Validation and Utility of the Free Light Chain Assay in Pleural Effusions of Patients With Multiple Myeloma

Thomas U. Marron,[1] Matthew G. Hanna,[2] Lakshmi Ramanathan,[3] Ajai Chari[1]

Clinical Practice Points

- Myelomatous pleural effusions are rare, portend a poor prognosis, and require rapid clinical intervention.
- The results of the present case series have validated the use of the commercially available free light chain (FLC) assay to detect the presence of free light chains in pleural fluid.
- Myelomatous pleural effusions have greater levels of involved FLCs than do paired serum specimens, likely because of local production.
- Serial measurements of FLCs in pleural effusion fluid might allow for more precise monitoring of localized pleural disease.

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Introduction

Although multiple myeloma (MM) is a bone marrow disorder, ≤ 20% of patients will ultimately develop extramedullary disease that can involve virtually any organ.[1,2] Pleural effusions are also a relatively uncommon occurrence in MM (incidence of 6%)[1] and are most often complications of infection or dysproteinemia, such as nephritic syndromes, congestive heart failure secondary to amyloidosis, or mediastinal lymphatic obstruction. The findings from case reports and larger studies have suggested myelomatous pleural effusions (MPEs) will be present in only 1% to 2% of MM patients,[3,4] and the diagnosis of MPE is ominous, with a median life expectancy of only 4 months.[3,5] The diagnosis of MPE currently depends on a cytologic evaluation of pleural fluid, which is often performed by nonhematopathologists and can also be difficult to perform, given the often anaplastic appearance of extramedullary plasma cells. Immunofixation of the drained pleural fluid is not routine, and it has not previously been determined whether the presence of free light chains (FLCs) in pleural fluid correlates with the diagnosis of MPE.

FLCs are secreted immunoglobulin light chains that are typically bound to the immunoglobulin heavy chains and are normally present at very low levels in the serum, because they are cleared renally 10 to 60 times faster than the usual rate of production.[6] Monoclonal neoplastic plasma cells produce an excess of either kappa or lambda FLCs, and the commercially available assay for FLCs has resulted in significant improvements in the diagnosis and monitoring of MM.[7] We have also shown that the FLC assay appears to be useful in diagnosing MM involvement in the central nervous system, because the FLC levels in the cerebrospinal fluid correlated dynamically with treatment and relapse.[8] To the best of our knowledge, only 2 single-patient case reports have measured FLCs in fluid from a patient with an MPE; however, the assay has neither been validated nor systemically evaluated for use in pleural effusions from patients with MM.[4,9] In 1 of these cases, a significantly greater concentration of involved FLCs and a significantly higher FLC ratio was found in the pleural fluid, which the investigators suggested was indicative of local production of FLCs in the pleural space by plasmacytoma.[4] The other study demonstrated a lower FLC ratio in the MPE fluid, countering the suggestion that the FLC assay might be useful in diagnosing MPE.[9] We, therefore, sought to first validate the use of the FLC assay in pleural effusions of control specimens to which the FLCs from MM patients were added and to then systematically examine the utility of the FLC assay in a larger series of MM patients with pleural effusions.

[1]Division of Hematology and Oncology, Department of Medicine
[2]Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY
[3]Department of Laboratory Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY

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Address for correspondence: Ajai Chari, MD, Division of Hematology and Oncology, Department of Medicine, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, Box 1185, New York, NY 10029.
E-mail contact: ajai.chari@mountsinai.org

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Table 1 Demographic Data of Patients at Pleural Effusion Drainage

