

Detecting FH-Anti-FH Immune Complexes in MGRS-C3G

Stephanie N Cook¹, Sydney S Jellison¹, Sarah M Roberts¹, Dingwu Shao¹, Andrea Reparaz²,
Carla M Nester¹, Santiago Rodriguez de Cordoba², Richard JH Smith¹, Yuzhou Zhang¹

¹Molecular Otolaryngology and Renal Research Laboratories, Carver College of Medicine, University of Iowa

²Complement Diagnostic Laboratory, Centro de Investigaciones Biológicas Margarita Salas, Consejo Superior Investigaciones Científicas (CSIC), Madrid, Spain

Background

C3 glomerulopathy (C3G) is a rare complement-mediated kidney disease characterized by dominant glomerular C3 deposition and driven by dysregulated activation of the alternative pathway (AP). Dysregulation can arise when complement factor H (FH), the major fluid-phase AP regulator, is bound by FH autoantibodies (FHAAs), inhibiting its cofactor and decay-accelerating activities and impairing host-surface recognition. In a subset of patients, often older adults, monoclonal gammopathy of renal significance (MGRS) is present; small B-cell or plasma-cell clones produce monoclonal immunoglobulins (M-proteins, i.e., paraproteins) that can function as FHAAs in MGRS-C3G and form circulating FH-Ig immune complexes (FHICs). Standard indirect ELISA under-detects FHAAs and FHICs due to sequestration of antibody within complexes (little free antibody), limited recognition of IgM or mixed isotypes, and conformational/epitope loss on plate-bound FH. To overcome these limitations, we used a lateral flow assay (LFA) designed to capture FHICs ⁽¹⁾. In MGRS-C3G, the LFA identified additional FHIC-positive samples compared with ELISA, and Protein G pull-down with FH immunoblot confirmed FH in IgG eluates, consistent with circulating FHICs.

Methods

1. FHAA ELISA: Indirect ELISA with plate-bound FH.
2. Lateral flow assay (three-cassette format) to detect IgG- and IgM-class FHICs.
3. Serum immunofixation electrophoresis (IFE) for M-proteins.
4. Protein G pull-down to isolate IgG and co-bound FH.

Study Cohorts

The study included three patient cohorts based on FHAA ELISA and M-proteins status.

Table 1: Study Cohorts

Cohort	Purpose	FHAA ELISA (AU)	C3G (n)	MGRS-C3G (n)	Total (n)
I	Validation	>1000	8	3	11
II	Validation	200-1000	10	4	14
III	Exploratory	Negative	0	32	32

Example of M-protein in an MGRS-C3G patient

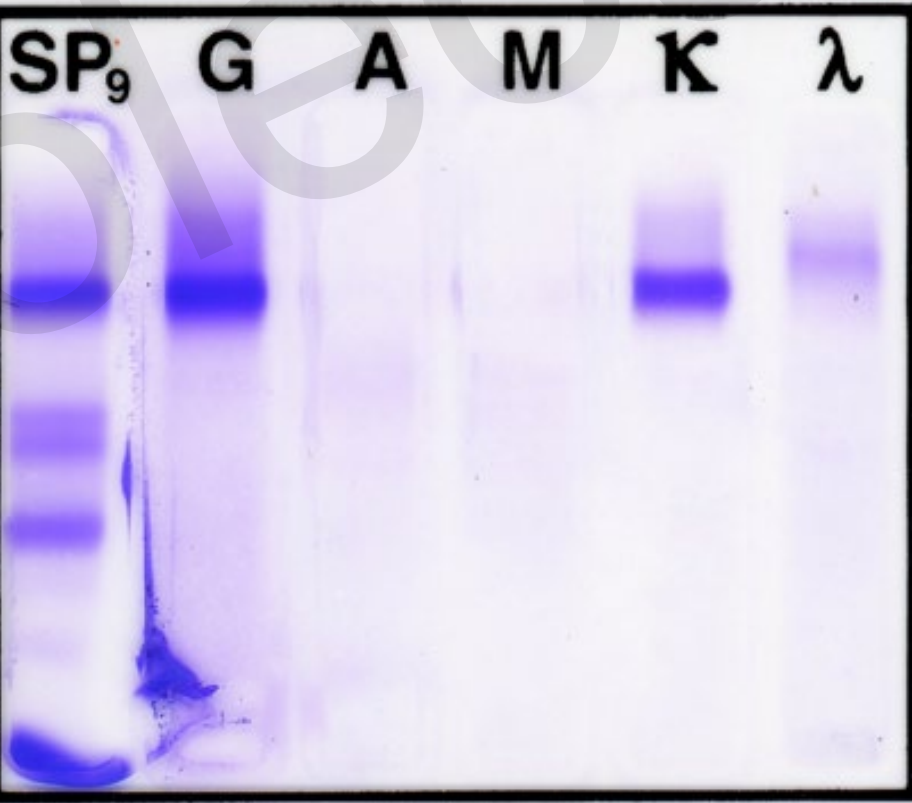


Figure 1. Immunofixation Electrophoresis. Lane SP shows the full serum protein profile after electrophoresis. Lanes G, A, and M correspond to immunofixation with anti-IgG, anti-IgA, and anti-IgM antibodies, respectively. Lanes κ and λ contain anti-kappa and anti-lambda light-chain reagents. A discrete band is observed only in the G and κ lanes, indicating the presence of a monoclonal IgG κ M-protein.

