BIOMARKERS: STATE OF THE ART, WHERE WE NEED TO GO, STANDARDIZATION

Elena Goicoechea de Jorge

DISCLOSURES

- EGJ has received lecture fees from Alexion, Vifor, Sobi, Samsung and Astellas, and has been a consultant for Q32 Bio, Sobi and Arrowhead.

LAYOUT

- Background (Complement, complement-mediated disease & samples)
- Overview of the available panel of complement biomarkers

  - Clinical relevance and the potential impact on the management of the patients.

- Unresolved issues and unmet needs in the field.

THE COMPLEMENT SYSTEM

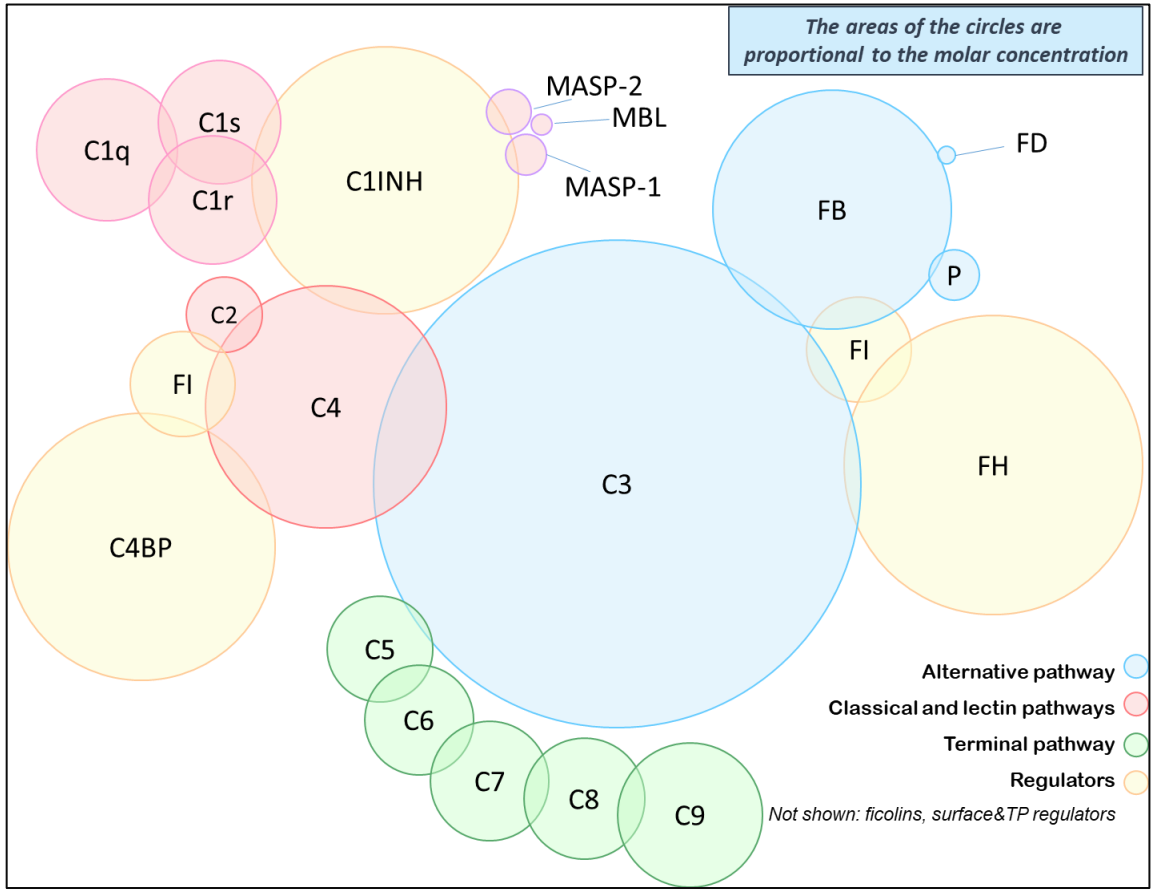
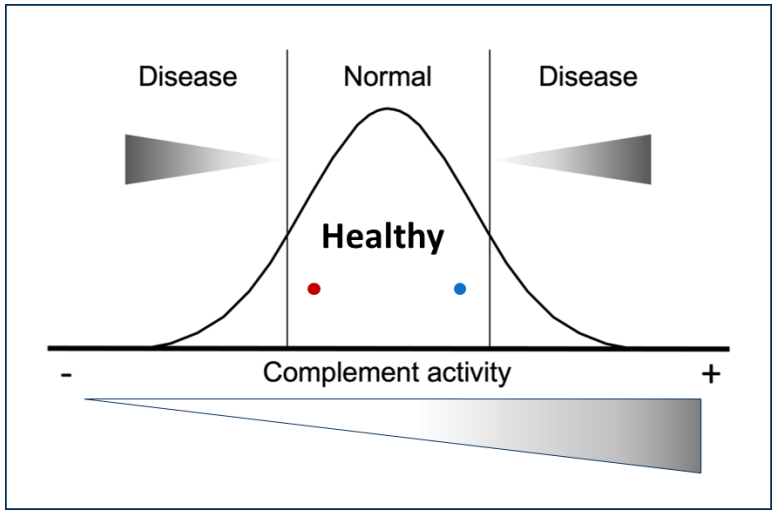


Image courtesy of Dr. Zoltán Prohászka

Wide range of variation in the general population:
up to 5-fold concentration difference (e.g. FH)



