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Complement Activation in Primary IgA Nephropathy (CAIGAN): study protocol for an international prospective multicenter observational cohort study

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Study protocol

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Abstract

Background

IgA nephropathy (IgAN) is the most common primary glomerular disease worldwide and a leading cause of chronic kidney disease and kidney failure. Increasing evidence supports a central role for complement activation in disease severity and crescent formation. With the emergence of complement-targeted therapies, biomarkers identifying patients with active complement dysregulation are increasingly needed.

Methods

CAIGAN (Complement Activation in IgA Nephropathy) is an international, prospective, observational multicenter cohort study involving adult and pediatric nephrology centers in Switzerland, Italy, France, and Spain. Consecutive patients undergoing clinically indicated native kidney biopsy for suspected IgAN will be screened, and those with biopsy-proven primary IgAN will be enrolled. Clinical, laboratory, and histopathological data will be collected at baseline, with clinical and laboratory follow-up at 1, 2, and 3 years. Blood and urine samples for complement analyses will generally be obtained after histopathological confirmation of IgAN, within 4 weeks after kidney biopsy and before initiation of immunosuppressive therapy. In patients with a rapidly progressive clinical course requiring prompt immunosuppressive treatment, sampling may precede histopathological confirmation and will be performed after informed consent and before administration of immunosuppressive agents. Complement profiling will include circulating complement proteins and activation products, functional pathway assessment, and optional complement-related genetic analyses. Kidney biopsies will be characterized using the Oxford MEST-C classification, with intrarenal complement deposition and exploratory transcriptomic analyses of residual kidney tissue performed in a subgroup. The primary objective is to validate prospectively the association observed in our retrospective pilot study between circulating sC5b-9 concentrations and the percentage of active glomerular crescents. Secondary exploratory objectives include characterization of the mechanisms underlying complement activation and dysregulation and evaluation of their associations with clinical phenotype, histopathological findings, renal tissue complement activation, genetic and transcriptomic profiles, and longitudinal kidney outcomes.

Discussion

CAIGAN will integrate complement phenotyping, genetics, histopathology, and prospective clinical follow-up to characterize complement activation across the spectrum of primary IgAN and possibly support the development of biomarker-guided therapeutic strategies.

Background

IgA nephropathy (IgAN) is the most frequent glomerular disease worldwide and remains a leading cause of chronic kidney disease (CKD) and end-stage kidney failure (ESKF) in young adults [1]. IgAN is characterized by mesangial deposition of galactose-deficient IgA1 (Gd-IgA1)-containing immune complexes, which initiate glomerular inflammation and progressive kidney injury [2]. Interestingly, mesangial IgA deposition may remain clinically silent for many years, as demonstrated by incidental findings in donor kidneys and autopsy studies, suggesting that additional pathogenic mechanisms are required for clinically overt disease [3-4].

The mechanisms responsible for the transition from silent mesangial IgA deposition to clinically overt nephropathy remain incompletely understood. Increasing evidence suggests that complement activation represents one of the key amplifiers of this transition [5]. Activation of the lectin pathway (LP) by Gd-IgA1-containing immune complexes is thought to amplify the alternative pathway (AP), resulting in the generation of complement component 3a (C3a), complement component 5a (C5a), and the membrane attack complex (C5b-9), which promotes inflammation, mesangial proliferation, and tissue injury [6-7].

Clinical manifestations of IgAN range from mild urinary abnormalities, such as hematuria and/or proteinuria, to CKD and severe forms of rapidly progressive glomerulonephritis leading to end-stage kidney failure (ESKF) [8]. The degree of complement activation seems to correlate with the severity of histopathological findings at the time of kidney biopsy, as well as the severity of the clinical presentation [9].

Among all histopathological lesions, active extra-capillary proliferation (glomerular crescents) identifies patients with the highest inflammatory burden and the worst kidney prognosis [10]. Patients with IgAN and active glomerular crescents showed higher urinary levels of C3a, C5a, and soluble C5b-9 (sC5b-9), as well as a linear association between urinary levels of mannose-binding lectin (MBL) and complement component 4d (C4d), markers of LP activation, and the proportion of crescents [11].