Lateral Flow Assay

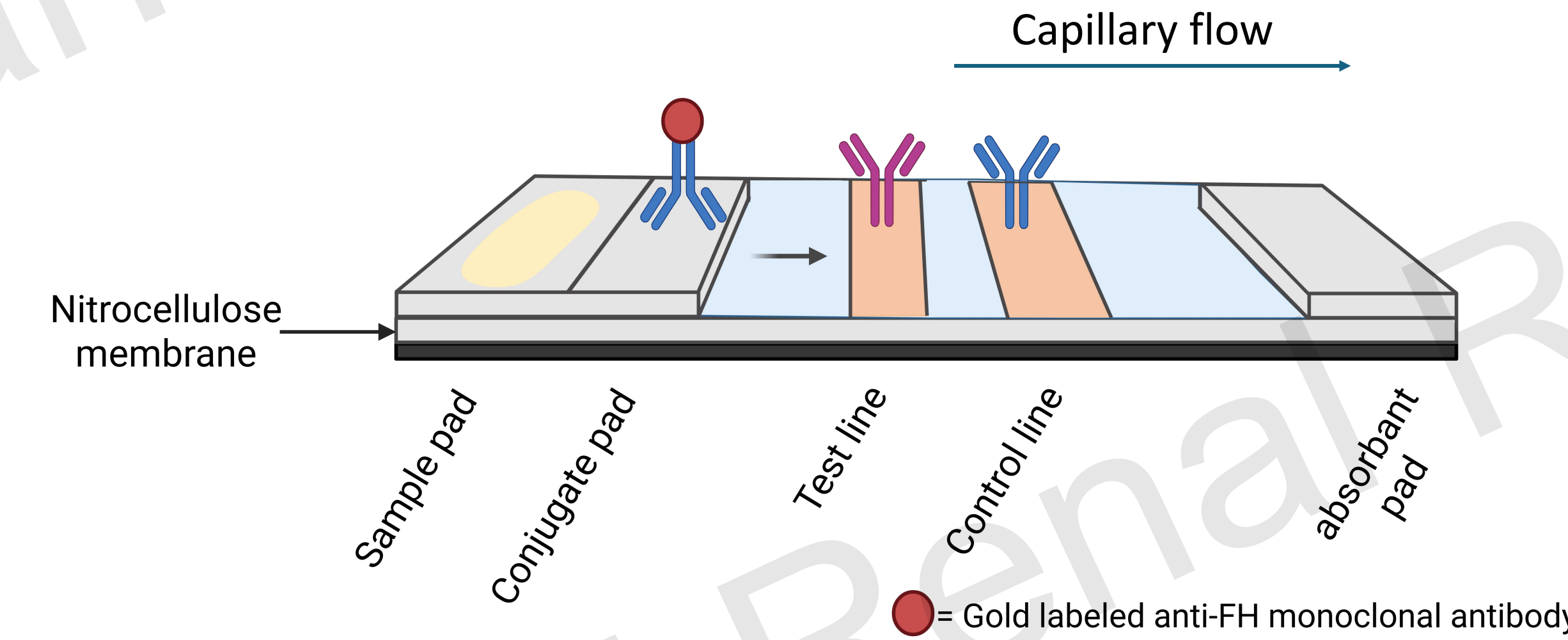


Figure 2. Lateral Flow Assay. Three cassettes (designated as A, B and C) share the same architecture but use different reagents for detecting IgG- and IgM- form FH immune complexes (see Table 2).

