

Three Cases Showing False Results in the Detection of Monoclonal Components Using Capillary Electrophoresis

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DOI: 10.1309/LMHZLIMXVYLWT7SQ

Abstract

In the detection of monoclonal proteins using capillary electrophoresis (CE), several problems have been described, especially for small amounts of paraprotein, light chain (LC) disease, and cases associated with contrast interference. In this report, we describe 3

unusual cases that showed false-positive peaks due to radiologic contrast interference and the escape of monoclonal LC in CE. In these cases questionable for monoclonal components, conventional gel tests and free LC assays could be helpful to confirm monoclonal gammopathy. Therefore, multiple methodological modalities

should be combined for diagnosing and monitoring plasma cell neoplasms and related disorders.

Keywords: capillary electrophoresis, contrast interference, sensitivity, monoclonal gammopathy

Capillary electrophoresis (CE) is an automated method for protein separation in blood or other body fluids.¹ It is increasingly used in clinical laboratories because of its high throughput, speed, and technical convenience.² In addition to electrophoretic mobility, electroendosmosis is an important phenomenon improving the resolution of CE.³ A sample can be subsequently precipitated with specific antisera, and the presence of monoclonal components reduces the peak (immunosubtraction [IS]), which is known as immunotyping (IT).⁴

However, problems with the detection of monoclonal proteins by CE have been described, especially for small amounts of paraprotein, light chain (LC) disease, and cases

associated with contrast interference.⁵⁻⁷ We have been performing CE/IT (Sebia, Lysse, France) for 15 months, and here we discuss 3 unusual cases that recently occurred in our laboratory.

Case Report 1

A 56-year-old Korean male with a history of diabetes mellitus, hypertension, and dyslipidemia was transferred to our hospital on August 10, 2010. He complained of weakness on his left side that began 4 days prior to hospitalization. A brain computed tomography (CT) with contrast enhancement and cerebral angiography revealed cerebral infarctions and arterial occlusion in the right-middle cerebral artery. Medical therapy and radiologic intervention were performed. About 48 hours after contrast injection, CE was used to analyze serum and urine specimens to quantify protein fractions. Results of serum CE showed a suspicious peak that was more visible in the urine CE (**Figure 1**). However, subsequent IS failed to identify a monoclonal component in either the serum or urine specimens. Moreover, results from the quantitative assays of immunoglobulin (Ig) and Ig free light chain (FLC) were also within the reference ranges. To consider every possibility, we asked the clinician about the sampling time and learned that the blood sample had been drawn immediately after the contrast injection for the brain CT. This raised suspicion that the peak could have resulted from interference by the iodinated contrast agent. Because the patient did not have any other clinical or laboratory findings associated with paraproteinemia, we decided to follow up on the CE results later, and we notified the clinicians about the possibility of contrast

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Abbreviations

CE, capillary electrophoresis; LC, light chain; IS, immunosubtraction; IT, immunotyping; CT, computed tomography; Ig, immunoglobulin; FLC, free light chain; rFLC, ratio of free light chain; EP, electrophoresis; IFE, immunofixation electrophoresis; UV, ultraviolet

