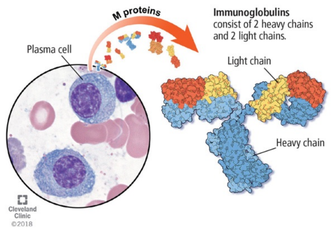
Detection of Patient Monoclonal Serum Free Light Chains by On-Probe Extraction coupled with High-Resolution Mass Spectrometry (OPEX-MS)

Priscilla S.-W. Yeung[1,2], Yajing Liu[1], Ashley Ruan[1], Christina Kerr[1], Run-Zhang Shi[1,2], David J. Iberri[3], Ruben Y. Luo[1,2]

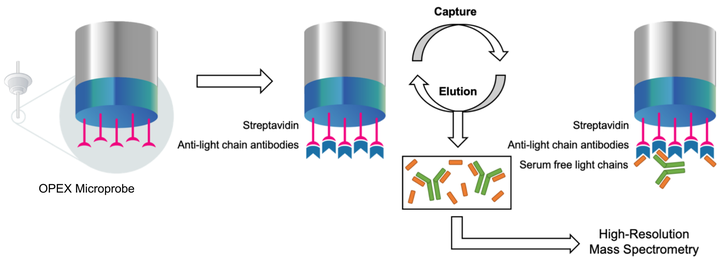
[1]Department of Pathology, Stanford University, Stanford, CA, USA; [2]Clinical Laboratories, Stanford Health Care, Palo Alto, CA, USA; [3]Department of Medicine, Division of Hematology, Stanford University, Stanford, CA, USA

1. Introduction



One of the essential clinical markers of plasma cell neoplasms, including multiple myeloma (MM) and amyloidosis, is serum free light chains (sFLC), which are circulating antibody light chains that are unbound to heavy chain [1, 2]. The current widely used immunoassay method quantifies total serum FLCs, including the polyclonal background. Although a skewed kappa/lambda sFLC ratio is often used as a proxy for clonality, some patients, such as those with renal disease, can have ambiguous results that can benefit from a direct measurement of clonality [3-5]. We developed an assay that couples an on-probe extraction immunocapture step with high-resolution mass spectrometry (OPEX-MS) to determine the clonality of sFLCs.

2. Materials and Methods



Sample Preparation by On-Probe Extraction

Remnant patient samples from the Stanford Clinical Chemistry Laboratory were collected and processed according to IRB protocols approved by Stanford Health Care.

Microprobes (1 mm diameter, Gator Bio) coated with streptavidin were used to immobilize biotinylated anti-kappa light chain or anti-lambda light chain capture antibodies (Abcam) onto the probe surface. The probes were introduced into 20X diluted patient serum. Captured proteins were eluted using 0.3% formic acid to break the antibody-antigen association and rinsed with phosphate buffered solution containing Tween. The analyte capture and elution steps were repeated 30 times for optimal signal to noise ratio.

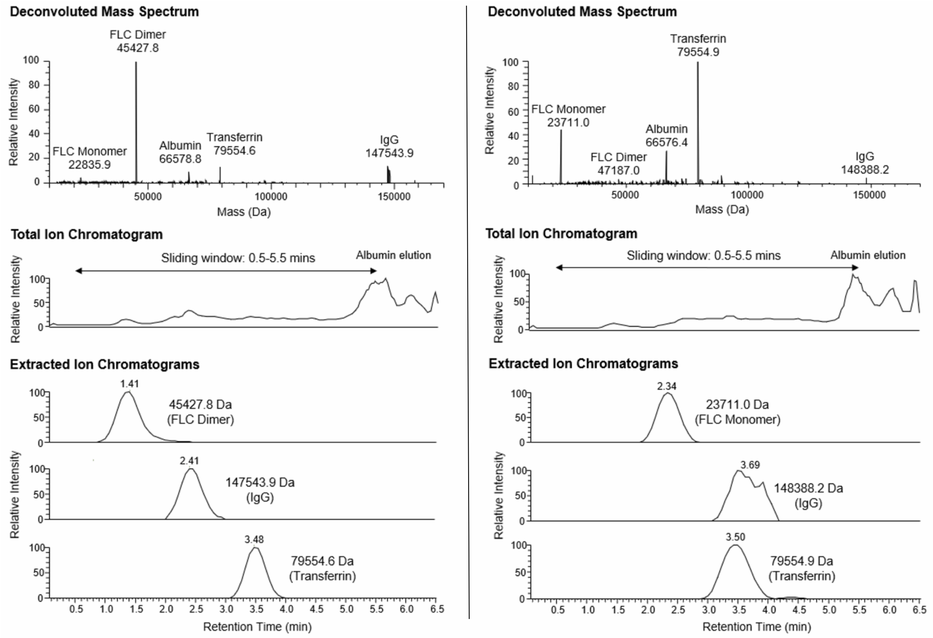
Analysis by High-Resolution Mass Spectrometry (HR-MS)