Our group recently demonstrated in a retrospective cohort study that plasma sC5b-9 concentrations were significantly higher in patients with crescentic IgAN and strongly correlated with the percentage of active glomerular crescents [12]. We also observed a trend toward higher plasma complement factor B activation fragment (Bb) levels in crescentic IgAN, a marker of AP activation. Another study using in situ immunofluorescence to assess complement protein deposition demonstrated a significant association between mesangial factor B deposition and active glomerular crescents in IgAN [13].

We hypothesize that complement activation in IgAN is highly heterogeneous among patients, with variable contributions of the lectin and alternative pathways. This biological heterogeneity may explain the inconsistent associations observed between individual complement biomarkers and disease severity in previous studies.

The recent development of complement-targeted therapies has profoundly changed the therapeutic landscape of IgAN as well as other glomerular diseases such as C3 glomerulopathy [14]. Several agents targeting different components of the complement cascade have recently entered clinical practice or are

undergoing advanced clinical evaluation [15-17]. Consequently, identifying biomarkers capable of accurately characterizing complement activation in individual patients has become an essential step towards precision medicine.

The CAIGAN study is a prospective multicenter cohort study of adult and pediatric patients with primary IgAN. The primary objective is to prospectively validate the association observed in our retrospective pilot study between circulating sC5b-9 concentrations and the percentage of active (cellular and/or fibrocellular) glomerular crescents. Secondary exploratory objectives include characterization of complement activation and dysregulation through circulating biomarkers, functional assessment of the alternative and lectin pathways, and acquired complement abnormalities, as well as their associations with clinical and histopathological features. Complement-related genetic variants, intrarenal complement deposition, renal transcriptomic signatures, and the association between baseline complement activation profiles and longitudinal kidney outcomes will also be explored. This integrated approach may help characterize the heterogeneity of complement activation in IgAN and provide a translational platform for future biomarker-guided therapeutic strategies.

Methods

Study design

CAIGAN is an international, prospective, observational multicenter cohort study designed to investigate complement activation in adult and pediatric patients with newly diagnosed primary IgAN. An overview of the CAIGAN study design and longitudinal follow-up is presented in Fig. 1.

The results of the CAIGAN study will be reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations [18]. Where applicable, reporting of data derived from routine clinical care and medical records will also follow the RECORD statement [19].

Study setting

The study will be conducted in university and regional hospitals across Switzerland, Italy, France, and Spain, with recognized expertise in the diagnosis and management of glomerular diseases. Participating centers will recruit patients over a 3-year inclusion period using a harmonized study protocol.

Study population

Consecutive adult and pediatric patients undergoing a clinically indicated native kidney biopsy for suspected IgA nephropathy will be screened for eligibility.

Clinical suspicion of IgAN will be based on one or more of the following clinical presentations:

- rapidly progressive glomerulonephritis;

- chronic kidney disease of unknown origin, frequently associated with persistent microscopic hematuria, with or without mild proteinuria;
- isolated urinary abnormalities, including microscopic hematuria and mild proteinuria detected during routine evaluation;
- recurrent episodes of macroscopic hematuria, typically occurring in association with upper respiratory or gastrointestinal infections; or
- nephrotic-range proteinuria, defined as a urine protein-to-creatinine ratio >300 mg/mmol or a 24-hour urinary protein excretion >3.5 g/day.

Inclusion criteria

Eligible participants will include adult and pediatric patients undergoing a clinically indicated native kidney biopsy with histopathologically confirmed primary IgA nephropathy. Kidney biopsy specimens must contain at least 10 glomeruli available for histopathological assessment, with pathology records available for review. The presence of TMA lesions on kidney biopsy will not constitute an exclusion criterion in the absence of an identifiable secondary cause of TMA.

Written informed consent must be obtained from the participant and/or legal representative, as applicable.

