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Clinical significance of serum triple monoclonal components: A report of 6 cases and a review of the literature

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ABSTRACT

A serum multiple monoclonal component (MC) is very rare. We here report 6 patients with 3 MCs. The triple MC was detected in all of them by immunofixation. 2/6 patients did not present hematological or oncological associated disease, while in the remaining 4, Waldenström macroglobulinaemia (2 cases), Polycythemia Vera and non-Hodgkin lymphoma were diagnosed. Of the 49 global patients reported in the literature (6+43), 64.6% had a lymphoproliferative disorder and only in 3 cases there was no associated disease. Therefore, the detection of such laboratory evidence should propel physicians to a deeper investigation.

1. Introduction

The occurrence of multiple serum monoclonal components (MCs) is rare. We recently observed that a double MC is often associated with another disease, especially a hematological malignancy [1]. Serum triple MCs are even rarer, and very little is known about their clinical significance. We here report our experience with patients who have three serum MCs. In addition, we have reviewed all of the international literature concerning patients with serum triple MCs, focusing on the associated diseases.

2. Patients and methods

Six patients (3M; 3F) with 3 serum MCs were included in this study. Four of these patients (#1, #2, #3, and #4 in Table 1) were discovered during our previous study on 34 patients with serum double monoclonal components observed from January 1996 to December 2011 [1]. Their ages ranged between 67 and 88 years (median age: 77.1). All patients underwent a routine laboratory work-up, bone marrow aspiration and biopsy, and skeleton X-ray. For patients in whom the diagnosis was made more recently, cytogenetic analysis and immunofluorescence studies were also performed. It is remarkable that in each case, the single or multiple MC was suspected—or evidenced—by high-resolution serum protein electrophoresis (on agarose gel) and subsequently confirmed and characterized by immunofixation (IF). IF was performed using an agarose film and antisera monospecific for the heavy and light chains of human immunoglobulins (anti-γ, anti-α, anti-μ, anti-δ, anti-ε, anti-κ, and anti-λ). Antisera were tested for specificity against known cases of multiple myeloma. In patients #4, 5, and 6 (Table 1), an intracellular immunofluorescence study was also performed using fluorochrome-conjugated goat antibodies specific for human μ, γ, or α immunoglobulin heavy chains and κ or λ light chains. Briefly, 1×10^5 cells in 0.1 ml obtained from nucleated bone marrow cells, were spun onto glass slides by cytocentrifugation, fixed in an ethanol–acetic acid solution (1:20) at 20 °C for 20 min, and rehydrated in PBS. The cytological preparations were sequentially incubated with 10 μL of diluted tetramethylrhodamine isothiocyanate and fluorescein isothiocyanate-conjugated antibodies to human heavy or light chains for 20 min at room temperature. After washing, the slides were examined with fluorescence microscopy using selective filters for rhodamine or fluorescein (Table 2).

3. Results

Two of the 6 considered patients (patients #1 and #5 of Table 1) did not present hematological or oncological associated diseases. Specifically, they did not have hypercalcemia, osteolytic lesions, anemia, renal failure, or other evidence of myeloma, nor did they meet the accepted criteria for the diagnosis of Waldenström macroglobulinemia (WM), amyloidosis, lymphoma, or other chronic lymphoproliferative diseases. Moreover, they did not present autoimmune or infectious disorders. For the remaining 4/6 subjects (patients #2, #3, #4, and #6 of Table 1), the associated diseases were WM, WM, polycythemia vera, and non-Hodgkin

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Table 1
Patients with three serum monoclonal components.

Pt #	Sex (age)	Serum MCs (IF)	Urine MCs (IF)	Associated disease
1	♀ (88)	IgGκ IgGλ IgMκ	κ	None
2	♂ (67)	IgMκ IgMκ IgMλ	None	WM
3	♂ (70)	IgMκ IgMκ IgMλ	κ	WM
4	♂ (77)	IgGκ IgGλ IgAκ	κ	PV
5	♀ (82)	IgGκ IgGλ IgAλ	None	None
6	♀ (79)	IgGκ IgGλ IgMκ	λ	NHL