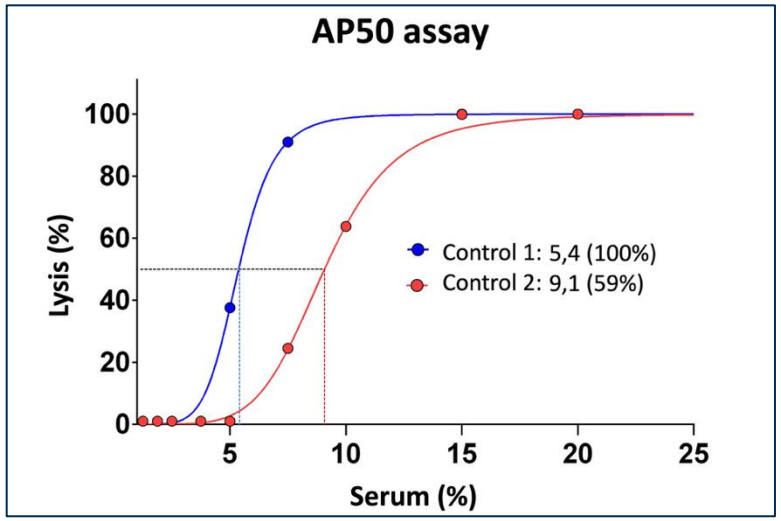
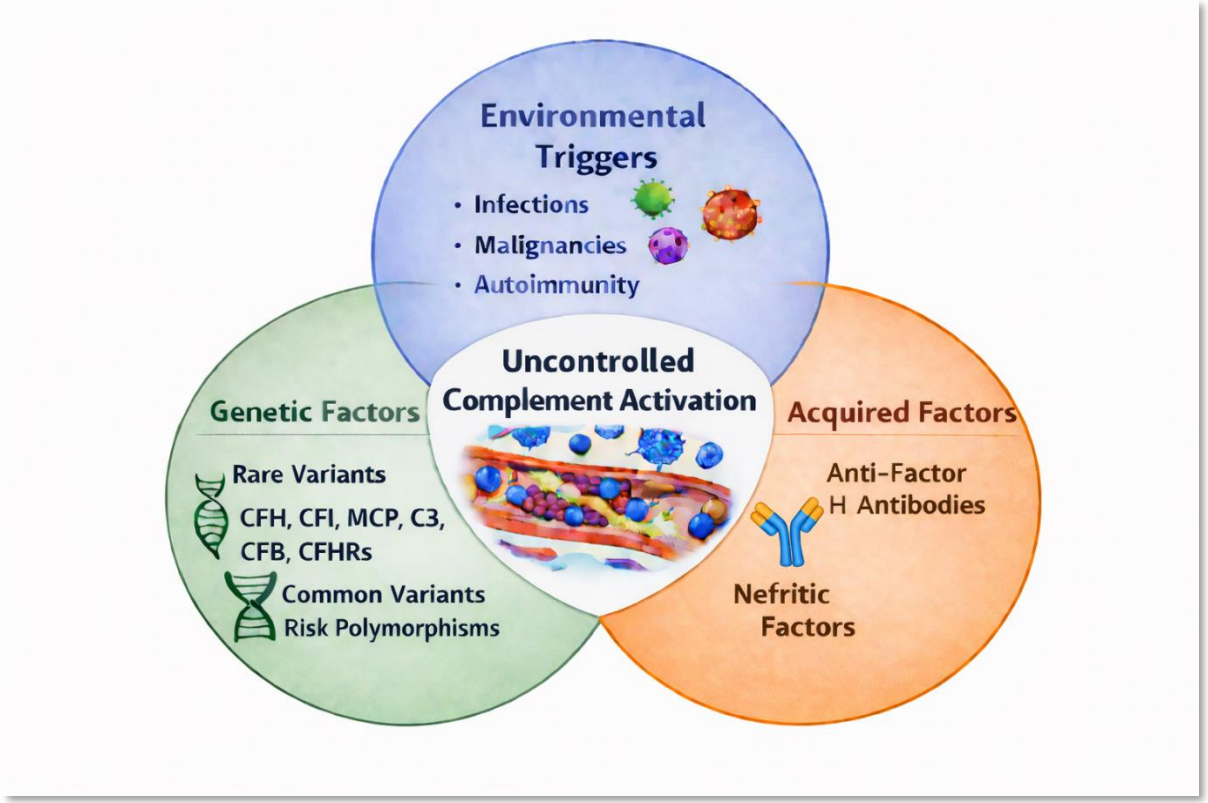


Image courtesy of Dr. Santiago Rodríguez de Córdoba

COMPLEMENT-MEDIATED KIDNEY DISEASES

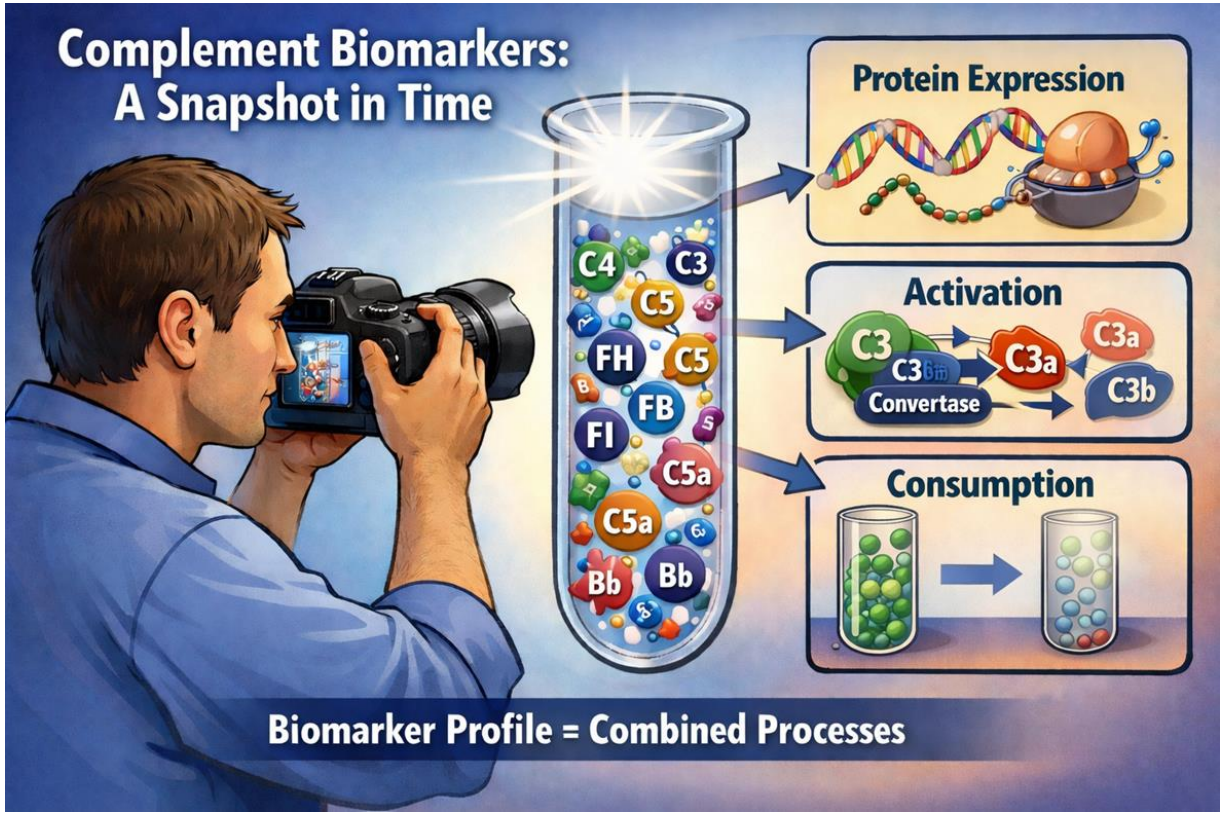
- Atypical HUS, C3 glomerulopathy and Immunoglobulin-associated MPGN are **complex, multifactorial diseases** that result from an uncontrolled complement activation through the alternative pathway.

