

Validation Study on the Utility of Immunoglobulin Heavy/Light Chain Immunofluorescence in Kidney Biopsies With Potential Monoclonal Gammopathy of Renal Significance Lesions



Satoru Kudose¹, Geetha Jagannathan¹, Dominick Santoriello¹, Miroslav Sekulic¹, Ibrahim Batal¹, M. Barry Stokes¹, Vivette D. D'Agati¹ and Glen S. Markowitz¹

¹Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, New York, USA

Correspondence: Satoru Kudose, Department of Pathology and Cell Biology, Columbia University Irving Medical Center, Room VC14-238, 630 West 168th Street, New York, New York 10032, USA. E-mail: sk4521@cumc.columbia.edu

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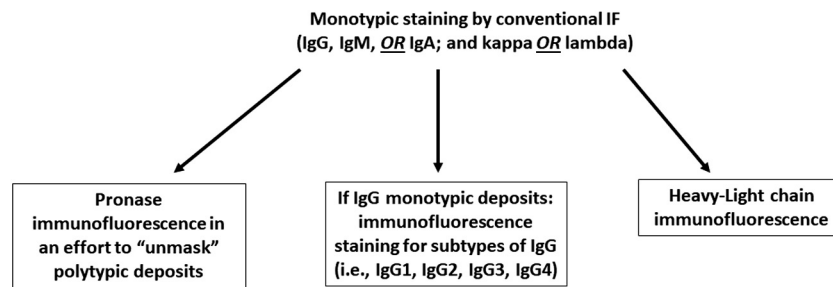
Conventional immunofluorescence (IF) for kidney biopsies is generally performed on frozen sections and uses a panel of polyclonal antibodies directed against the constant regions of immunoglobulin heavy chains and light chains (LCs). Diagnosis of monoclonal gammopathy of renal significance (MGRS) currently relies on the detection of isotype and LC-restricted (monotypic) staining for heavy and LCs, supplemented by IgG subclass staining and paraffin IF in selected cases. Recently, Nasr *et al.*¹ described the potential use of IF using polyclonal antibodies directed against the conformational epitope at the junction of the heavy and LC (HLC) constant regions (HLC-IF), including IgG κ , IgG λ , IgM κ , IgM λ , IgA κ , and IgA λ , for the evaluation of MGRS or other conditions with monotypic deposits.

Using these new antibodies, Nasr *et al.*¹ found that some cases with apparent monotypic glomerular deposits by conventional IF (i.e., cases that stain for only 1 heavy chain isotype and only 1 LC) may have polypotypic staining by HLC-IF with reactivity for both κ - and λ -heavy chain pairs. Therefore, HLC-IF could be used to confirm or exclude monotypic composition of deposits in these cases, similar to IgG subclass staining.¹ Furthermore, they recommended that some cases that would be classified as MGRS based on conventional IF, such as proliferative glomerulonephritis (GN) with monoclonal immunoglobulin deposits (PGNMIDs), should not be categorized as MGRS if HLC-IF reveals staining for both IgG κ and IgG λ .¹

Given the potentially utility of HLC-IF in the diagnosis of MGRS (Figure 1), we sought to broaden the clinical experience with HLC-IF in an independent series of kidney biopsies, enriched with entities that had relatively small sample size in the study by Nasr *et al.*¹ Specifically, we evaluated 43 cases by direct HLC-IF for IgG κ and IgG λ and 22 cases by direct HLC-IF for IgA κ and IgA λ (including 12 controls; see Supplementary Methods).

Results are summarized in Table 1. There were 20 biopsies with PGNMID which exhibited, by definition, monotypic deposits by conventional IF, including 12 with IgG3 κ , 5 IgG1 κ , 1 IgG1 λ , 1 IgG2 κ , and 1 IgG3 λ . The cohort was unintentionally enriched with patients with malignancy or paraproteinemia including 1 patient with multiple myeloma and PGNMID-IgG3 κ , 3 patients with B-cell lymphoma (not further classified) including 2 with PGNMID-IgG3 κ and 1 with PGNMID-IgG2 κ , and 5 additional patients with a documented paraprotein (all PGNMID-IgG3 κ). HLC-IF results revealed both IgG κ and IgG λ in only 2 of 20 patients with PGNMID (10%), both of whom had IgG1 κ deposits by conventional IF. Of the 2 patients, 1 was 23 years old without detectable paraprotein or hematology malignancy and the other was 69 years old with free κ LCs on urine immunofixation but no hematology malignancy.

