibody study, as summarized in **Table 3**. Median IgG4 level for patients with positive ANA studies (at or above our positive cutoff titer of 1:160) was 381 mg/dL (range, 208–1,530 mg/dL). With one exception, cases with positive autoantibody studies had serum IgG4 levels of at least 300 mg/dL. Two cases had histories of positive ANCA, including one patient with a diagnosis of granulomatosis with polyangiitis (Wegener granulomatosis) and crescentic glomerulonephritis (serum IgG4 level of 661 mg/dL) and one patient with acute renal

failure, crescentic glomerulonephritis, and necrotizing vasculitis with a perinuclear ANCA pattern at a 1:80 titer (serum IgG4 level of 411 mg/dL).

Absolute eosinophil counts were available for 84 patients and ranged from 0 to  $12.8 \times 10^9/L$  (median,  $0.15 \times 10^9/L$ ). Only two patients had absolute eosinophil counts in excess of  $5 \times 10^9/L$ : one of these patients was on allergy desensitization therapy with absolute eosinophil count of  $12.8 \times 10^9/L$ , elevated IgE level, and serum IgG4 level of 996 mg/dL; the other had a history of stage III chronic renal insufficiency and serum IgG4 level of 717 mg/dL. Interestingly, the patient with the highest IgG4 level (6,670 mg/dL; case 265 as listed in Table 2), who also had a lymph node biopsy specimen showing increased IgG4-positive plasma cells and a diagnosis of possible IgG4-RD, had an absolute eosinophil count at the time of the SPEP study of only  $0.1 \times 10^9/L$ .

Serum C3 and C4 complement levels were available in only 11 cases and CH50 level in only three cases. Only one case (case 265 as listed in Table 2, with the highest measured serum IgG4 level in the study) had decreased C3, C4, or CH50 levels (C3 of 26 mg/dL [reference range, 79–152 mg/dL]; C4 of 9 mg/dL [reference range, 15–46 mg/dL]; CH50 of <30 complement activity enzyme immunoassay (CAE) units [reference range, 54–144 CAE units]). Increased C3 levels were seen in three cases and normal levels in seven cases. Normal C4 levels were seen in 10 cases. Normal CH50 was seen in two cases.

In one case with a pseudoclonal IgG4 peak, the patient had a history of treated plasma cell myeloma. Following treatment of the myeloma, the patient developed an IgG4 peak in the same region of the electropherogram as the original M protein. Identification as a polyclonal IgG4 peak was made due to the difference in shape of the peak, the characteristic IgG- $\kappa$  pseudoclonal pattern (in contrast to the original IgG- $\lambda$  M protein), and demonstration of an elevated IgG4 level of 1,640 mg/dL **Figure 5**.

## Discussion

This study highlights the fact that elevated IgG4 levels may form discrete peaks on capillary electrophoresis, closely mimicking monoclonal restrictions. This pitfall is exacerbated by the apparent  $\kappa$  light chain restriction in a subset of cases, potentially leading to a false conclusion of clonality. This phenomenon was observed in a report of three patients with IgG4-RD by Jacobs et al,<sup>21</sup> who speculated but did not formally demonstrate that this was a monotypic but polyclonal expansion of IgG4, citing previous work documenting abnormally elevated serum free light chain ratios in patients with IgG4-RD.<sup>23</sup> The current study goes further by formally