Table 2. Reagents and Setting

Cassette	Conjugate pad	Test line	Control line
A	Gold-anti-FH mAb 214 (binds middle region of FH)	Anti-human IgG antibody	Anti-FH polyclonal
B	Gold-anti-FH mAb 35H9 (binds N-terminal FH)	Anti-human IgG antibody	Anti-FH polyclonal
C	(same as A)	Anti-human IgM antibody	Anti-FH polyclonal

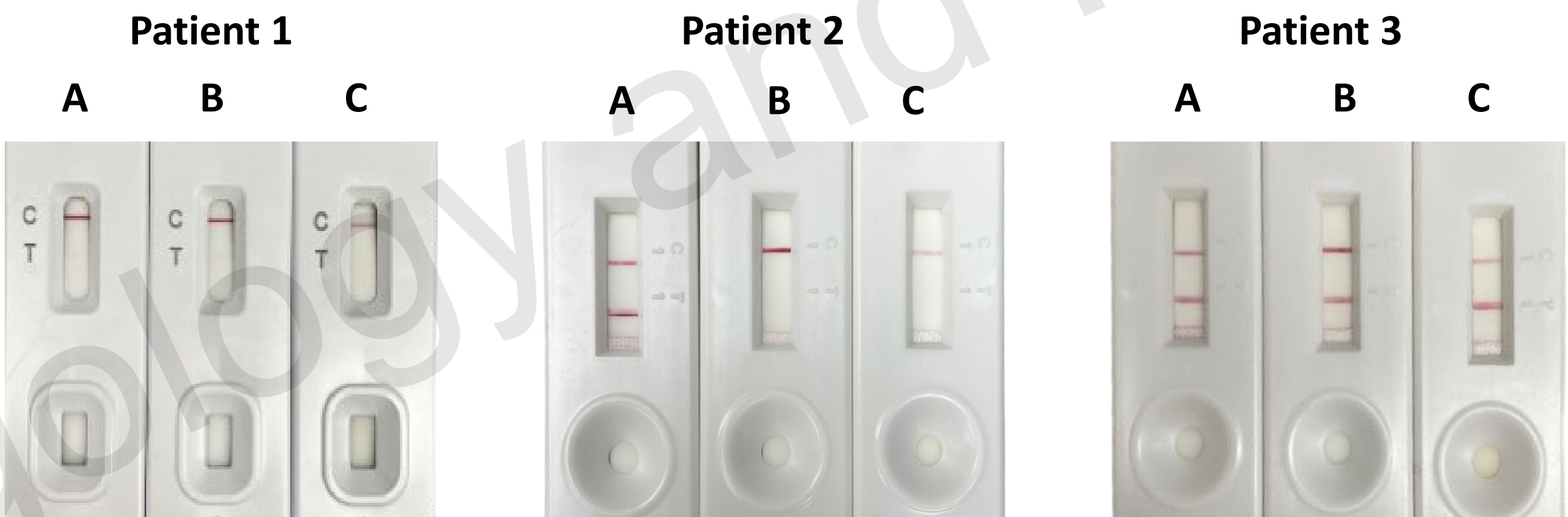


Figure 3. Examples of FHIC LFA results. Patient 1 (negative): Control line (C) present; no test line (T) on any cassette. Patient 2 (IgG FHIC-positive): Only Cassette A shows C+T, consistent with an N-terminal targeting FHAA; Cassette C (IgM) is negative. Patient 3 (IgG and IgM FHIC-positive): T lines present on IgG cassettes A/B and on the IgM cassette C. Note: A valid result must display the C line; any visible T line is considered positive.

Table 3. Lateral Flow Assay and M-Protein Results by Cohort

Cohort	Patient (n)	LFA+		MGRS (n)	LFA+ in MGRS(%)
		A+/B+ (%)	A/B/C+ (%)		
I	11	11 (100%)	6 (55%)	3	3 (100%)
II	14	11 (79%)	0 (0%)	4	4 (100%)
III	32	12 (38%)	4 (33%)	32	12 (38%)
Overall	57	34 (60%)	10 (29%)	39	19 (49%)

Confirmation of FH in FHIC

Protein G affinity purification was used to confirm circulating FHICs in an MGRS-C3G patient positive for LFA but negative for FHAA ELISA.