Exclusion criteria

Patients will be excluded if kidney biopsy does not confirm IgA nephropathy or if secondary causes of IgA deposition are identified. Specifically, exclusion criteria include:

- secondary IgA nephropathy related to chronic liver disease or systemic vasculitis, including IgA vasculitis (Henoch–Schönlein purpura);
- IgA nephropathy superimposed on diabetic nephropathy or ANCA-associated vasculitis;
- crescents attributable to another glomerular disease, including lupus nephritis, membranous nephropathy, anti-glomerular basement membrane disease, or post-infectious glomerulonephritis;
- equivocal cases suggestive of IgA-dominant post-infectious glomerulonephritis, characterized by predominant C3 deposition relative to IgA together with documented recent Staphylococcus infection;
- de novo or recurrent IgA nephropathy in a kidney allograft;
- thrombotic microangiopathy (TMA) secondary to another underlying disorder, including Shiga toxin-associated hemolytic uremic syndrome, pregnancy-associated hemolytic uremic syndrome, infection, drug-induced TMA (e.g., calcineurin inhibitors or gemcitabine), malignancy, transplantation, autoimmune disease, malignant hypertension, sickle cell disease, glucose-6-phosphate dehydrogenase deficiency, folate deficiency, or other recognized secondary causes [20].

- Refusal to receive information for clinically relevant incidental findings during the study which may contribute to the prevention, diagnosis, and treatment of an existing or future disease.

Study procedures

Baseline demographic, clinical and laboratory data will be collected at the time of diagnostic evaluation and kidney biopsy. Recorded variables will include age, sex, gender, body weight, mean arterial pressure (MAP), relevant comorbidities, current medications, serum creatinine, estimated glomerular filtration rate (eGFR) calculated using the CKD-EPI equation in adult participants and a Schwartz-based equation in pediatric participants, proteinuria, albuminuria and hematuria. Proteinuria and albuminuria will be assessed using the urine protein-to-creatinine ratio (UPCR) and urine albumin-to-creatinine ratio (UACR), respectively, preferably on first-morning urine specimens. Hematuria will be assessed by urine microscopy and quantified as the number of red blood cells per high-power field (RBC/HPF). Longitudinal clinical and laboratory data will be collected at 1, 2, and 3 years after inclusion, from routine clinical follow-up and medical records. These data will include serum creatinine, eGFR, proteinuria, albuminuria, and information on kidney replacement therapy and kidney transplantation. Major clinical events occurring during follow-up will also be recorded, including hospitalization, new diagnosis of malignancy, serious infections, major surgeries, and major cardiovascular events. Treatment exposure will be recorded at baseline and during follow-up, including initiation, discontinuation, or relevant changes in renin–angiotensin system blockade, sodium–glucose cotransporter-2 (SGLT2) inhibitors, systemic corticosteroids and other immunosuppressive therapies, and complement-targeted therapies. At each follow-up time point, the occurrence of components of the prespecified composite kidney outcome will be recorded. A ≥50% decline in eGFR from baseline or an eGFR <15 mL/min/1.73 m² will be considered an endpoint only if confirmed by a subsequent measurement performed at least 2 weeks later. No additional study-specific clinical visits, blood collections, or urine collections will be required for longitudinal follow-up; available data will be obtained from routine clinical care and medical records.

Because immunosuppressive therapy may influence circulating complement biomarkers, blood and urine samples intended for complement analyses will be collected before initiation of immunosuppressive treatment.

As a general rule, blood and urine samples for complement analyses will be obtained after histopathological confirmation of IgA nephropathy and informed consent, within a maximum of 4 weeks after kidney biopsy. In patients presenting with a rapidly progressive clinical course for which prompt initiation of immunosuppressive therapy is anticipated, blood and urine samples will be obtained after informed consent and before administration of immunosuppressive agents. In these exceptional cases, complement sampling may precede histopathological confirmation and may be performed at or shortly before the time of kidney biopsy. The study workflow for kidney biopsy and biospecimen collection is summarized in Fig. 2.

The time interval between kidney biopsy and complement sampling will be recorded for each participant. All biological samples will be processed locally according to standardized pre-analytical protocols.

Aliquots will be stored at −80°C in the Valais Hospital Biobank. Baseline complement analyses will be centralized at the Laboratory of Lausanne University Hospital.