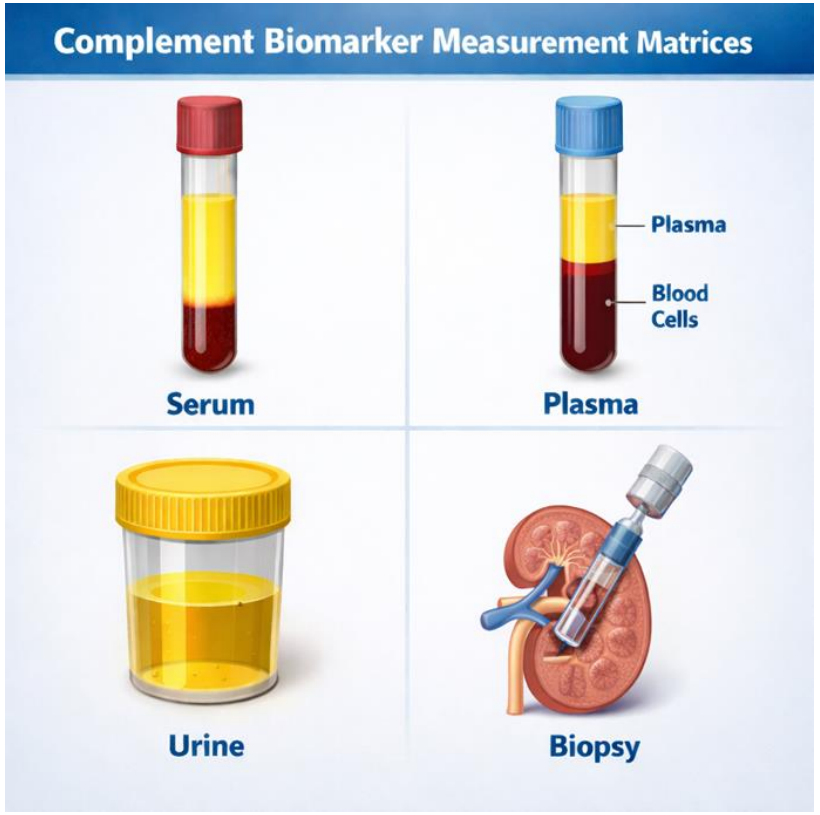


- Disease result as a combination of multiple factors.
- Different pathogenic mechanisms involved.
- Complement profiles are heterogeneous:

  Systemic vs Glomerular microenvironment

THE SAMPLES





Each matrix has its own advantages and limitations for the different complement biomarkers

How can the complement system be assessed?

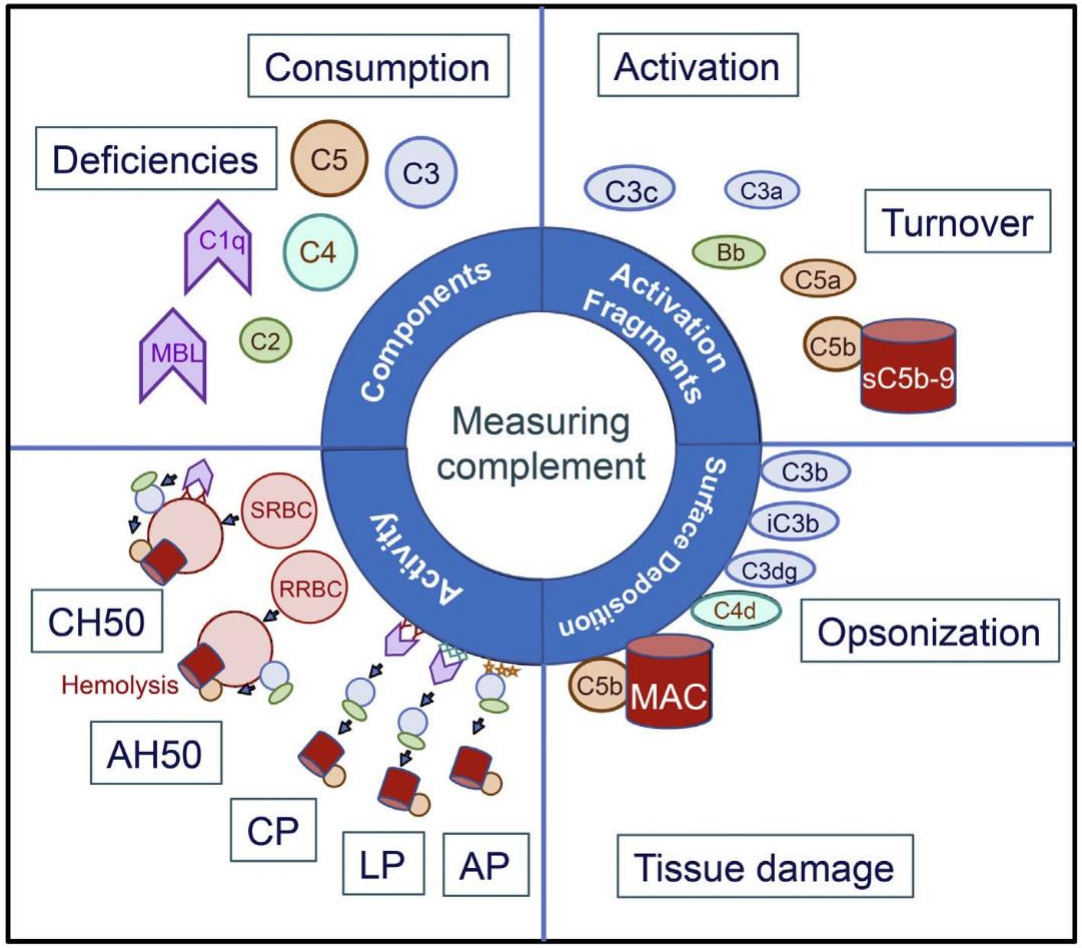


Figure from Ricklin D, et al. *Mol Immunol.* 2017;89:10-21

+ Autoantibodies

+ complement genetics

THE UTILITY OF COMPLEMENT BIOMARKERS IN THE CLINICAL SETTING

Key Applications of Complement Biomarkers

Diagnosis

- ✓ Determination of complement involvement
- ✓ Distinguish between different complement-mediated diseases
- ✓ Identify pathway-specific abnormalities