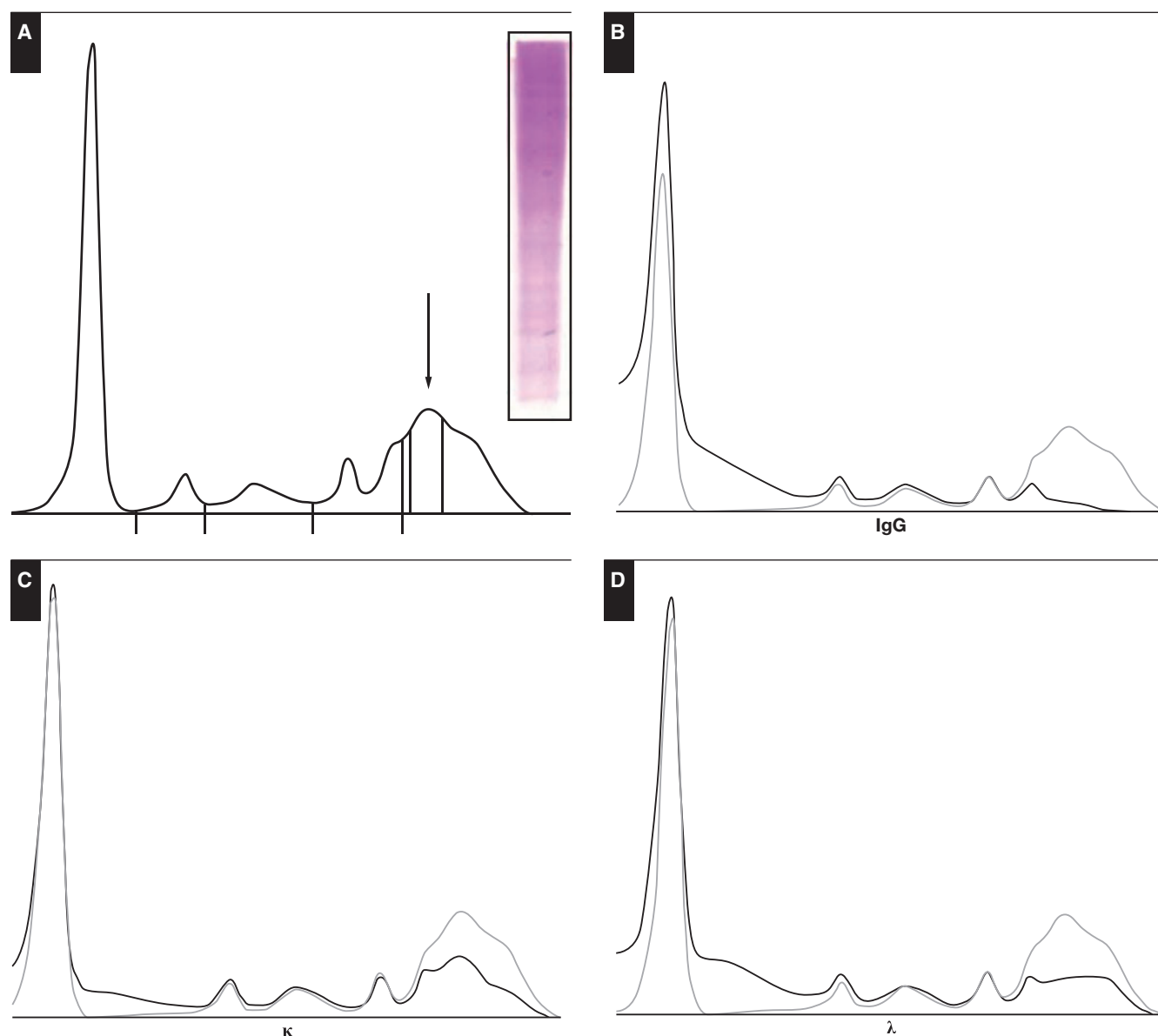


**Figure 4** Of 11 cases with pseudoclonal immunoglobulin G4 (IgG4) patterns, only one showed apparent  $\lambda$  restriction. The polyclonal nature of this IgG4 increase was confirmed by isoelectric focusing (inset). **A**, Capillary electrophoresis pattern, with IgG4 peak highlighted by the arrow. **B**, Immunotyping pattern for immunoglobulin G. **C**, Immunotyping pattern for  $\kappa$  light chain. **D**, Immunotyping pattern for  $\lambda$  light chain.

demonstrating the polyclonal nature of this apparently monotypic IgG4 increase in a subset of cases by isoelectric focusing. As a practical matter, we also demonstrated a consistent pattern of migration for the polyclonal IgG4 peak, which on the Sebia platform used in our laboratory had a predictable pattern between 2.6 and 2.8 times the x-axis channel number relative to the serum albumin peak. This may be of use to laboratories using similar analytical platforms.

The observed  $\kappa$ -restricted pseudoclonality of serum IgG4 increases may be attributable to the biochemistry and physiology of IgG4 rather than to analytical artifact.<sup>24</sup> While the overall ratio of  $\kappa$ - to  $\lambda$ -expressing IgG is

commonly cited as approximately 2 to 1, this ratio actually varies depending on IgG subclass. IgG2, for example, has a nearly even  $\kappa/\lambda$  ratio, while the natural  $\kappa/\lambda$  ratio of IgG4 is about 8 to 1—heavily skewed toward the  $\kappa$  light chain.<sup>24</sup> Since IgG1 is the dominant IgG subclass and has a  $\kappa/\lambda$  ratio of 2.4 to 1, the overall ratio of IgG- $\kappa$  to IgG- $\lambda$  is closer to 2 to 1. This may also explain the observation by previous investigators that cases of IgG4-expressing plasma cell myeloma are disproportionately weighted toward  $\kappa$  light chain expression<sup>25,26</sup> and the observation of increased serum free light chain ratio in some patients with IgG4-RD.<sup>23</sup> While we observed apparent  $\kappa$  restriction in many cases on

