Relatively Restricted Migration of Polyclonal IgG4 May Mimic a Monoclonal Gammopathy in IgG4-Related Disease

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ABSTRACT

Objectives: IgG4-related disease (IgG4-RD) is an increasingly recognized syndrome of unknown etiology that can affect a wide variety of organs. The commonly shared features include tumor-like swelling of the involved organs, a lymphoplasmacytic infiltrate enriched with polyclonal IgG4-positive plasma cells, variable degree of fibrosis, and elevated serum concentrations of polyclonal IgG4.

Methods: In a qualitative retrospective study, the electrophoretic characteristics of serum from patients with increased polyclonal IgG4 were studied to see if a reproducible pattern could be identified.

Results: We demonstrate that a characteristic focal band bridging the β and γ fraction by serum protein electrophoresis may be a first serologic indication for IgG4-RD. We further demonstrate that significant κ:λ skewing can occur in the polyclonal IgG4 fraction.

Conclusions: The focal band detected by electrophoresis in sera from patients with IgG4-RD can be confirmed as polyclonal by immunofixation or immunosubtraction. Because these bands may be predominately of one light chain isotype, they could be misinterpreted as monoclonal gammopathies.

IgG4-related disease (IgG4-RD) is an increasingly recognized syndrome of unknown etiology comprising a collection of disorders that share specific pathologic, serologic, and clinical features.1

Before it was recognized as a systemic condition in 2003, the seemingly disparate conditions observed in multiple organs were thought to be unrelated.2,3 The commonly shared features in IgG4-RD include tumor-like swelling of involved organs, a lymphoplasmacytic infiltrate enriched with IgG4-positive plasma cells, and a variable degree of fibrosis. In addition, elevated serum concentrations of polyclonal IgG4 are found in 60% to 70% of patients with IgG4-RD.4 Although the overall disease epidemiology remains largely undefined, most patients are older than 50 years and respond to glucocorticoids within weeks, particularly in early stages of disease.5

Because of the wide variety of clinical manifestations of IgG4-RD and a lack of familiarity with this relatively new and rare syndrome, diagnosing a new patient is quite a challenge. There is usually a delay of more than 1 year from presentation to IgG4-RD diagnosis.6 During the workup of such complex conditions, serum protein electrophoresis (SPE) may be performed.

In general, SPE is performed to screen for symptoms suggesting the presence of a monoclonal gammopathy. An additional homogeneous peak in a focal region of the SPE spectrum is indicative of a monoclonal gammopathy. Monoclonal gammopathies are associated with a clonal plasma cell proliferative process that is malignant or potentially malignant, including multiple myeloma, Waldenström macroglobulinemia, solitary plasmacytoma, smoldering multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), and amyloid light-chain amyloidosis.
In contrast, polyclonal gammopathies may be caused by any reactive or inflammatory process. Differentiation of a monoclonal from a polyclonal increase in immunoglobulins is critical and can be done with immunofixation electrophoresis (IFE) or immunosubtraction (ISUB). A monoclonal gammopathy is characterized by the presence of a sharp, well-defined band on the SPE with a single heavy chain and a similar band with a κ or λ light chain by IFE or ISUB. A polyclonal gammopathy is characterized by a broad diffuse increase of the γ-globulin zone caused by one or more heavy chains and both κ and λ light chains.

Here we demonstrate that with SPE, polyclonal IgG4 antibodies migrate to a well-defined, focal, yet relatively broad electrophoretic area. In patients with relatively high concentrations of polyclonal IgG4, a characteristic focal band bridging the β and γ fraction in the SPE spectrum is observed, which may be the first indication that the underlying process may be IgG4-RD. Furthermore, we demonstrate that significant κ:λ skewing can occur in the polyclonal IgG4 fraction. The focal band detected by electrophoresis in sera from patients with IgG4-RD could be misinterpreted as a monoclonal gammopathy, which could lead to unnecessary other invasive diagnostic tests and cause diagnostic delay.