HLC-IF was performed on 7 biopsies with immunotactoid GN (ITG) with monotypic deposits based on conventional IF with IgG subclass staining, including 3 with IgG1 λ , 2 IgG1 κ , and 2 IgG2 κ , and 1 with bitypic staining



***Additional modalities of importance in evaluating biopsies with monotypic deposits:** A) Congo red stain to diagnose light & heavy chain amyloidosis; B) immunohistochemical staining for DNAJB9 to diagnose monotypic fibrillary glomerulonephritis; C) electron microscopy to diagnose light & heavy chain amyloidosis, monotypic fibrillary, immunotactoid, and cryoglobulinemic glomerulonephritis.

Figure 1. A general workflow for evaluation of kidney disease with monotypic staining by conventional IF. In individual cases, clinical history, pathologic findings, and availability of ancillary tests must be carefully considered. IF, immunofluorescence.

for IgG1κ and IgG2κ. Among the 7 with monotypic ITG by conventional IF, 4 had chronic lymphocytic leukemia (including 2 patients with IgG1κ, 1 with IgG1λ, and 1 with IgG2κ deposits) and 2 had monoclonal gammopathy

in the absence of chronic lymphocytic leukemia (1 with IgG1λ deposits and 1 with IgG2κ deposits). The remaining patient with monotypic ITG, and the patient with bitypic ITG, had no detectable paraprotein or hematology

Table 1. HLC-IF on independent samples of glomerular diseases with monotypic or bitypic deposits; comparison of results at Columbia University and Mayo Clinic

Pathology	CUIMC		Mayo	
	Monotypic deposits by conventional IF	Number of cases polytypic by HLC-IF	Monotypic deposits by conventional IF	Number of cases polytypic by HLC-IF
PGNMID	IgG1κ	2/5		
	IgG1λ	0/1		
	IgG2κ	0/1		
	IgG3κ	0/12	IgG3κ	2/7
	IgG3λ	0/1	IgG3λ	0/3
	Total	2/20	Total	2/10
Immunotactoid GN	IgG1κ	2/2		
	IgG1λ	0/3		
	IgG1κ and IgG2κ	1/1		
	IgG2κ	2/2		
	Total	5/8	Not evaluated	
Monotypic or bitypic MN	IgG1κ	1/3		
	IgG3λ	0/1		
	IgG1κ and IgG3κ	2/2	IgG3κ	0/1
	Total	3/6	Total	0/1
Monotypic fibrillary GN	IgG1κ	2/3	IgGλ	3/6
	Total	2/3	Total	3/6
Monotypic or bitypic anti-GBM disease	IgG1κ	2/2	IgGκ	0/1
	IgG1λ	0/3	IgGλ	0/2
	IgG1λ and IgG4λ	1/1	IgMκ	0/1
			IgAλ	1/2
	Total	3/6	Total	1/6
LC-restricted IgA nephropathy/vasculitis ^a	IgAκ	0/8		
	IgAλ	3/14	IgAλ	6/12
	Total	3/22	Total	6/12

CUIMC, Columbia University Irving Medical Center; GBM, glomerular basement membrane; GN, glomerulonephritis; HLC, heavy and light chain; IF, immunofluorescence; LC, light chain; MN, membranous nephropathy; PGNMID, proliferative glomerulonephritis with monoclonal immunoglobulin deposits.

^aThese cases were stained with IgAκ and IgAλ HLC-IF.

malignancy. HLC-IF results revealed both IgG κ and IgG λ in 4 of the 7 with monotypic ITG (57%), including 2 with IgG1 κ and 2 with IgG2 κ , and in the single patient with bitypic ITG. Not surprisingly, in 4 of the 5 cases with polytypic staining by HLC-IF, there was greater intensity of staining for the IgG-LC pair that had been originally identified in the monotypic deposits by conventional IF. Of note, in 3 of the 4 patients with chronic lymphocytic leukemia, the deposits were polytypic with HLC-IF.