**Table 1**

**Results of Serum Free Light Chain Measurements in 16 Cases, With Comparisons to Total Serum IgG4 Level and Results of Clonality Assessment**

| Case No. | IgG4 Level, mg/dL | $\kappa$ SFLC, mg/dL | $\lambda$ SFLC, mg/dL | Free $\kappa/\lambda$ Ratio | Immunotyping Pattern             |
|----------|-------------------|----------------------|-----------------------|-----------------------------|----------------------------------|
| 97       | 1,640             | 4.14                 | 1.02                  | 4.05                        | $\kappa$ Restricted              |
| 111      | 361               | 3.91                 | 6.35                  | 0.62                        | Polyclonal                       |
| 122      | 3,300             | 4.17                 | 3.26                  | 1.28                        | Polyclonal                       |
| 156      | 257               | 4.38                 | 2.74                  | 1.60                        | ND                               |
| 158      | 232               | 1.70                 | 1.47                  | 1.16                        | Polyclonal                       |
| 167      | 248               | 4.54                 | 2.92                  | 1.56                        | Polyclonal                       |
| 171      | 361               | 2.91                 | 2.63                  | 1.10                        | Polyclonal                       |
| 181      | 215               | 15.40                | 6.79                  | 2.26                        | ND                               |
| 204      | 1,230             | 13.30                | 15.90                 | 0.84                        | $\kappa$ Restricted <sup>a</sup> |
| 219      | 254               | 1.13                 | 1.03                  | 1.10                        | ND                               |
| 222      | 342               | 8.05                 | 8.50                  | 0.95                        | ND                               |
| 249      | 717               | 10.70                | 9.38                  | 1.14                        | ND                               |
| 260      | 237               | 3.57                 | 3.14                  | 1.14                        | ND                               |
| 292      | 302               | 2.04                 | 1.43                  | 1.43                        | ND                               |
| 294      | 507               | 13.30                | 8.46                  | 1.57                        | ND                               |
| 297      | 858               | 6.04                 | 4.78                  | 1.26                        | Polyclonal                       |

IgG4, immunoglobulin G4; ND, not done; SFLC, serum free light chain.

<sup>a</sup>Case 204 also was analyzed by isoelectric focusing, which demonstrated a polyclonal pattern.

**Table 2**

**Presumptive Evidence of IgG4-Related Disease in Six Cases**

| Case No. | Age, y | Sex | Serum IgG4 Level, mg/dL | Description of Clinical Findings                                                                                                                               |
|----------|--------|-----|-------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 263      | 81     | M   | 6,670                   | Lymph node biopsy specimen with dense plasmacytic infiltrate and 40% IgG4-positive plasma cells by immunohistochemistry                                        |
| 31       | 69     | M   | 3,290                   | Common bile duct obstruction with pancreatic head mass on ERCP; no confirmed neoplasm                                                                          |
| 61       | 65     | M   | 1,790                   | End-stage renal disease due to acute interstitial nephritis; pelvic lymph node biopsy specimen with lymphoplasmacytic infiltrate; polyclonal by flow cytometry |
| 6        | 54     | M   | 1,420                   | Mediastinal lymphadenopathy; lung mass diagnosed on biopsy specimen as scar with chronic inflammation                                                          |
| 7        | 79     | F   | 1,250                   | Ampullary pancreatic mass on CT scan; no biopsy diagnosis on record                                                                                            |
| 87       | 63     | F   | 1,150                   | Interstitial lung disease with pulmonary hypertension and multifocal lymphadenopathy                                                                           |

CT, computed tomography; ERCP, endoscopic retrograde cholangiopancreatography; IgG4, immunoglobulin G4.