LC-MS analysis was performed on a TLX-2 multi-channel HPLC coupled with a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific). Proteins were separated on a reverse phase HPLC column (MAbPac, 2.1 mm X 5 mm) (Thermo Fisher Scientific). The sample injection volume was 20 µL, and the analytical column temperature was set to 60ºC. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile. The LC method consisted of starting at 10% MPB, ramp to 25% MPB over 1 minute, ramp to 35% MPB over 7 minutes, ramp to 98% MPB over 3 minutes, and followed by 8 minutes of column washing steps. The high-resolution mass spectrometer was set to positive electrospray ionization with full-scan data acquisition mode during a 6.5 minute window from 4-10.5 minutes of the LC method. The resolution was set to 17,500, AGC of target 3e6, maximum IT of 300 ms, and scan range of 1000-4000 m/z. For the heated ESI, the settings were the following: sheath gas 30 psi, auxiliary gas 15 psi, spray voltage 3.5 kV, capillary temperature 320ºC, auxiliary gas heater 320ºC, and S-lens RF level 50. Data analysis was performed on BioPharma Finder 5.1 software using a sliding window ReSpect deconvolution algorithm.

References

1. Rajkumar, S. V. Am J Hematol 97, 1086-1107, doi:10.1002/ajh.26590 (2022).
2. Larsen, J. T. et al. Leukemia 27, 941-946, doi:10.1038/leu.2012.296 (2013)
3. Lutteri, L. & Jacobs, J. F. M. Clin Chem Lab Med 56, e232-e234, doi:10.1515/cclm-2018-0040 (2018)
4. Sarto, C. et al. Clin Biochem 95, 28-33, doi:10.1016/j.clinbiochem.2021.05.006 (2021)
5. Sepiashvili, L. et al. Clin Chem 65(8): p. 1015-1022, doi: 10.1373/clinchem.2018.299461 (2019)
6. Dispenzieri, A. et al. Leukemia 34, 2749-2753, doi:10.1038/s41375-020-0940-8 (2020)

3. Detection of Monoclonal FLCs and IgGs



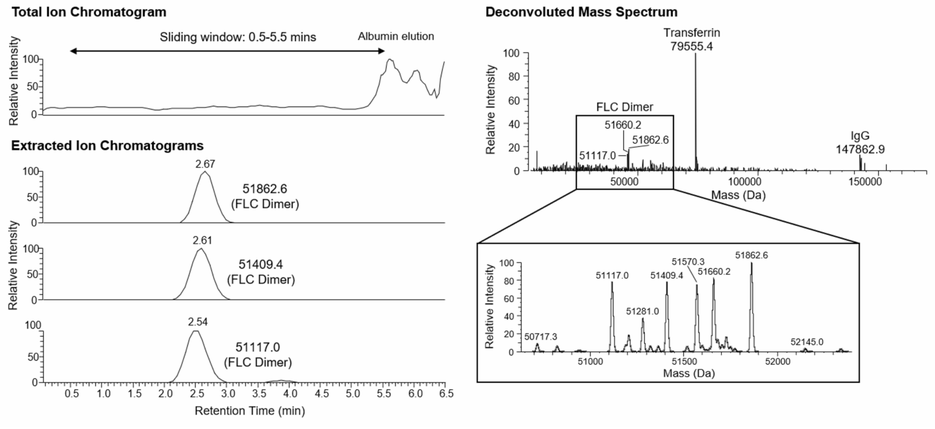
OPEX-MS deconvoluted mass spectra and chromatography traces of a IgG lambda MM patient (left) and a IgG kappa MM patient (right). The monoclonal FLC peaks are chromatographically separated from albumin and transferrin. Positive peaks met three criteria: 1) peaks were within the 20-30 kDa and 40-60 kDa deconvoluted mass ranges corresponding to the expected masses of FLC monomers and dimers, 2) peaks intensities were statistical outliers (>Q3 + 1.5*IQR) within the mass windows, and 3) chromatograms showed Gaussian peaks expected of true positive samples.