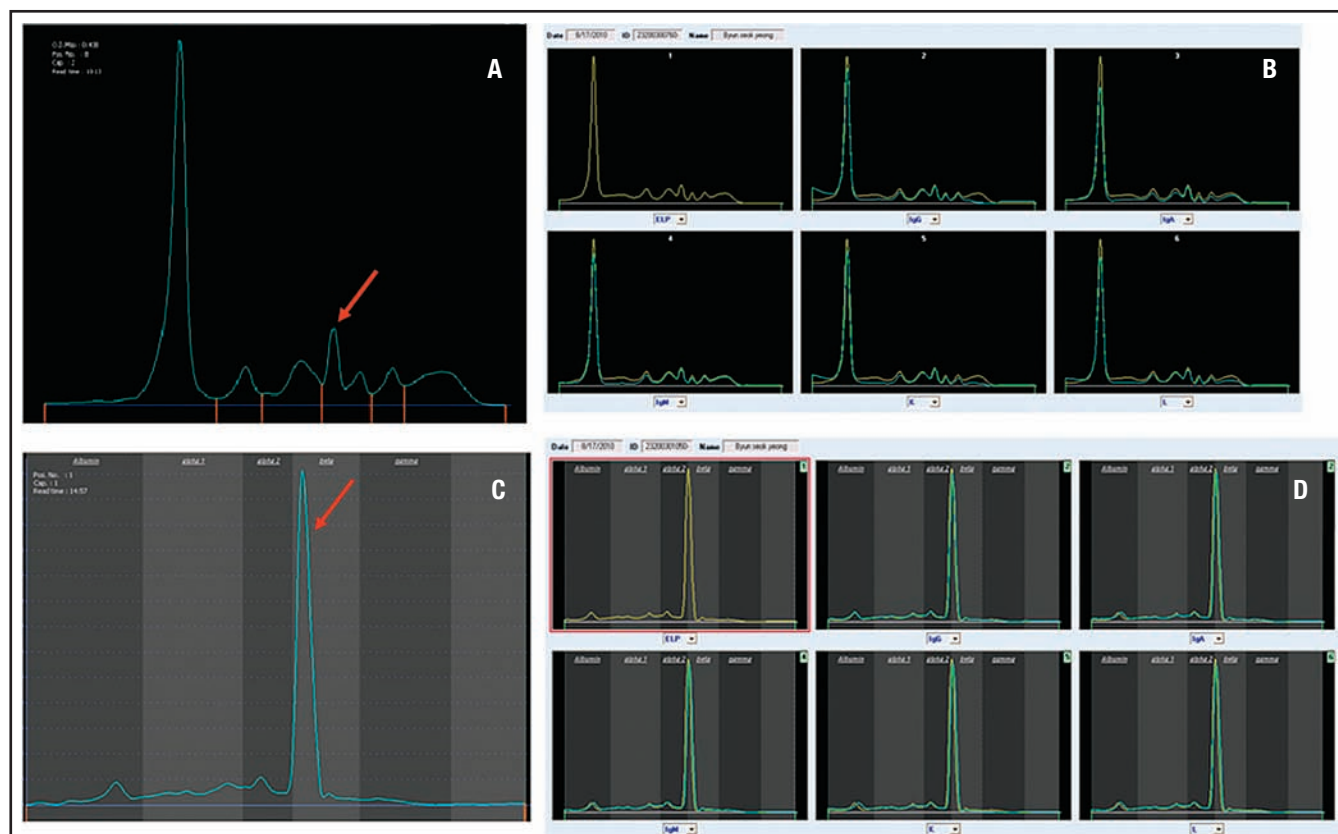


Figure 1 Capillary electrophoresis (CE) and immunosubtraction (IS) results for Case 1. CE in serum (A) and urine (C) samples showed discrete peaks suspected to be the M-component (arrows). However, IS findings of serum (B) and urine (D) specimens presented no subtraction in all of immunotypings (IgG, IgA, IgM, kappa, and lambda light chains), suggesting that these peaks did not show the monoclonality.

interference. After 6 days, the complete disappearance of those peaks was confirmed in serum and urine specimens. Because clinical improvement was noted, the patient was discharged after 10 days for follow-up at the outpatient clinic.

Case Report 2

On August 15, 2010, a 50-year-old male was transferred to our hospital for sudden left-side weakness and an altered mentality that occurred 1 day prior to hospitalization. A brain CT with contrast enhancement was immediately performed and showed an acute cerebral infarction in the right-middle cerebral artery territory. Medical therapy, including anti-hypertensive and anti-platelet drugs, was started in the intensive care unit. About 1 week later, CE/IT was used for protein analysis of serum and urine. Although the serum tests showed no remarkable findings, the urine CE showed a small but discrete peak that was not subtracted in the IT (Figure 2). Based on other laboratory results, such as normal urine FLC assays and a history of CT examination with contrast enhancement (like case 1), we suspected contrast interference again. After we informed the clinicians about the possibility of contrast interference, we waited a few days so the contrast materials could be completely eliminated by the kidneys. Six days later, the small peak from the urine specimen had completely disappeared, just as it had in Case 1.