**Table 3**

**Summary of Autoantibody Serologic Studies in 45 Patients for Whom These Studies Were Ordered by the Clinical Service<sup>a</sup>**

| Autoantibody Type    | No. Positive/No. Tested (% Positive) |
|----------------------|--------------------------------------|
| ANA ( $\geq 1:160$ ) | 12/44 (27)                           |
| Anti-SSA             | 2/11 (18)                            |
| Anti-SSB             | 1/11 (9)                             |
| Anti-dsDNA           | 2/11 (18)                            |
| Anti-RNP             | 2/9 (22)                             |
| Anti-Sm              | 0/5 (0)                              |
| Anti-Scl70           | 0/3 (0)                              |
| Anti-CCP             | 1/7 (14)                             |
| AMA                  | 0/2 (0)                              |
| Anti-GBM             | 0/1 (0)                              |
| Rheumatoid factor    | 1/8 (13)                             |
| ANCA                 | 2/13 (15)                            |

AMA, anti-mitochondrial antibody; ANA, antinuclear antibody; ANCA, anti-neutrophil cytoplasmic antibody; CCP, cyclic citrullinated peptide; dsDNA, double-stranded DNA; GBM, glomerular basement membrane; RNP, ribonucleoprotein; Scl70, scleroderma 70 antigen (topoisomerase I); Sm, Smith antigen; SSA, Sjögren syndrome A; SSB, Sjögren syndrome B.

<sup>a</sup>Sixteen (36%) of 45 patients had at least one positive autoantibody study. The total number of positives in the table exceeds 16 since some patients had more than one positive autoantibody study.

immunotyping, we noted increased serum free light chain ( $\kappa/\lambda$ ) ratios in only two of 16 patients evaluated—most patients had increases in both serum free  $\kappa$  and serum free  $\lambda$  light chains, with normal  $\kappa/\lambda$  ratios. This observation initially appears discordant from the study of Grados et al,<sup>23</sup> who demonstrated abnormal serum free light chain ratios in IgG4-RD. However, we only had serum free light chain data in just over 5% of our study population. Furthermore, the portion of the present cohort for which serum free light chain studies were ordered by the clinical service included only two patients with pseudoclonal ( $\kappa$ -restricted) patterns on immunotyping—one of these two had an abnormal serum free light chain ratio. Thus, it is possible that the free serum  $\kappa$  skew may be less reproducible when surveyed over the broader group of individuals with increased serum IgG4 level, rather than individuals with known or presumed IgG4-RD. It is also likely that the precise mechanism(s) for the apparent expansion of  $\kappa$  light chain-expressing IgG4 in some clinical settings needs further study.

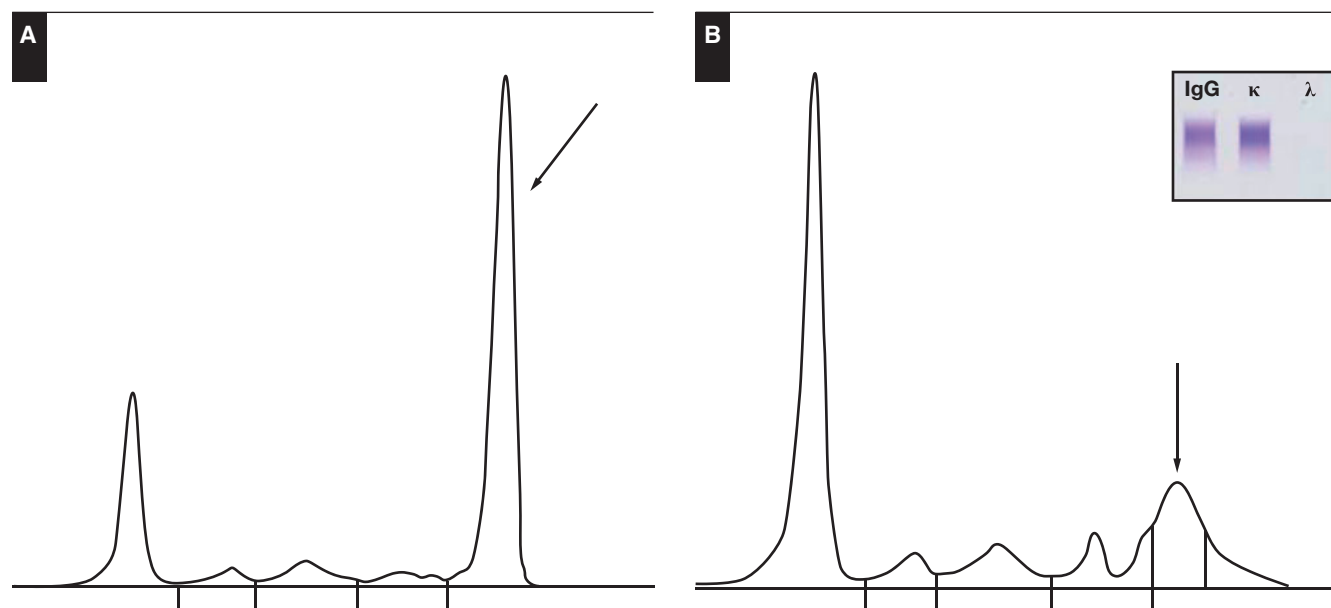


**Figure 5** **A**, Capillary electrophoresis patterns from a patient who initially had an immunoglobulin G4 (IgG)– $\lambda$  monoclonal gammopathy (highlighted by the arrow) associated with plasma cell myeloma. **B**, Four years later, an IgG4 peak is demonstrated (highlighted by the arrow), mimicking the initial M protein. However, gel immunofixation (inset) confirmed an IgG– $\kappa$  pseudoclonal pattern different from the original IgG– $\lambda$  and consistent with the measured IgG4 level of 1,640 mg/dL (reference range, 3–201 mg/dL).