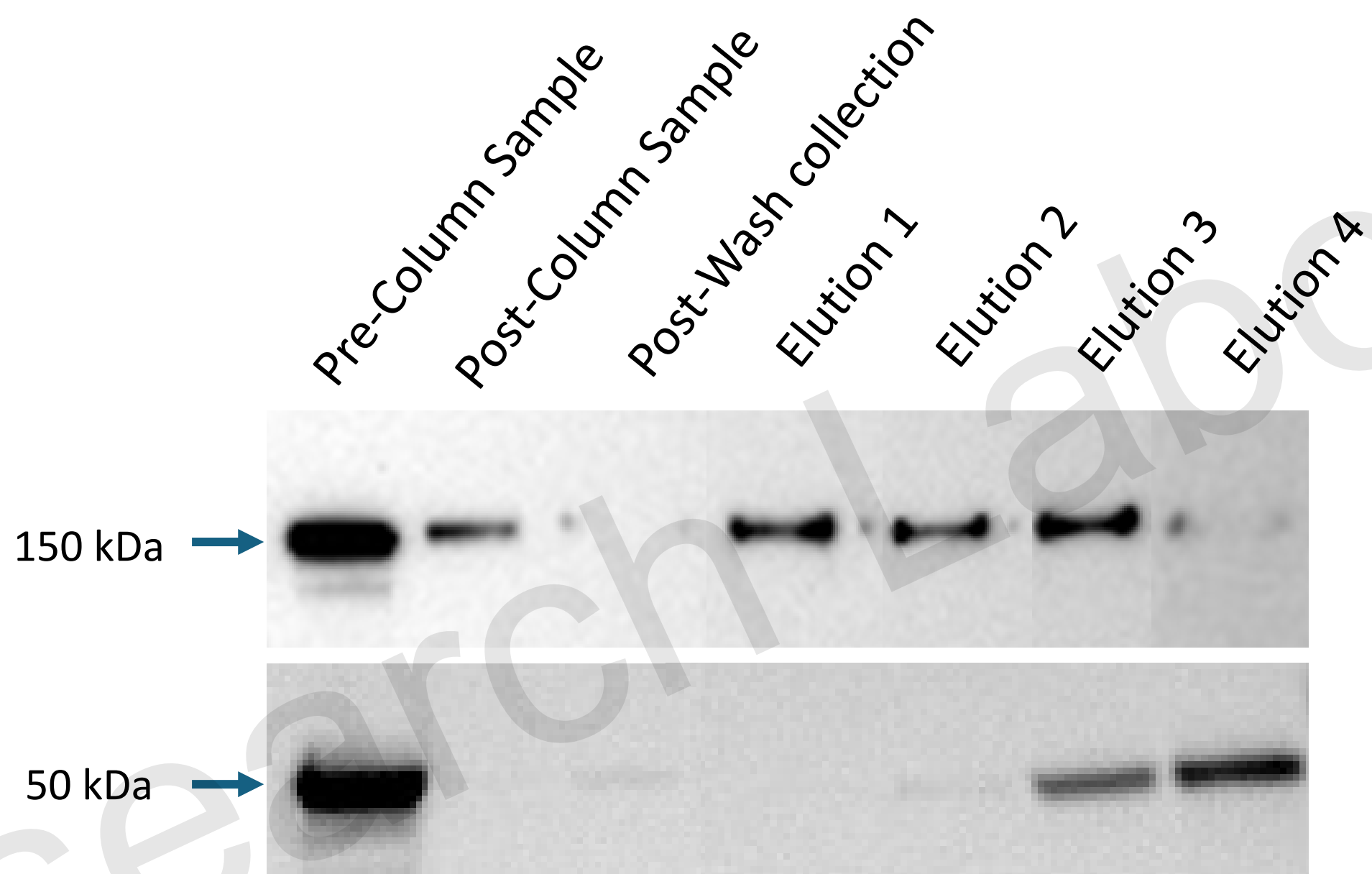


Figure 4. Confirmation of FH-Ig immune complexes by Protein G pull-down. Patient serum was diluted, applied to Protein G resin, washed stringently, and eluted with low-pH buffers. Fractions were resolved by reducing SDS-PAGE and immunoblotted for factor H (FH; top) and human IgG heavy chain (bottom).

FH (~155 kDa) and IgG heavy chain (~50 kDa) are present in the input. FH is depleted from flow-through/wash and reappears in the eluates, co-eluting with IgG. Earlier FH release (elution 1) is expected because the FH-FHAA (antigen-antibody) interaction is weaker than IgG-Protein G binding. Because Protein G does not bind free FH, co-elution with IgG demonstrates FH-anti-FH immune complexes, corroborating the LFA FHIC positivity in this MGRS-C3G patient.

Discussion and Conclusions

- The LFA detects disease-relevant anti-FH activity. LFA was positive in 34/57 (60%) overall, of which 10 (29%) were co-positive for IgM FHAA. Importantly, in cohort III, 12 of 32 (38%) ELISA-negative samples were LFA-positive. Among patients with MGRS, 19 of 39 (49%) were LFA-positive. No patient was positive for IgM only.
- The FHIC-targeted LFA outperforms standard FHAA ELISA for detection, particularly in MGRS and IgM-co-positive cases.
- LFA is a rapid, low-cost tool that complements ELISA and should be considered in the diagnostic work-up of MGRS-C3G.
- For suspected MGRS-C3G, an algorithm of LFA→IFE (monoclonality)→ELISA→functional FH assays can (a) identify FH-directed paraproteins as a driver of AP dysregulation, and (b) provide a rapid screen to guide hematologic work-up and therapy monitoring.
- Future work: multi-site validation, calibration to quantitative metrics, interference controls, and correlation with biomarkers and clinical outcomes.

Reference

(1) Rodriguez de Cordoba S *et al. Front Immunol.* 15:1527016.

Acknowledgments

Supported in part by National Institutes of Health R01 DK110023.