Case Report 3

A 61-year-old Korean female with nephritic syndrome was admitted to our hospital on September 3, 2010, because of aggravated back pain that had persisted for several months. An initial complete blood count revealed a hemoglobin level of 10.7 g/dL (reference range: 12-16 g/dL), a platelet count of $483 \times 10^9/L$ (reference range: $150-350 \times 10^9/L$), and a WBC count of $7.1 \times 10^9/L$ (reference range: $4-10 \times 10^9/L$) with 62% segmented neutrophils, 29% lymphocytes, 8% monocytes, and 1% eosinophils. Blood biochemistry tests were unremarkable: total protein of 6.0 g/dL (reference range: 5.8-8.0 g/dL), albumin of 3.83 g/dL (reference range: 3.1-5.2 g/dL), BUN 27 mg/dL (reference range: 8-23 mg/dL), and a creatinine level of 1.1 mg/dL (reference range: 0.6-1.2 mg/dL). Urine protein test with 24-hour collected urine was increased up to 2260 mg/day (reference range: 40-150 mg/day). To rule out monoclonal gammopathy, serum CE/IT was completed. Because there was no definite peak or IS we could not find any evidence to imply the clonality. However, in the FLC assay, serum lambda FLC markedly increased to 2940 mg/L (reference range: 5.71-26.3 mg/L), and the kappa/lambda FLC ratio (rFLC) was markedly reversed to 0.005 (reference range: 0.26-1.65; Table 1). In urine, lambda FLC and rFLC also changed significantly to values outside of the reference ranges. Urine CE/IT showed a discrete peak and a definite IS in the lambda LC, suggesting a monoclonal gammopathy. Moreover, radiological findings revealed multiple