Prognosis

- ✓ Certain biomarker patterns correlate with:
  - Disease severity
  - Risk of progression or recurrence

Treatment Selection

- ✓ Guide use of:
  - Complement inhibitors
  - Immunosuppressants
- ✓ Identify patients needing targeted therapy vs supportive care

Monitoring

- ✓ Track:
  - Disease activity
  - Response to therapy
  - Relapse risk

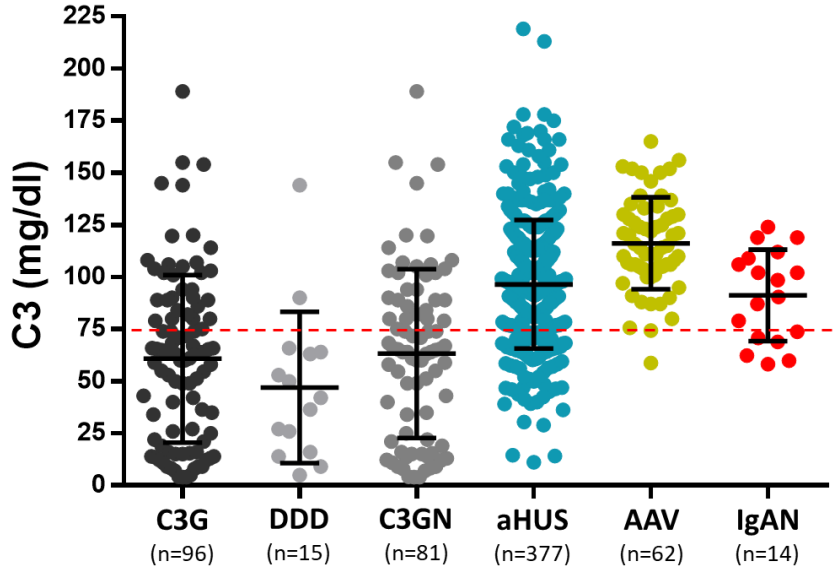
✓ Diagnosis | ✓ Prognosis | ✓ Treatment Selection | ✓ Monitoring

COMPLEMENT ASSAYS CURRENTLY AVAILABLE

	Biomarker	Method	Clinical relevance	Impact on management	Comments
Complement protein levels	**C3, C4** **FB** **FH, FI, MCP** **C5** **FHR-1** **FHR-5**	Nephelometry ELISA Luminex FACS	• **All**: Congenital deficiencies • **C3**: complement activation/AP activation • **C4**: CP, LP activation • **FH, FI, MCP**: suggestive of AP activation • **FB**: AP activation • **C5**: terminal pathway activation	**All:** • Determination of complement involvement • Useful for variant classification (e.g. null alleles) • **C5 only:** Monitoring of complement inhibition • **FHR-1 and -5**: Prognostic value	**C3, C4**: • Widely available in reference labs • Reference values • In combination may aid in differential diagnosis **FH, FI, FB, MCP**: Family studies often useful • **FHR-1:** Prognostic in C3G and IgAN • **FHR-5:** Prognostic in IgAN

Table modified from Vivarelli M, et al. (2024)

Normal plasma levels cannot rule out complement activation or functional defects



Dr. Goicoechea de Jorge.
Unpublished data

- The value of these markers increases when interpreted together.

COMPLEMENT ASSAYS CURRENTLY AVAILABLE

	Biomarker	Method	Clinical relevance	Impact on management	Comments
Activated proteins **or** **Complexes**	**C3a/iC3b/C3b/ C3c/C3dg/C3d** **C4d, C5a, Ba/Bb** **sC5b-9** **C1s:C1Inh** **MASP1:C1Inh**	ELISA Multiplex ELISA Luminex assay	• **C3a/iC3b/C3b/C3c/C3dg/C3d**: complement activation/AP activation • **C4d**: complement activation/CP, LP activation • **Ba#/Bb**: AP activation • **C5a, sC5b-9**: terminal pathway activation • **C1s:C1Inh**: early marker of CP activation • **MASP1:C1Inh**: early marker of LP activation	• Determination of complement activation status • Monitoring of active disease • Monitoring of complement inhibition (i.e., C5a, sC5b-9)	• Only available in specialized labs and for research purposes • Strict logistic requirements • Reference values • Proper sample collection is critical to avoid *ex vivo* complement activation

Table adapted from Vivarelli M, et al. (2024)

- *In vitro* complement activation.
- EDTA-plasma samples and a correct sample handling is required.
- A negative result in a plasma sample cannot rule out C activation in the glomerular microenvironment.
- Urine: C3d, Ba, Bb, C5a, sC5b-9
- The value of these markers increases when interpreted together.

COMPLEMENT ASSAYS CURRENTLY AVAILABLE

	Biomarker	Method	Clinical relevance	Impact on management	Comments
In vitro assays	**CH50, AP50** **SRBC hemolysis assay** **HMEC-1 (serum), HMEC-1 (activated plasma)** **mHam test**	• **CH50, AP50**: hemolysis assays, ELISA • **SRBC hemolysis assay**: hemolysis assay • **HMEC-1, mHam test**: tissue culture/immunofluorescence/confocal microscopy, flow cytometry	• **CH50**: overall complement activity • **AP50**: AP activity • **CH50, AP50**: Useful to identify global complement deficiency or consumption • **SRBC assay**: impaired FH-dependent surface-regulation of complement, predisposition to C' dysregulation • **HMEC-1**: complement activation with consequences to endothelial cells, predisposition to C' dysregulation • **mHam test**: complement activation with consequences to cell surfaces	• **CH50, AP50, HMEC-1, mHam test**: useful for screening complement dysfunction and monitoring of complement inhibition • **SRBC hemolysis assay**: detection of FH/FH related-1 abnormalities • **HMEC-1, mHam test**: detection of predisposition to C' dysregulation at cell surface level	• **CH50, AP50**: widely available, limited value • **SRBC hemolysis assay**: false negatives in activated serum • **HMEC-1, mHam test**: performed at selected labs and for research purposes, uncertain if there are false negatives in activated plasma

Table modified from Vivarelli M, et al. (2024)

- Low **CH50** and **AP** values suggest complement consumption or genetic deficiencies but do not provide mechanistic information.
- **HMEC-1** and **mHam** tests help identify patients who will benefit from targeted complement inhibition.
- **HMEC-1** test has been shown to detect complement dysfunction in samples in remission.