Complement analyses

Comprehensive complement profiling will include functional and quantitative assessment of the classical, alternative, lectin and terminal complement pathways. Analyses will include complement components C3 and C4; complement factor B (FB) and its activation fragment Bb; the regulatory proteins factor H (FH) and factor I (FI); soluble C5b-9 (sC5b-9); functional assessment of the classical, alternative and lectin pathways using CH50, AP50 and mannose-binding lectin (MBL) pathway activity, respectively; anti-factor H (anti-FH) antibodies; and additional characterization of the lectin pathway, including mannose-binding lectin-associated serine proteases (MASPs) and other lectin-pathway components, where available.

Frozen serum samples will be used for determination of CH50, AP50 and MBL pathway activity and for detection of anti-FH antibodies. Functional pathway activity will be assessed using commercially available WIESLAB® enzyme-linked immunosorbent assay (ELISA) kits. EDTA plasma samples will be used to quantify sC5b-9, Bb, FH and FI. Soluble C5b-9 and Bb will be measured using commercially available MicroVue® ELISA kits (Quidel), whereas FH and FI concentrations will be determined using commercially available ELISA kits from Hycult Biotech.

Urinary sC5b-9 and other selected complement components may also be assessed as exploratory biomarkers of complement activation in IgAN. Complement analyses will be centralized at the Laboratory of Lausanne University Hospital.

In addition, the CAIGAN study will provide a framework for the development and validation of additional assays aimed at characterizing acquired mechanisms of complement dysregulation. These will include nephritic factors targeting complement convertases (C3 nephritic factor [C3NeF], C5 nephritic factor [C5NeF], and C4 nephritic factor [C4NeF]) and complement-directed autoantibodies, including antibodies against FB, C3b, and properdin. Additional complement autoantibodies may be investigated as assay development and validation progress. These exploratory analyses will aim to identify acquired mechanisms contributing to alternative and lectin/classical pathway dysregulation and downstream terminal complement activation.

Genetic analyses

Genetic analyses will be optional and performed only in participants who provide specific informed consent for genetic testing. Peripheral blood collected in EDTA tubes after histopathological confirmation of primary IgAN will be used for genomic DNA extraction. As genetic analyses are not affected by immunosuppressive treatment, genetic sampling may be performed at any time following confirmation of the diagnosis.

Genetic analyses will be performed by whole-exome sequencing (WES); however, bioinformatic analysis and interpretation for the purposes of this study will be strictly restricted to a predefined panel of genes involved in complement regulation and complement-associated thrombotic microangiopathy: ADAMTS13, C3, CD46, CFB, CFD, CFH, CFHR1, CFHR2, CFHR3, CFHR4, CFHR5, CFI, CFP, DGKE, HOXA2, MMACHC, and THBD. Complementary analysis for genomic rearrangements involving CFH, CFHR1, and CFHR3 will be performed by multiplex ligation-dependent probe amplification (MLPA).

DNA extraction and genetic analyses will be centralized at the Genetic Medicine Service of Lausanne University Hospital (CHUV).

Because of resource constraints, WES will initially prioritize participants with active crescentic IgAN. Depending on resource availability, genetic analyses may subsequently be extended to other participants with confirmed primary IgAN.

Histopathology

Kidney biopsy specimens will be evaluated by experienced renal pathologists at each participating center according to routine clinical practice. Histopathological lesions will be classified using the Oxford MEST-C classification, including mesangial hypercellularity (M), endocapillary hypercellularity (E), segmental glomerulosclerosis (S), tubular atrophy/interstitial fibrosis (T), and the presence of crescents (C) [21,22]. Cellular and fibrocellular crescents will be considered active crescents, whereas fibrous crescents will be considered chronic lesions. The absolute number and percentage of active and fibrous crescents will be recorded separately relative to the total number of evaluable glomeruli. The percentage of active glomerular crescents will be calculated as the number of glomeruli containing cellular or fibrocellular crescents divided by the total number of evaluable glomeruli × 100. Only kidney biopsies containing at least 10 glomeruli will be included in the study. The presence and histological characteristics of TMA lesions will also be systematically documented.

In the subgroup of participants whose kidney biopsies are assessed at Lausanne University Hospital (CHUV), local complement activation will be further investigated in kidney tissue by immunofluorescence staining, including assessment of C3, C5b-9 and C4d deposition. Associations between renal complement deposition, circulating complement biomarkers, histopathological lesions and clinical phenotype will be explored.