There were 6 patients who had LC-restricted membranous nephropathy,² including 3 with IgG1 κ monotypic deposits, 1 with IgG3 λ monotypic deposits, and 2 with bitypic IgG1 κ and IgG3 κ deposits. One patient with monotypic deposits (IgG1 κ) had chronic lymphocytic leukemia and 1 patient with bitypic deposits had lymphoma of mucosa-associated lymphoid tissue (with IgM paraprotein). The remaining 4 patients did not have paraproteinemia or hematolymphoid malignancy. HLC-IF results revealed staining for both IgG κ and IgG λ in 1 patient with monotypic IgG1 κ deposits and in both patients with bitypic deposits by conventional IF.

Six patients had LC-restricted atypical anti-glomerular basement membrane disease,³ including 2 with IgG1 κ -restriction, 3 with IgG1 λ -restriction, and 1 with bitypic IgG1 λ and IgG4 λ . One patient with IgG1 κ deposits had a serum IgG κ and free κ paraprotein, the remaining 5 had no evidence of paraproteinemia, and none had evidence of hematolymphoid malignancy. HLC-IF results revealed both IgG κ and IgG λ in both cases with IgG1 κ restriction and in the case of bitypic IgG1 λ and IgG4 λ .

HLC-IF was performed on 3 biopsies with monotypic DNAJB9-associated fibrillary GN and IgG1 κ -restricted deposits.^{4,5} One patient had an IgG κ serum paraprotein, but none had evidence of hematolymphoid malignancy. HLC-IF results revealed both IgG κ and IgG λ in the 2 patients who lacked a detectable paraprotein and, oddly, were negative for both IgG κ and IgG λ in the other patient.

We evaluated 22 biopsies with LC restricted, IgA-dominant staining including 16 cases of apparent IgA nephropathy and 6 with IgA vasculitis. Among the 22 patients, only 1 had a serum M-spike (IgG κ and free κ) and none had a history of malignancy. HLC-IF result was positive solely for IgA κ in all 8 cases with IgA κ restriction by conventional IF. In contrast, HLC-IF results revealed both IgA κ and IgA λ in 3 of 14 biopsies (21%) with IgA λ restriction by conventional IF. Notably HLC-IF results in 12 control cases revealed findings concordant with conventional IF.

Our results extend and refine the findings by Nasr *et al.*¹ In our cohort, HLC-IF detected polytypic glomerular deposits in approximately 30% of cases that

display monotypic deposits by conventional IF. HLC-IF seems most useful in monotypic or bitypic forms of membranous nephropathy, fibrillary GN, atypical anti-glomerular basement membrane disease, and in a few cases of λ -restricted IgA nephropathy, because almost all with polytypic HLC-IF results had no evidence of hematolymphoid malignancy or paraproteinemia that matched the glomerular deposits. This was particularly notable for monotypic DNAJB9-associated fibrillary GN, as recent reports suggest that this entity should not be considered a form of MGRS.^{4,5}

HLC-IF was of limited utility in excluding monotypic deposits in PGNMID, with detection of both IgG κ and IgG λ -heavy chain pairs in only 2 of 20 patients with PGNMID (10%), similar to the finding by Nasr *et al.*¹ (2 of 10; 20%). This entity remains something of an enigma in that deposits reproducibly seem to have a monotypic composition despite the absence of a detectable paraprotein in 70% of patients. Similarly, HLC-IF was of limited utility in cases of κ -restricted IgA nephropathy/vasculitis.

Importantly, our findings also suggest that the presence of both κ - and λ -heavy chain pairs by HLC-IF does not in and of itself exclude an association with paraproteinemia or hematolymphoid malignancy. Although coincidental associations cannot be excluded, this finding highlights the need for careful interpretation of HLC-IF results in the context of specific disease categories. In particular, among patients with monotypic ITG (most of whom have paraproteinemia or hematolymphoid malignancy), the combined results of conventional IF and HLC-IF suggest that, although monotypic deposits predominate in 2/3 cases, some of these cases have a smaller component of polytypic deposits that are best identified by HLC-IF.⁶

Limitations of our data include the relatively small sample size, the limited availability of clinical data, and the inability to verify our data by an orthogonal non-antibody based method.

In summary, we report an independent series of kidney biopsy specimens evaluated with HLC-IF. Our findings largely validate the findings by Nasr *et al.*¹ and expand the literature on the utility of HLC-IF in the evaluation of selected kidney diseases. More experience is needed to define the role of HLC-IF in routine kidney biopsy practice.

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTAL MATERIAL

[Supplementary File \(PDF\)](#)

Supplementary Methods.

Supplementary References.

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