Interestingly, we observed a single case in which the pseudoclonal pattern appeared to be  $\lambda$  restricted—this case was shown to be polyclonal by isoelectric focusing (Figure 4). Jacobs et al<sup>21</sup> observed apparent  $\lambda$  restriction in one of their three reported cases. In light of the known increase in the  $\kappa/\lambda$  ratio within the IgG4 subclass relative to other IgG subclasses, as well as the observed  $\kappa$  restriction in all but one of our evaluated cases, the reason for this one example of apparent  $\lambda$  restriction is not clear and likely warrants further study.

Although initial examination of capillary electropherograms in the setting of increased IgG4 could lead to the conclusion that a monoclonal restriction (M protein) is present, distinct, albeit subjective, characteristics should lead to the consideration of polyclonal IgG4. The IgG4 peak has a distinct location in the anodal portion of the  $\gamma$  region just cathodal to the  $\beta$ – $\gamma$  junction (sometimes mimicking the  $\beta$ – $\gamma$  bridging of serum immunoglobulin A (IgA) increases such as may be seen in liver disease or mucosal inflammation), with a fairly consistent migration location relative to the serum albumin peak, as detailed above. Furthermore, there is a uniquely rounded, “bell-shaped” quality to the IgG4 peak (Figures 1 and 2). While this pattern may be mimicked by true M proteins (particularly of the IgM or IgA type), its presence should lead to at least consideration of a polyclonal IgG4 increase mimicking a monoclonal gammopathy. Further evaluation may include nephelometric determination of serum IgG4 concentration (a routine procedure in our

laboratory) or immunosubtraction/immunotyping/immunofixation to demonstrate either the polytypic or  $\kappa$ -skewed nature of the peak in most cases (with one exception in our study). The current study further demonstrated a lack of clonality in such cases by isoelectric focusing, but this method is not widely validated for clinical determination of clonality in the setting of serum gammopathy and may be less practical for routine use by clinical immunology laboratories.

The retrospective nature of the current study and the inconsistent availability of detailed medical records for review precluded a formal statistical clinicopathologic analysis of SPEP IgG4 patterns. However, we did observe that in patients with the highest levels of IgG4 (at least 1,150 mg/dL) as initially identified by SPEP pattern, the medical record contained some level of clinical, radiographic, or biopsy evidence of conditions believed to be on the spectrum of IgG4-RD. Due to the incomplete availability of clinical correlation in this study, we cannot rule out additional cases of IgG4-RD. However, previous studies have indicated that increased serum IgG4, while approximately 90% sensitive for the presence of IgG4-RD, has low specificity and low positive predictive value.<sup>27</sup> This reinforces the notion that modest increases in serum IgG4 may accompany a number of clinical conditions.

We observed positive autoimmune serologic results in more than one-third of the 45 patients who had these tests

ordered as part of their medical workup by the clinical service. One case with a positive ANA study had minimally elevated IgG4 level (208 mg/dL); however, all other cases with positive autoantibody studies had levels of at least 300 mg/dL. Autoimmune disorders, including microscopic vasculitides, have been associated with increased serum IgG4 levels. Our clinical cohort included two documented cases of ANCA-positive microscopic vasculitis, including one case of granulomatosis with polyangiitis—conditions known to be associated with increased IgG4 levels but not generally included in the category of IgG4-RD.<sup>28</sup>

In summary, this study documents a series of more than 300 cases in which a distinctive SPEP pattern led to suspicion of increased serum IgG4 level—a pattern that at times may mimic monoclonal gammopathy, even on immunosubtraction/immunofixation. This may pose a substantial diagnostic pitfall for pathologists and laboratory professionals providing medical interpretation of SPEP patterns. Although the literature suggests this is likely to be an inefficient screening method for conditions related to increased serum IgG4, this distinct finding on SPEP may herald the presence of disorders known to be associated with increased IgG4 levels and, as such, may provide useful information to the medical record.

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## References

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