In this subgroup, exploratory transcriptomic analyses may also be performed on residual formalin-fixed paraffin-embedded (FFPE) kidney biopsy tissue available after completion of the diagnostic assessment. These analyses will aim to characterize renal gene-expression signatures associated with complement pathway activation, active glomerular injury, histopathological lesions and clinical phenotype. No additional kidney biopsy or tissue sampling will be performed for this purpose.

Study objectives

The primary objective is to prospectively validate the association observed in our retrospective pilot study between circulating sC5b-9 concentrations and the percentage of active glomerular crescents in patients with biopsy-proven primary IgA nephropathy.

The secondary exploratory objectives are to:

- Characterize activation and dysregulation of the alternative and lectin complement pathways through assessment of complement components, activation products, regulatory proteins, functional pathway activity, and acquired complement abnormalities, and investigate their relationships with circulating sC5b-9 concentrations.
- Evaluate the associations between complement pathway activation profiles and histopathological lesions and clinical presentation at diagnosis.
- Evaluate associations between circulating complement biomarkers and renal tissue complement deposition in the subgroup of participants whose kidney biopsies are assessed at Lausanne University Hospital.
- Explore renal transcriptomic signatures associated with complement pathway activation, active glomerular injury, histopathological lesions, and clinical phenotype in residual FFPE kidney biopsy tissue from the CHUV subgroup.
- Explore complement-related genetic variants in relation to circulating complement activation, clinical phenotype, and histopathological findings.
- Evaluate the associations between baseline complement activation profiles, histopathological findings, and longitudinal kidney outcomes assessed at 1, 2, and 3 years of follow-up.
- Establish a prospective multicenter biobank of biological samples from patients with IgA nephropathy to support future translational research.

Kidney outcomes will be assessed using a composite kidney endpoint defined, among participants without kidney failure at baseline, as the first occurrence of a ≥50% decline in eGFR from baseline, an eGFR <15 mL/min/1.73 m², initiation of kidney replacement therapy, or kidney transplantation. A ≥50% decline in eGFR from baseline or an eGFR <15 mL/min/1.73 m² will require confirmation by a subsequent measurement performed at least 2 weeks later. Kidney failure is defined by an eGFR <15 mL/min/1.73 m² and/or requirement for kidney replacement therapy. Participants presenting with an eGFR <15 mL/min/1.73 m² and/or requiring kidney replacement therapy at baseline will be analyzed separately with respect to kidney function recovery, persistence of kidney replacement therapy, and kidney transplantation during follow-up.

Sample size calculation

The sample size calculation was primarily based on the study objective of prospectively validating the association between circulating sC5b-9 concentrations and the percentage of active glomerular crescents. In our retrospective pilot study, a strong positive correlation was observed between plasma sC5b-9 concentrations and the percentage of active crescents (Spearman's rs = 0.73) [12]. To account

for the uncertainty associated with the small sample size of the pilot cohort, a more conservative correlation coefficient of 0.50 was assumed. With a two-sided α level of 0.05 and 80% statistical power, approximately 30 evaluable participants would be sufficient for the primary correlation analysis.

Although approximately 30 evaluable participants would be sufficient for the primary correlation analysis, CAIGAN is designed not only to validate this association but also to characterize the biological mechanisms underlying terminal complement activation. The study therefore aims to recruit 120 adult participants to enable comprehensive exploratory phenotyping of complement activation and dysregulation, including quantitative assessment of complement components, activation products and regulatory proteins, functional characterization of complement pathways, complement-directed autoantibodies, nephritic factors, and complement-related genetic variants. This larger cohort will improve the precision of biomarker estimates, allow characterization of distinct complement activation profiles across the clinical and histopathological spectrum of IgAN, provide a framework for the development and validation of novel complement assays, and account for unavailable or non-evaluable biological samples or histopathological data. Based on the reported incidence of IgAN of approximately 0.4 per 100,000 inhabitants [23], the population served by the participating adult centers, and the expected recruitment capacity of each center, we anticipate enrolling approximately 120 adult participants during the 3-year recruitment period. Because IgA nephropathy also affects children, recruitment will be extended to pediatric nephrology centers. Based on the estimated pediatric incidence of up to 0.2 cases per 100,000 children per year [24], we anticipate enrolling at least 30 pediatric patients during the recruitment period.