Materials and Methods

In this retrospective study, routine diagnostic samples from patients with IgG4-RD were selected that gave a characteristic focal band by SPE of which the laboratory specialist found it difficult to differentiate if the focal band was of monoclonal or polyclonal origin. Further cases to illustrate the electrophoretic migration properties of IgG4 antibodies were selected from the Radboud University Medical Center, Nijmegen, The Netherlands. All experiments were conducted in accordance with the Helsinki Declaration of 1975.

Electrophoretic Techniques

Agarose gel electrophoresis (AGE) and IFE were performed on the Hydrasys (Sebia, Evry, France) combined with reagents from Sebia according to the manufacturer’s protocol. For AGE, 10 µL of serum was applied to agarose gel in a proprietary buffer and subjected to electrophoresis. Proteins were stained with amido black, and an electropherogram was generated by scanning densitometry. For IFE, serum proteins were separated on Sebia 4IF gels in a proprietary buffer and then precipitated in the presence of specific antisera to γ, α, and μ heavy chains as well as κ and λ light chains. To further type IgG subclass, we performed IFE using monoclonal antisera for the IgG subclasses as described previously. Proteins were stained with acid violet following the removal of unprecipitated protein.

Capillary electrophoresis (CE) and ISUB were performed on the Capillaries (Sebia, Norcross, GA) combined with reagents from Sebia. Manufacturer’s guidelines were followed in performing the analysis, as described previously. For both CE and ISUB, proteins were separated using a proprietary borate buffer (pH 9.9) through a narrow-bore fused silica capillary at constant high voltage. Diluted patient serum was injected into the capillaries and directly detected by monitoring absorbance to generate an electropherogram. ISUB was performed as for CE, except that sera were preincubated with specific antisera to γ, α, and μ heavy chains as well as κ and λ light chains.

Results

AGE and IFE in Patients With IgG4-RD

In Figure 1, we show SPE results using AGE of three patients before they were diagnosed with IgG4-RD. All three patients have an abnormal spectrum because a focal band bridging the β and γ fraction of the spectrum is observed (Figure 1D for comparison with a healthy control). In addition to their elevated IgG4 subclass value of, respectively, 390 mg/dL, 2,420 mg/dL, and 3,060 mg/dL (reference value, 3-200 mg/dL), all three patients have hypergammaglobulinemia. Since the hypergammaglobulinemia of these three patients is strongly skewed toward the β fraction, it can be clearly differentiated from the more typical form of hypergammaglobulinemias (Figure 1E). It differs from the typical β/γ bridging found with a polyclonal increase in IgA because the increase is in the γ and not the β/γ region. The AGE results of the three patients with IgG4-RD can strongly resemble patterns observed in patients with a monoclonal protein (Figure 1F-1H for comparison). The IFE data presented in demonstrate that the focal band observed in AGE of all three patients with IgG4-RD consists of IgG antibodies. The connecting light chains are mainly κ in patient A, normally distributed in patient B, and mainly λ in patient C. To identify the IgG subclasses of the focal IgG, we performed IFE using monospecific antisera for each subclass. In all three patients, the focal band consisted of IgG4 antibodies Figure 3A. Figure 3B visualizes the IgG subclasses in four additional patients with IgG4-RD using IFE. These findings indicate that IgG4 overwhelmingly predominates in the anodal end of the γ fraction.

CE and ISUB in Patients With IgG4-RD

Similar observations about the position and skewing of the κ:λ ratio in sera from patients with a polyclonal increase in IgG4 subclass can be made using CE. In Figure 4A and Figure 4B, the CE and ISUB on serum from two patients diagnosed with IgG4-RD and a marked increase in IgG4...
subclass of, respectively, 5,080 mg/dL and 6,680 mg/dL are shown (reference value, 3-200 mg/dL). The marked increase in the anodal portion of the γ region is in the same position as seen in the SPE (Figure 1A-1C). The ISUB shows considerably greater removal with the anti-κ antisera than by the anti-λ antisera in both patients. In addition to IgG4, we have found that individuals with an isolated increase in polyclonal IgM may have a similar restriction in the anodal end of the γ fraction. In Figure 4C, the CE and ISUB results of a patient with a polyclonal increase in IgM are shown.
Electrophoretic Migration Properties of IgG Subclasses

It is well known that human IgG, IgA, and IgM antibodies each have a unique electrophoretic migration pattern. In a schematic overview, we show that the IgG subclasses also each have their own unique electrophoretic distribution. IgG4 subclass and IgA and IgM immunoglobulins overlap in their migration with similar, although not identical, midpoints.