Pt. No.	MM Isotype	Age at MM Diagnosis (Years)	Time to First Study Fluid (mo)	Lines of Therapy (n)	Systemic Disease Status	Durie-Salmon Stage	International Staging System	Molecular High Risk	Time to Death (mo)
1	IgA-λ	54	46	8	PD	IIIA	Unknown	t(14:16)	6
2	κ-LC	64	8	1	PD	IIIB	III	None	4
3	IgA-κ	63	<1	0	NDMM	IIIA	III	Amp Ch1	20
4	IgA-λ	72	34	3	PD	IIIA	Unknown	Del p53	2
5	IgG-λ	68	96	7	PD	NA	Unknown	Del 17p	3
6	IgA-λ	64	49	8	PD	IIIB	Unknown	None	1
7	λ-LC	53	32	2	CR	IIIA	II	NA	Alive
8	IgG-λ	67	9	1	sCR	IA	I	None	Alive
9	IgG-κ	58	34	5	PD	IIIB	Unknown	None	1
10	κ-LC	69	16	2	PD	IIIA	I	None	16
11	κ-LC	62	18	3	PD	IA	Unknown	None	Alive
Median		64	32	3					

Abbreviations: Amp = amplification; Del = deletion; LC = light chain; MM = multiple myeloma; NA = not available; NDMM = newly diagnosed MM; PD = progressive disease; sCR = stringent complete response.

Materials and Methods

A validation study was performed using 8 patients who had no known diagnosis of a B-cell or plasma cell malignancy. Given the role of renal excretion in maintaining FLC homeostasis, patients with a diagnosis of chronic or end-stage kidney disease were excluded. FLC analysis was performed on remaining fluid in the Clinical Chemistry Laboratory of the Department of Pathology, Mount Sinai Hospital, using the SPAplus Automated Analyzer and the Freelite Serum Free Light Chain Assay (Binding Site, Birmingham, UK) according to the manufacturer's instructions for serum samples. After baseline pleural fluid FLC measurements, the pleural fluid was spiked with the serum remaining after routine clinical testing from patients with diagnosed MM with high concentrations of FLC. The spiked pleural fluid was then tested for kappa and lambda FLCs at 1:10 and, subsequently, 1:100 dilution factors. Kappa and lambda FLCs were tested separately to distinguish whether the pleural fluid matrix had any effect on the detection sensitivity of each FLC individually.

In those MM patients who had undergone thoracenteses for routine clinical indications, the FLCs were measured in the pleural fluid remaining after all routine testing had been performed. For each patient, the involved FLCs, uFLCs, FLC ratio, and difference between the involved and uFLCs were calculated in the serum and pleural fluid. All additional clinical data, including demographic data, cytology, and Light's criteria, were obtained from the electronic medical record. The institutional review board at the Icahn School of Medicine at Mount Sinai approved the present study, in accordance with federal regulations.

Results

The Freelite assay reliably measured the FLCs in the control pleural fluid specimens spiked with FLC-rich serum from a MM patient with active disease at 1:10 and 1:100 dilutions, which confirmed that the chemical composition of the pleural fluid matrix does not impair the assay's ability to accurately assess the FLC levels (data not shown).

The FLCs were then measured in 15 pleural effusions from 11 MM patients (patient 1 had had the pleural fluid drained at 5 different times). Generally, these patients had either relapsed or refractory MM after many lines of therapy. The median age of the initial MM diagnosis was 64 years; and all the patients, except for 1, whose initial presentation was with an MPE (patient 3), had failed $\geq$ 1 line of therapy, and all but 1 (patient 7) had active systemic disease (Table 1). All the patients had a normal creatinine clearance at the time of thoracentesis, with the exception of patients 7 and 9 (Supplemental Table 1; available online).

Of the 15 pleural effusions, 10 were confirmed to be MPEs based on positive cytology results (Table 2), and 4 of the 6 patients with MPEs had known high molecular risk findings. In these 10 specimens, the involved FLCs (iFLCs; Table 2) were consistently greater than the uninvolved FLCs (uFLCs). Furthermore, the difference between the iFLCs and uFLCs was more pronounced in the pleural fluid than in the serum in all cases of MPE, as demonstrated both by the FLC ratio (iFLC divided by uFLC) and the absolute difference (iFLC minus uFLC [dFLC]).