Left: Detection of lambda FLC monomer (22.8 kDa), FLC dimer (45.4 kDa), and IgG M-protein (147.5 kDa).

Right: Detection of kappa FLC monomer (23.7 kDa), FLC dimer (47.2 kDa), and IgG M-protein (148.4 kDa).

4. Monoclonal FLC Glycosylation

A recent publication [6] showed that MGUS patients with glycosylated light chains had an increased risk of progression to MM (HR 6.4), demonstrating that post-translation modifications in these proteins can be used as prognostic biomarkers. Consistent with the previously reported prevalence of light chain glycosylation, OPEX-MS was able to identify light chain glycosylation in 8 out of 103 (7.7%) of positive samples [5, 6].



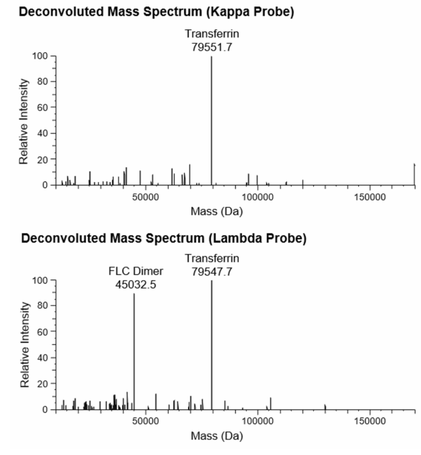
5. Comparison with FLC Immunoassay

Four cohorts of samples from unique patients were tested based on Binding Site FreeLite FLC immunoassay results according to the manufacturer's reference ranges: negative (n = 50), kappa elevated (n = 49), lambda elevated (n = 45), and dual elevated with normal ratio (n = 100). Among the 50 samples in the immunoassay negative cohort, OPEX-MS identified 2 kappa positive and 5 lambda positive samples, all of which were from patients with a previous history of monoclonal gammopathy, suggesting a potentially improved sensitivity in residual clones in treated patients. In the kappa elevated cohort, 16 out of 49 (33%) were negative by the OPEX-MS method. For these 16 discrepant samples, the average kappa/lambda ratio was 2.8, indicating that these were mostly borderline kappa elevated samples with unclear underlying clonality. There was good overall concordance between the immunoassay and OPEX-MS methods for the lambda elevated samples.

		FLC Immunoassay			
		Negative	Kappa Elevated	Lambda Elevated	Dual Elevated
OPEX-MS	Negative	43	16	1	83
	Kappa Positive	2	32	0	0
	Lambda Positive	5	1	44*	16
	Both Positive	0	0	0	1
	Total	50	49	45	100

*This included five high lambda FLC (>100 mg/dL) samples that displayed <20% crossover binding to the kappa probe

6. Clone Detection in Dual Elevated Samples



FLC immunoassays can yield ambiguous results in patients with chronic kidney disease or inflammatory conditions [3-4]. In the dual elevated, normal ratio cohort, 17% that had monoclonal FLCs detected. All of these patients had histories of monoclonal gammopathy, implying that OPEX-MS was able to detect their residual disease within the dual elevated polyclonal FLC background.

Left: Residual lambda clone detected in a patient with chronic kidney disease and lambda light chain amyloidosis on treatment. FLC immunoassay results: kappa 5.4 mg/dL, lambda 7.3 mg/dL (both elevated, normal ratio). Serum protein immunofixation electrophoresis result: negative.

7. Summary

- On-probe extraction coupled with high-resolution mass spectrometry (OPEX-MS) can be used as a complementary method to FLC immunoassay to directly detect monoclonal sFLCs in patient samples.
- Monoclonal sFLCs can be identified by their deconvoluted masses and retention times.
- In line with previous studies, light chain glycosylation was detected in 7.7% of positive samples at high resolution, which allows for the detailed study of glycosylation patterns.
- Residual clones can be identified in dual elevated samples with normal kappa/lambda ratios, demonstrating the value of OPEX-MS in elucidating FLC immunoassay results.
- By using different capture antibodies, the OPEX-MS workflow can be applied to other clinical protein biomarkers.