COMPLEMENT ASSAYS CURRENTLY AVAILABLE

	Biomarker	Method	Clinical relevance	Impact on management	Comments
Anti-factor antibodies	**Anti-FH** **anti- FB** **Nefritic factors**	ELISA LFA (FH) Hemolysis assays	• **Anti-factor H**: impaired complement regulation by FH • **Anti-factor B**: increased formation of C3 convertase • **Nefritic factors**: stabilisation of convertases	• **All**: Identify acquired complement dysregulation. Guide treatment decision • **Anti-factor H**: diagnosis of complement-mediated TMA. Help predict risk of recurrence • **Anti-factor B**: diagnostic • **Nefritic factors**: aid diagnosis of C3G	• **Anti-factor H**: rather widely available, standardization required • **Anti-factor B**: more prevalent in acute PIGN than in C3G or IC-MPGN • **Nefs**: common drivers of C3G

Table modified from Vivarelli M, et al. (2024)

- Nefritic factors are very heterogenous (e.g. type of NeFs, epitope, mechanism of action)
- Complexity and variability of detection methods (e.g. Autoantibody vs Ag-Ab complex detection in ELISA and LFA, respectively).

LFA FOR ANTI-FACTOR H DETECTION

frontiers | Frontiers in Immunology

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Novel immunochromatographic test for rapid detection of anti-factor H autoantibodies with an assessment of its clinical relevance

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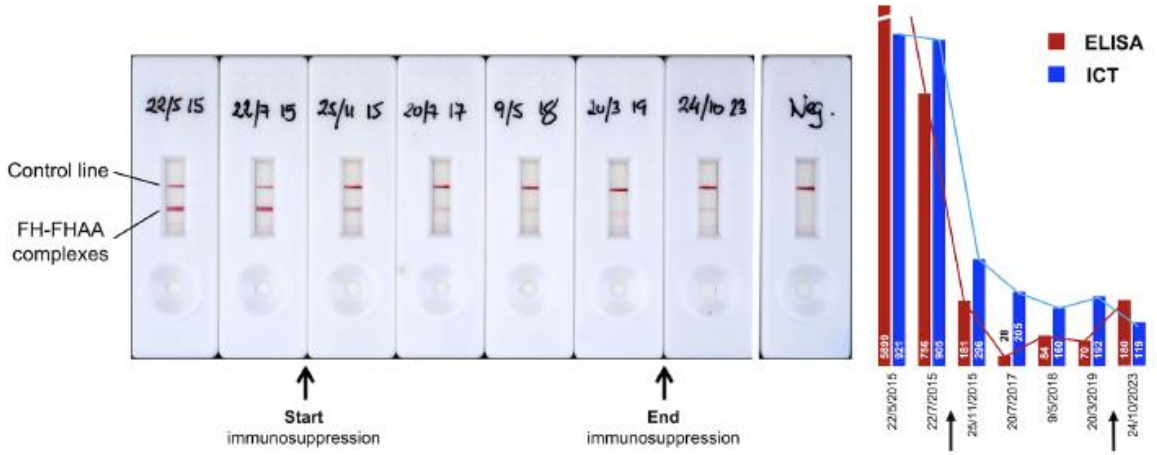
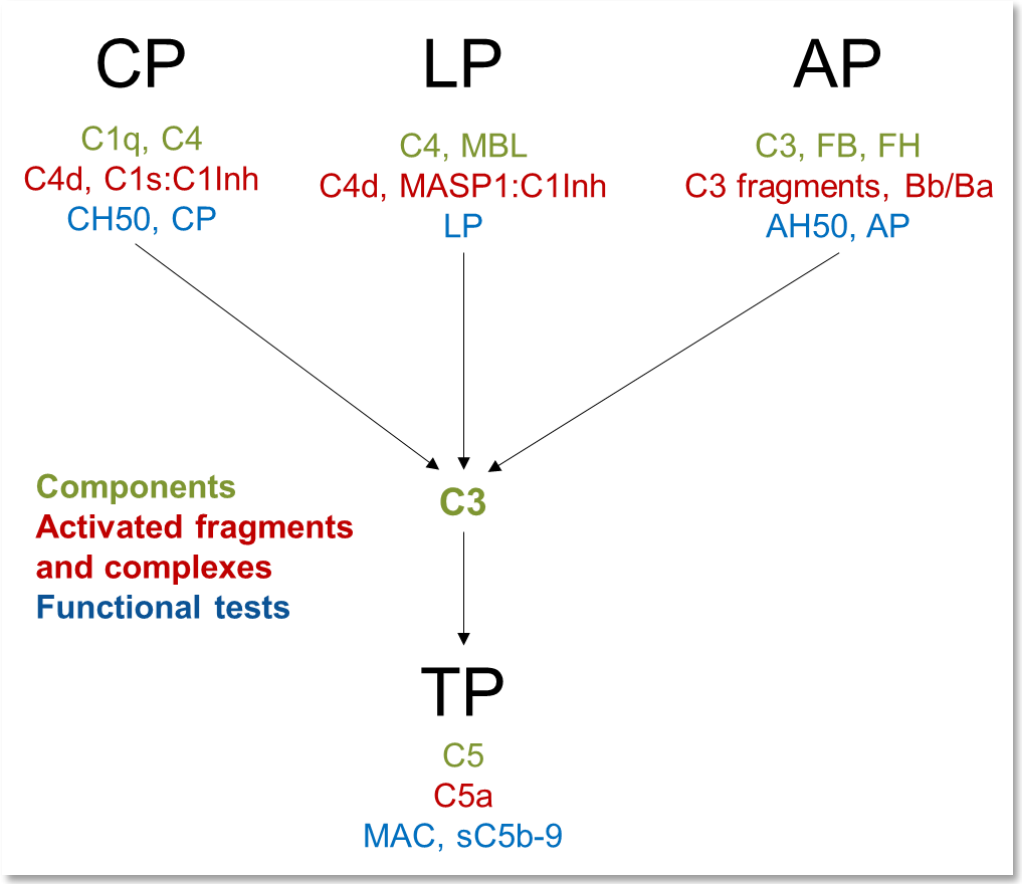


FIGURE 8
Use of ICT to monitor FH-FHAA complexes in patients under immunosuppressive treatment. Serial samples from a patient originally diagnosed with high free FHAA titers, as determined by ELISA, were obtained at different dates (hand written in the cassettes), before, during, and after immunosuppression. For each sample, an ELISA and ICT were performed to determine titers of free FHAA and levels of FH-FHAA complexes.