Discussion

The primary objective of this retrospective study is to create awareness of the analytical challenge of interpreting SPE data in patients with IgG4-RD. For laboratory specialists who interpret SPE patterns, it is important to realize that each of the four IgG subclasses have their own unique electrophoretic position. Consequently, a restricted mobility that resembles a monoclonal gammopathy may be seen in sera that have a polyclonal increase in the IgG4 subclass.

Soon after the introduction of gel electrophoresis for serum protein fractionation, it was documented that human IgG, IgA, and IgM antibodies each have a unique electrophoretic migration pattern. IgA monoclonal proteins, for example, often comigrate with the β region transferring and C3 bands during electrophoresis, which makes detection and quantification of these monoclonal proteins more difficult. Although it is technically feasible to perform subclass typing of IgG monoclonal proteins by IFE, this is not done in routine laboratory diagnostics since IgG subclass typing of monoclonal gammopathies has no prognostic or therapeutic consequences. The consequence is that many laboratory specialists are not aware that IgG subclasses each have their own unique electrophoretic migration pattern.
In this report, we have shown that polyclonal IgG4 antibodies migrate to a well-defined, focal, yet relatively broad electrophoretic area. A relatively high serum concentration of IgG4 will, therefore, always give rise to a focal band at the anodal end of the γ fraction, irrespective of whether it is polyclonal or monoclonal IgG4. One should be aware, however, that a polyclonal increase in IgM can produce a similar restriction since, as shown in Figure 5, the normal IgM and IgG4 subclass immunoglobulins overlap in their migrations with similar, although not identical, midpoints. Strong skewing of the polyclonal IgG4 antibodies toward either IgG4-κ or IgG4-λ, visualized by either IFE or ISUB, can further mimic a monoclonal appearance in SPE analysis.

With the emergence in the past decade of IgG4-RD as a new clinical entity, we argue that awareness and correct interpretation of the specific electrophoretic migration of IgG4 are important. On one hand, for patients with relatively high concentrations of polyclonal IgG4, this characteristic focal band bridging the β and γ fraction in the SPE spectrum may be a first indication that the patient’s symptoms may be related to IgG4-RD. On the other hand, because this focal band may be
misinterpreted as a monoclonal gammopathy, this could be a trigger for needless invasive diagnostic tests that create risk for the patient and may delay the correct diagnosis.

Since patients with newly diagnosed IgG4–multiple myeloma often have a more discrete M-spike and a suppressed concentration of polyclonal immunoglobulins (as visualized in Figure 1H), this may help to differentiate them from patients with IgG4-RD. However, this does not hold true for patients with IgG4-MGUS or IgG4–multiple myeloma who have a low tumor burden or are in remission.

Recently, Grados et al15 have shown that the serum free light-chain (FLC) κ:λ ratio can be strongly abnormal in patients with IgG4-RD. This has two important implications. First, it suggests that our finding of strong skewing toward either IgG4-κ or IgG4-κ is not uncommon in patients with IgG4-RD. Second, since nephelometric FLC measurements often have an abnormal FLC κ:λ ratio in IgG4-RD, FLC analysis may not be helpful to further distinguish between polyclonal or monoclonal IgG4. Because of these findings, when we detect a fast γ restriction due to IgG4 subclass, which also has a disproportionate predominance of one light-chain class, we correlate our findings with the clinical picture prior to making a final diagnosis of a monoclonal gammopathy.

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