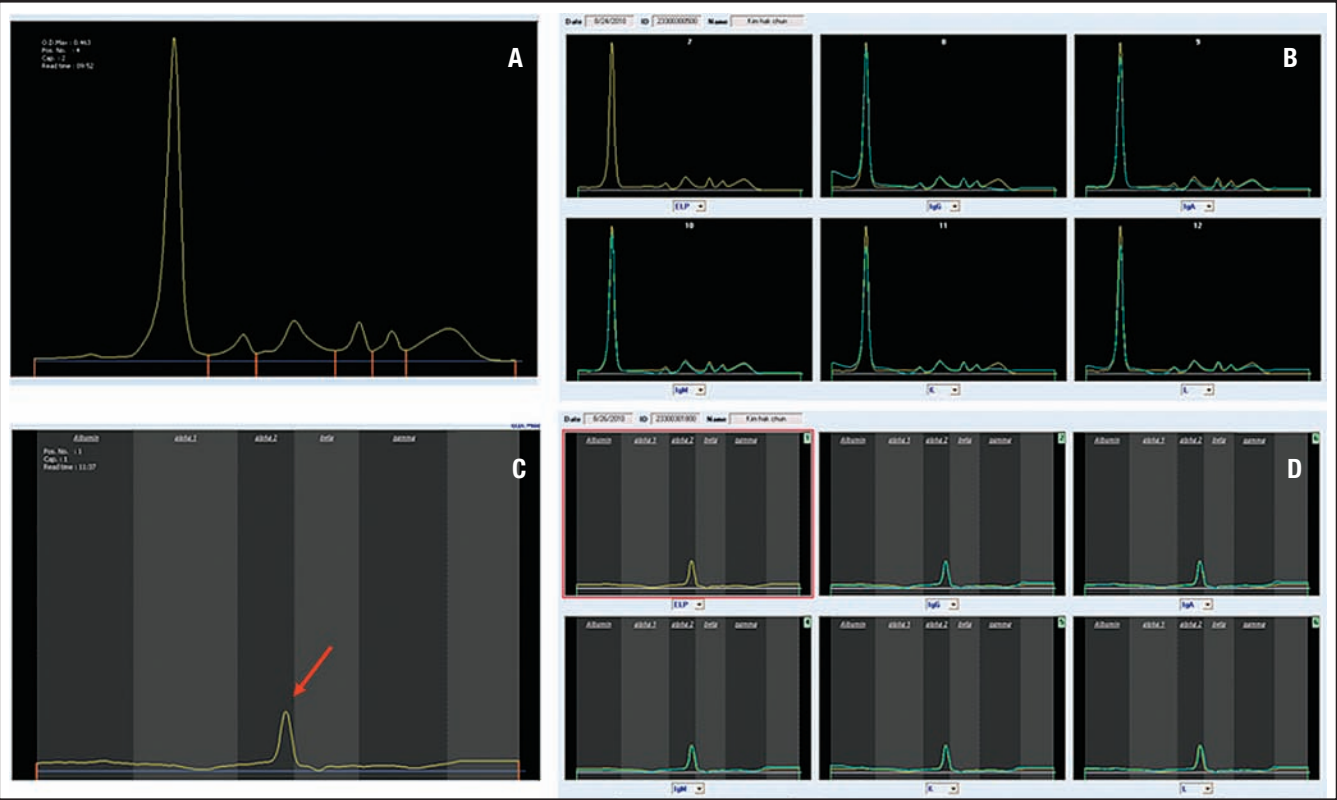


Figure 2 Capillary electrophoresis (CE) and immunosubtraction (IS) results for Case 2. Serum CE (A) and IS (B) showed no remarkable findings. Contrast with Case 1 that showed peaks in serum and urine samples. Case 2 showed the peak only in the urine specimen, suspected to be the M-component. However, urine (D) IS findings showed no subtraction for all of immunotypings (IgG, IgA, IgM, kappa, and lambda light chains), suggesting that these peaks did not show the monoclonality.

punched-out bone lesions, and a bone marrow study showed that increased plasma cells accounted for 20% in all nucleated cells. To solve the discrepancy in these results, conventional gel electrophoresis (EP) was performed with high-resolution gel EP in a Hydrasys analyzer (Sebia) using Hydragel 15 HR gels (Sebia). Gel EP revealed the presence of a discrete peak in blood and urine specimens, and immunofixation electrophoresis (IFE) subsequently identified it as lambda LC. Finally, the patient was diagnosed with a plasma cell

neoplasm. The patient started chemotherapy, and autologous peripheral stem cell transplantation was planned.

Discussion

The identification of paraproteins plays important roles in the diagnosis, classification, and treatment of plasma cell neoplasm.⁸ The method of choice for the detection of para-

Table 1 Serial Follow-up Results in the False-Negative Case of the Monoclonal-Free Light Chain in Capillary Electrophoresis

Results (Reference Range)	2010.09.03	2010.09.06	2010.09.24	2010.11.01
Serum IgG (694-1618 mg/L)	NA	599	NA	843
Serum IgA (68-378 mg/L)	NA	36.8	NA	38.5
Serum IgM (60-263 mg/L)	NA	26.8	NA	39.7
Serum IgE (~100 mg/L)	NA	NA	28.5	<18
Serum kappa FLC (5.71-26.3 mg/L)	15.7	NA	NA	15.7
Serum lambda FLC (3.3-19.4 mg/L)	2940	NA	NA	1560
Serum rFLC (0.26-1.65)	0.005	NA	NA	0.010
Urine kappa FLC (2.04-10.37 mg/L)	NA	15.4	NA	36
Urine lambda FLC (1.35-24.19 mg/L)	NA	3470	NA	4190
Urine rFLC (0.24-6.66)	NA	0.004	NA	0.008

NA, not available; Ig, immunoglobulin; FLC, free light chain; rFLC, ratio of free light chains.