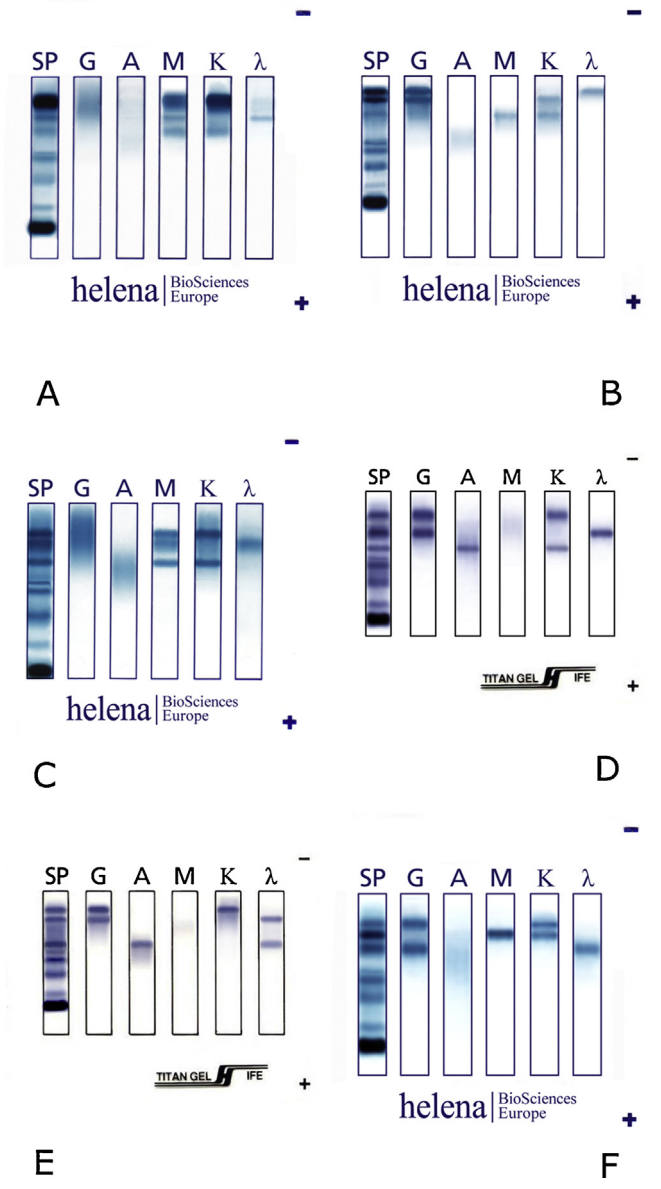

Fig. 1. (A–F) Immunofixation detecting and characterizing the triple MC in the reported patients (1–6).

Table 3
Immunofluorescence analysis of bone marrow plasma cells.

Combination of class-specific antibodies			Plasma cells in the bone marrow (%)		
RITC-conjugated		FITC-conjugated	Pt #4	Pt #5	Pt #6
Anti-γ	+	Anti-α	0	0	0
Anti-γ	+	Anti-μ	0	0	0
Anti-α	+	Anti-μ	0	0	0
Anti-γ	+	Anti-κ	70	25	70
Anti-γ	+	Anti-λ	30	75	30
Anti-α	+	Anti-κ	100	0	0
Anti-α	+	Anti-λ	<1	100	0
Anti-μ	+	Anti-κ	0	0	100
Anti-μ	+	Anti-λ	0	0	<1

lymphoma, respectively. In patients #4 and #6, the 3 MCs were detected before diagnosis of the hematologic malignancy, whereas in patients #2 and #3, the two diagnoses were concomitant. Serum electrophoresis showed three different picks in the γ-region only in one case (patient #6). In 2/6 cases (patients #1 and 2), only one localized band was detected, whereas 2 bands were observed in 3/6 patients (patients #3, 4, and 5). The triple MC was detected and characterized by IF in all 6 patients (Fig. 1A–F). The 18 MCs detected in our patients were 8 IgG, 8 IgM, and 2 IgA. There was no significant difference in the κ and λ chain distribution. Intracellular immunofluorescence staining (Table 3) demonstrated that 3 MCs were produced by 3 different plasma cell populations because there was no simultaneous detection of immunoglobulins of different classes in their cytoplasm. In patient #4, 100% of plasma cells positive for α heavy chains were simultaneously positive for κ light chains but negative for λ light chains; moreover, 70% of γ-positive plasma cells reacted with anti-κ chain antibodies and 30% with anti-λ chain antibodies. In patient #5, 100% of plasma cells positive for α-chains were also positive for λ-chains but negative for κ-chains; in addition, 75% and 25% of γ-positive plasma cells reacted with anti-λ and anti-κ antibodies, respectively. In patient #6, 100% of plasma cells that were μ-positive were simultaneously κ-positive, but λ-negative; furthermore, 70% and 30% of γ-positive plasma cells were κ- and λ-positive, respectively. Bone marrow infiltration by plasma cells ranged between 1% and 4%. In the 2 WM patients (patients #2 and 3 in Table 1), lymphoplasmacytoid cells were also observed (13.8% and 12%, respectively).

4. Discussion

The simultaneous occurrence of 3 different serum monoclonal components (MCs) in one patient is rare, and its exact incidence is not known. Pruzanski [32] reported 1 triclonal component (TC) out of 789 monoclonal gammopathies, whereas Kyle [21] reported 1 TC and 57 biclonal components out of 3447 patients with serum MCs. Most of the reported cases in the English literature (Table 4) concern a single patient, and only one author [13] described 4 cases of serum TC. To the best of our knowledge, this represents the first description of 6 consecutive cases. Four of these patients had a hematological malignancy, and in particular, 3/4

Table 2
Bone marrow plasma cells and biochemical values in patients with serum triple paraprotein.

Pt #	PC (%)	Hgb (g/dl)	Crea (mg/dl)	β_2MG (mg/l)	LDH (U/l)	Ca (mg/dl)	SP (g/dl)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)
1	3.0	9.8	1.6	7.4	370	8.3	7.7	2532	117	498
2	3.2[a]	12.9	1.2	2.5	355	9.5	7.7	646	107	1275
3	1.0[a]	14.5	1.1	3.2	320	10.1	9.1	1352	421	1688
4	4.0	17.7	1.0	5	530	9.1	8.4	2727	563	86
5	3.3	10	0.9	2.7	290	8.8	7.4	822	595	22
6	4.0	12.7	1.0	2.9	530	9.1	7.3	1690	250	2090

[a] Patients # 2 and 3 had WM and in their bone marrow also lymphoplasmacytoid cells were present (13.8%, and 12%, respectively).