"This ICT offers improved detection of FHAAs compared to ELISA as demonstrated by cases where the ICT identifies FH-FHAA complexes in samples that tested negative with the free FHAA ELISA."

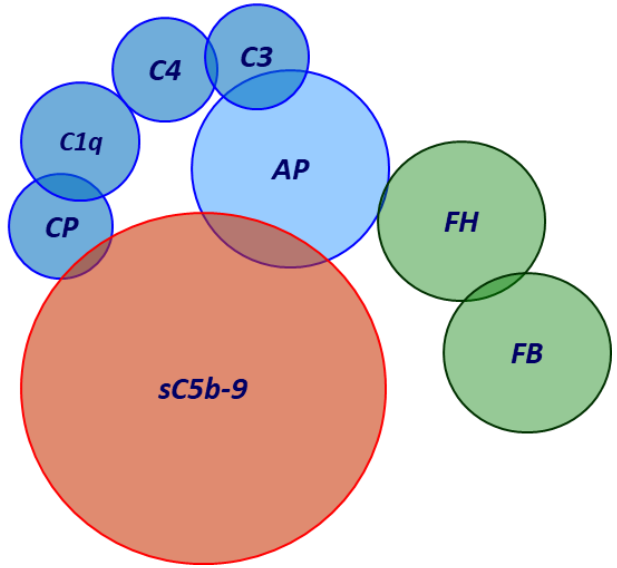
"This novel assay offers a simple, fast, cost-effective, and likely, more clinically relevant alternative for diagnosing FHAAs in at-risk populations."

PATTERNS OF COMPLEMENT ACTIVATION



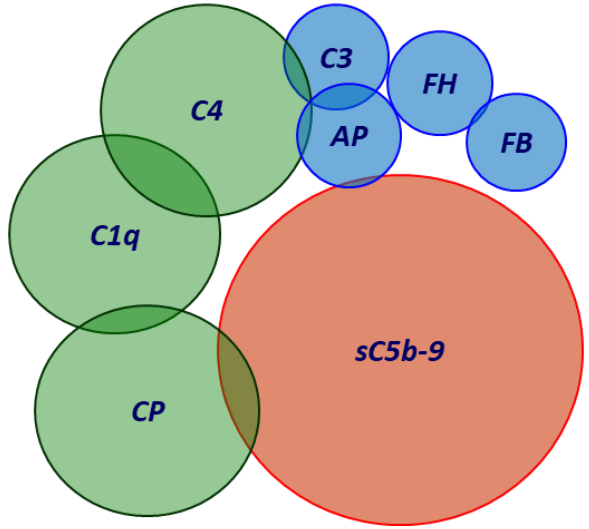
CP overactivation pattern

- *Infection, autoimmunity (immunocomplex)*
- *Active SLE*



AP dysregulation pattern

- *Alternative pathway dysregulation*
- *Het. factor H deficiency*

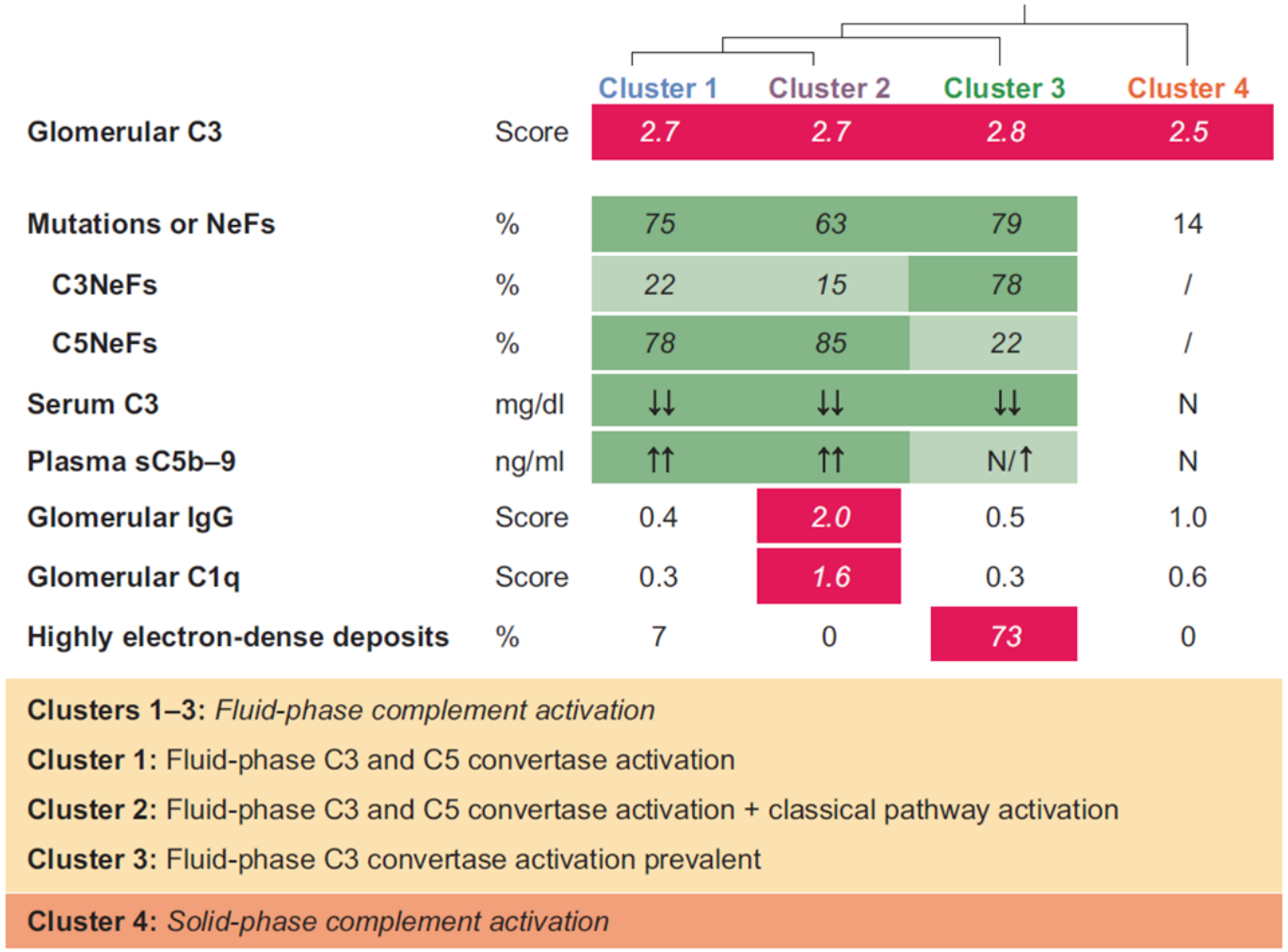


Increase
Normal
Decrease

Patterns help differentiate disease mechanisms:
↓C3 with normal C4 → **alternative pathway activation**
↓C3 and ↓C4 → **classical pathway activation**