Table 2 Characteristics of Four Cases of Pseudoparaproteinemia Due to Contrast Interference in Capillary Electrophoresis

	van der Watt and Colleagues	Vermeersch and Colleagues	Cho and Colleagues (Case 1)	Cho and Colleagues (Case 2)
Age (yr)	62	5	56	50
Gender	Male	Female	Male	Male
Protein (g/dL)	NA	NA	6.3	6.8
Albumin (g/dL)	NA	NA	3.7	3.8
BUN (mg/dL)	NA	NA	21	19
Creatinine (mg/dL)	NA	NA	0.6	0.6
Main diagnosis	Ischemic heart disease	Fontan operation for a univentricular heart	Cerebral infarction	Cerebral infarction
Radiologic examination using contrast	Coronary angiography	Coronary angiography	Brain CT	Brain CT
Sampling time (after contrast injection)	6 h	During the angiocardiology	48 h	~1 week
Contrast type	Iopamidol (Jopamiron)	Iomeprol (Iomeron)	Iopamidol	Iopamidol
Contrast dose	62 g (100 mL)	6-7 mL/kg	2 mL/kg	2 mL/kg
Instruments	Paragon 2000 (Beckman Coulter)	Capillarys (Sebia)	Minicap (Sebia)	Minicap (Sebia)
Peak region	Alpha2	Beta	Alpha2	Alpha2
Specimen	Serum	Serum	Serum and Urine	Urine
IFE or IS results	No monoclonal protein in IFE	No monoclonal protein in IFE	No subtraction in IS	No subtraction in IS
Reference	[6]	[7]	Present study	Present study

NA, not available; IFE, immunofixation electrophoresis; IS, immunosubtraction.

subclassify the monoclonal components.⁴ However, because these techniques are labor-intensive and lengthy, CE/IT has emerged as a useful alternative method because it is automated, fast, and highly reproducible.^{3,4}

In a conventional gel test, protein quantification is based on dye binding, whereas CE uses ultraviolet (UV) detection.^{9,10} Exogenous UV-absorbing substances do not cause problems in the gel-based methods, but they can interfere with protein detection in CE.⁹⁻¹² There were 2 previous cases that described contrast interference in CE, and, to our knowledge, the cases presented here are the third report. Iopamidol was involved in 3 of the 4 total cases, and all of the false-positive peaks were in the alpha2 or beta region (Table 2). It is important to note that, unlike a real monoclonal peak, the false-positive peak due to contrast was not immunosubtracted in IT. As shown in our cases, contrast interference might be more problematic, especially in urine specimens, because contrast materials become concentrated in urine.⁹ To avoid such interference, instructions should note that sufficient time is required before collecting blood for protein EP by CE after a patient receives iodinated contrast media.¹¹ The mean (SD) half-life of iomeprol in the elimination phase is 109 minutes, and it has been reported that no residual interference was observed in patients after 24 hours. However, in a patient with renal dysfunction, the interference could persist for more than 24 hours. In this situation, use of a desalting column or activated charcoal may be helpful in removing the interference.^{11,12}

The false-negative result in the detection of the M protein using CE has been reported in 5% of samples, with a few substances interfering with CE but not interrupting gel EP.⁵ The performance of CE/IT is similar to that of gel EP/IFE in terms of specificity, but the sensitivity is slightly less than that of gel EP/IFE, especially for small molecules and low concentrations of targeted molecules.¹³ It has been proposed that the monoclonal component could escape from the detection by

CE when it is made up solely of FLCs.¹⁴ Although the reason is not fully known, the UV detection method might be one of the reasons why FLCs are not detected. In these cases, assessment of the FLCs concentration and rFLC can aid CE to identify monoclonal FLCs.¹⁴ Thereafter, we did not discard the gel EP methods entirely, and we routinely perform gel EP/IFE as well as CE on questionable samples for monoclonal components. We also recommended that clinicians not collect blood for CE from those patients who had received contrast media during the preceding week. Based on previous reports and our experience, we suggest that multiple methodological modalities should be combined for diagnosing and monitoring plasma cell neoplasm and related disorders. LM

Both Hee Joo Lee, MD, PhD and Jin-Tae Suh, MD, PhD contributed equally to this work, and both are considered senior author.

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