Image courtesy of Dr. Zoltán Prohászka

Complexity of complement activation Patterns in C3G/IC-MPGN



		Cluster 1	Cluster 2	Cluster 3	Cluster 4
Glomerular C3	Score	*2.7*	*2.7*	*2.8*	*2.5*
Mutations or NeFs	%	*75*	*63*	*79*	14
C3NeFs	%	*22*	*15*	*78*	/
C5NeFs	%	*78*	*85*	*22*	/
Serum C3	mg/dl	↓↓	↓↓	↓↓	N
Plasma sC5b–9	ng/ml	↑↑	↑↑	N/↑	N
Glomerular IgG	Score	0.4	*2.0*	0.5	1.0
Glomerular C1q	Score	0.3	*1.6*	0.3	0.6
Highly electron-dense deposits	%	7	0	*73*	0

Clusters 1–3: *Fluid-phase complement activation*

Cluster 1: Fluid-phase C3 and C5 convertase activation

Cluster 2: Fluid-phase C3 and C5 convertase activation + classical pathway activation

Cluster 3: Fluid-phase C3 convertase activation prevalent

Cluster 4: *Solid-phase complement activation*

Marina Noris, Erica Daina and Giuseppe Remuzzi. Nephrol Dial Transplant (2023) 38: 283–290.

PRACTICAL CHALLENGES IN THE CLINICAL APPLICATION OF COMPLEMENT BIOMARKERS

Rizk et al. CJASN; 2025; doi: https://doi.org/10,2215/CJN,0000000967

Clinical Unmet needs in Complement Biomarkers

- **Current biomarkers are insufficient for precision medicine in complement-mediated kidney diseases.**

Laboratory and technical Unmet needs in Complement Biomarkers

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External quality assurance program for diagnostic complement laboratories: evaluation of the results of the past seven years

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- Assess the actual performance of laboratories
- Identify weaknesses in methods and procedures
- Generate comparable data over time
- This makes it an essential tool for standardising complement diagnostics

Laboratory and technical Unmet needs in Complement Biomarkers

- **Complement testing is not sufficiently standardized or reproducible.**

SUMMARY

- **We have several tools to assess the complement system…**

  … but expert knowledge is required to interpret the results correctly.

"We may still be far from the ideal, but the distance we have already covered brings us closer than ever to precision medicine in complement-mediated diseases."

THANK YOU

FUTURE DIRECTIONS

1. Integrated Biomarker Strategies

- Move from single markers → **composite biomarker panels**
- Combine: Functional assays, components, activation fragments, autoantibodies, genetic data, high-throughput & multi-Omics approaches, Advanced Data Integration with the use of AI and machine learning

2. Standardization for implementation in the clinical practice.

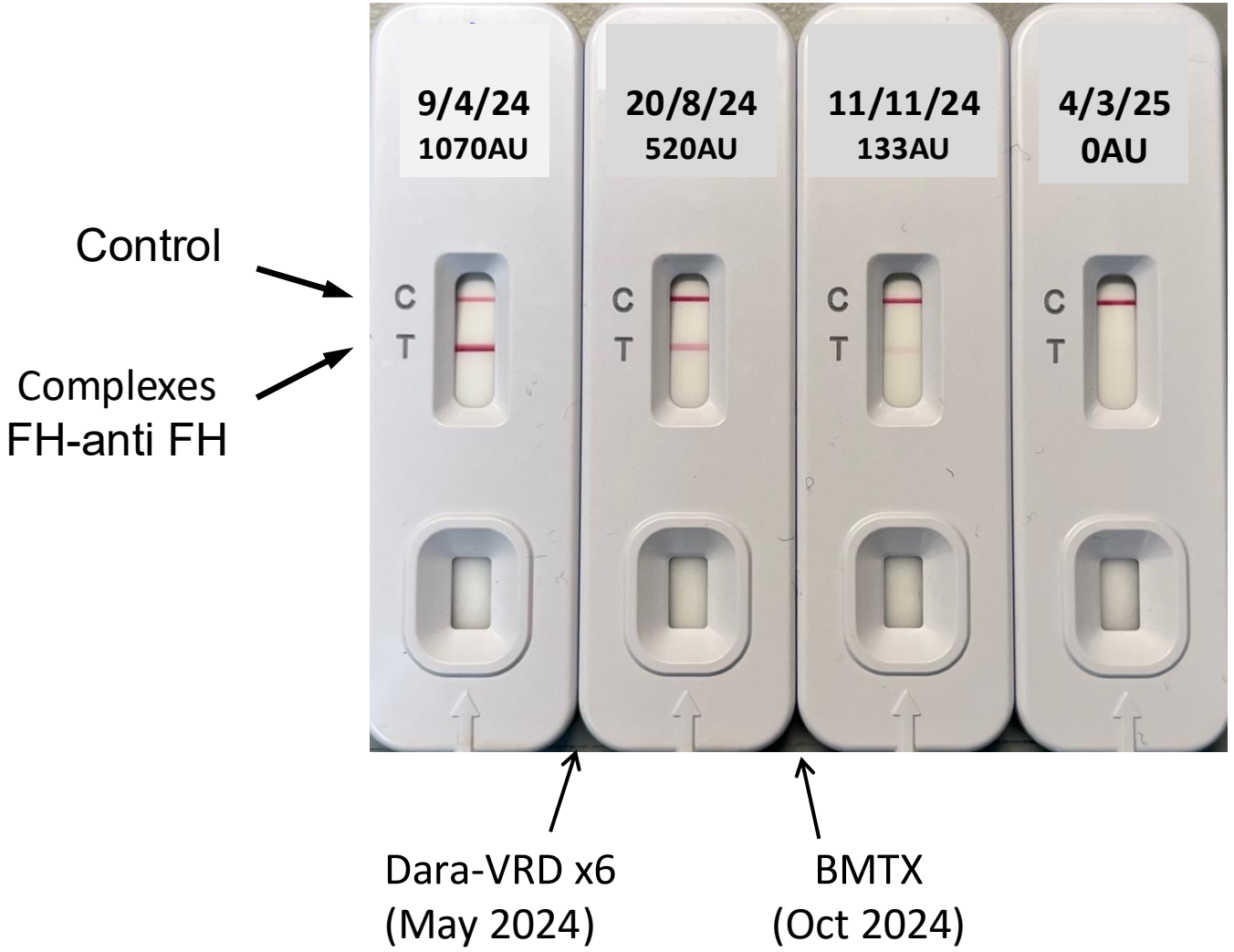
3. Clinical trials for complement inhibitors with a comprehensive complement and genetic workout.

- Impact of inhibitors on complement biology
- Identification of predictive tools to guide precission medicine

MONITORING OF COMPLEMENT INHIBITION STRATEGIES

Component or funtional test	anti-C5 (ecu/ravu)	Pegcetacoplan	Iptacopan
CP activity	Deficient/method dep.	Unchanged	Unchanged
AP activity	Deficient/Deficient	Deficient	Deficient
C3	No change	3-4x upper limit	No change (return to normal)
Additional parameters?	Free Ab Low free C5	Decrease in C3dg	-

DETECTION AND MONITORING OF ANTI-FH AUTOANTIBODIES IN C3G



- 55-year-old male with normal kidney function until May 2023. In January 2024, he presented with creatinine 1.5 mg/mL and proteinuria of 3.4 g/24h, associated with **IgG-kappa multiple myeloma**.
- Kidney biopsy showed granular C3 deposits (+++) without classical immune complexes, leading to a diagnosis of C3 glomerulopathy with focal and segmental glomerulosclerosis (collapsing variant) in the context of crystalline podocytopathy due to kappa light chains (MGRS).
- Low C3 levels, with no complement genetic variants.
- **ELISA negative for anti-FHAA, but strong positivity for IgG FH-anti-FH immune complexes on ICT**.

As many as 46% of MGRS-C3G patients test positive for FHAA

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

Sydney S Jellison[1]*, Sarah M Roberts[1]*, Samantha J Martin[1], Carla M Nester[1], Andrea Reparaz[2], Santiago Rodriguez de Cordoba[2], Richard JH Smith[1], Yuzhou Zhang[1]
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Background
C3 glomerulopathy (C3G) is a complement-mediated kidney disease driven by dysregulated alternative pathway (AP) activation. In some patients (typically >50 years of age), a diagnosis of monoclonal gammopathy of renal significance (MGRS) is made to reflect the presence of small B-cell or plasma cell clones that produce nephrotoxin monoclonal immunoglobulins. How these monoclonal proteins contribute to disease (MGRS-C3G) is an important knowledge gap to address. We hypothesize that Factor H autoantibodies (FHAAs), particularly when forming immune complexes FH (FHICs), significantly contribute to disease. Moreover, we believe traditional ELISA-based assays have limited sensitivity in detecting circulating FHICs, especially IgM-type FHAAs.

Methods
We evaluated a lateral flow assay (LFA) (1) consisting of three cassettes to detect both IgG- and IgM-type FHICs in serum samples from 58 C3G patients. Patients were categorized by FHAA ELISA results: Group A: FHAA >1000 Arbitrary Units (AU); n=10 (C3G = 8; MRGS-C3G = 2, including one patient IgM-only positive). Group B: ambiguous/equivocal FHAA levels (200–1000 AU); n=14 (C3G = 10; MGRS-C3G = 4). Group C: MGRS-C3G patients lacking known acquired or genetic disease drivers but showing fluid-phase complement hyperactivity; FHAA <200 AU; n = 33.

Results
Group A: LFA confirmed FHIC positivity in all 10 patients, including the single IgM-only case. Notably, six additional cases were IgM co-positive, highlighting enhanced LFA sensitivity over ELISA. Group B: LFA detected clear FHIC positivity in 11 of 14 patients (79%), clarifying the ambiguous ELISA results. All four MGRS patients are strongly positive for IgG-type FHICs. No IgM-type FHIC positives were identified in this group. Group C: Despite being ELISA-negative, LFA detected FHICs in 12 of 33 patients (36%); among these, four were weakly IgM-FHAA co-positive.
Overall, FHICs were identified 33 of 58 patients, including 18 of 39 MGRS-C3G cases.

Conclusion
In 18 of 39 MGRS patients (46%), FHAAs were detected as the monoclonal protein driving complement dysregulation. Conventional ELISAs identified only 6 of these cases, likely due to FHICs formation masking detection. These findings highlight both the pathogenic role of FHAAs in MGRS-C3G and the diagnostic value of LFA. Large-scale prospective studies are warranted to confirm and expand upon these observations.

Acknowledgements and References
Supported in part by National Institutes of Health R01 DK110023.
(1) Rodrıguez de Cordoba S *et al.* Front. Immunol. 15:1527016. doi: 10.3389/fimmu.2024.1527016

Clinical Unmet needs in Complement Biomarkers

➢ **Current biomarkers are insufficient for precision medicine in complement-mediated kidney diseases.**

- **Key limitations:**

  - Incomplete biological information: No single biomarker captures disease activity, pathway specificity and treatment response.

  - Poor clinicopathologic correlation: Weak association with histologic activity and disease progression.

  - Systemic vs intrarenal mismatch: Blood biomarkers ≠ kidney activity, lack of reliable noninvasive renal biomarkers

  - Limited role in therapy guidance: No standardized tools to select patients, to monitor complement inhibitors, and to define treatment duration

  - Disease heterogeneity is not captured: Especially in C3G and IC-MPGN. Need for mechanism-based stratification

➢ **Clinical need for Integrated, kidney-specific, dynamic biomarker panels**

Laboratory and technical Unmet needs in Complement Biomarkers

- **Complement testing is not sufficiently standardized or reproducible.**
- **Key limitations:**

  - Lack of standardization: No reference methods or universal calibrators. Results not interchangeable across labs

  - Significant inter-laboratory differences: Particularly in functional assays, sC5b-9 and C3NeFs

  - Greater dispersion in clinically relevant scenarios: Lower accuracy in pathological samples

  - Preanalytical challenges: Up to 50% of errors due to sample handling and ex vivo activation

  - Structural Limitations: Limited availability of advanced assays, autoantibody testing, lack of control materials and commercial standardization

- **Need for global harmonization + robust assays + strict preanalytical control**