Statistical analysis

Continuous variables will be assessed for normality using graphical methods and the Shapiro–Wilk test. Normally distributed variables will be reported as mean ± standard deviation (SD), whereas non-normally distributed variables will be presented as median with interquartile range (IQR). Categorical variables will be summarized as frequencies and percentages.

Comparisons between groups will be performed using the Student's t-test for normally distributed continuous variables or the Mann–Whitney U test for non-normally distributed variables. Categorical variables will be compared using the χ^2 test or Fisher's exact test, as appropriate.

The primary analysis will assess the association between circulating sC5b-9 concentrations and the percentage of active glomerular crescents using Spearman's rank correlation coefficient. This analysis corresponds to the primary study objective on which the sample size calculation is based. Given the inclusion of both adult and pediatric participants, potential heterogeneity in the primary association according to age group will be explored. If sample size permits, the primary analysis will be repeated separately in adult and pediatric participants as an exploratory subgroup analysis. In addition, the association between circulating sC5b-9 concentrations and the percentage of active glomerular crescents will be reassessed in a sensitivity analysis using a regression model including the time interval between kidney biopsy and complement sampling as a covariate. Given the expected proportion of

participants without active crescents, circulating sC5b-9 concentrations will also be compared between participants with and without active glomerular crescents (0% vs >0%) using the Student's t-test or Mann–Whitney U test, as appropriate.

All secondary analyses will be considered exploratory. These analyses will investigate associations between complement biomarkers, functional complement pathway activity, complement-related genetic variants, histopathological findings, renal tissue complement deposition, transcriptomic signatures, and clinical characteristics, as applicable. Multivariable regression models may be used, where appropriate, to explore whether associations between complement biomarkers and active crescentic lesions persist after adjustment for clinically relevant covariates, including age, sex, mean arterial pressure, eGFR, proteinuria, and albuminuria.

Exploratory longitudinal analyses will examine associations between baseline complement biomarkers, genetic variants, histopathological findings, and kidney outcomes during the 3-year follow-up period. Where appropriate, multivariable analyses will be adjusted for established prognostic factors in IgA nephropathy, and longitudinal treatment exposure may also be considered where the number of observed events permits.

All statistical tests will be two-sided, and a P value <0.05 will be considered statistically significant. Statistical analyses will be performed using Stata version 16.1 (StataCorp, College Station, TX, USA) or R (R Foundation for Statistical Computing, Vienna, Austria).

Ethics

The study received ethics approval with conditions from the Commission cantonale d'éthique de la recherche sur l'être humain du Canton de Vaud (CER-VD; Project ID: 2023-00720) on 23 July 2026. The requested amendments have subsequently been submitted to the Ethics Committee and are currently under review. Participant recruitment will not begin until the CER-VD has confirmed that all conditions have been fulfilled. Written informed consent will be obtained from all participants before the initiation of any study-specific procedures. For pediatric participants, written informed consent will be obtained from parents or legal guardians, with assent obtained from children when appropriate according to local regulations.

Participation in the genetic component of the study will be optional. Participants who decline genetic analyses will remain eligible for all other study procedures and follow-up assessments.

The study will be conducted in accordance with the principles of the Declaration of Helsinki [25], the Swiss Human Research Act (HRA) [26], the Human Research Ordinance (HRO) [27], and all applicable local regulatory requirements. The sponsor-investigator assumes overall responsibility for the conduct of the study.

Discussion

Our preliminary pilot study from a single-center case series demonstrated a significant positive correlation between circulating sC5b-9, a marker of terminal complement pathway activation, and the percentage of active glomerular crescents [12]. This observation is further supported by the recent demonstration that inhibition of factor B with iptacopan significantly reduces proteinuria in patients with IgAN, reinforcing the pathogenic importance of the alternative complement pathway [28].

The CAIGAN study has been designed to extend our findings in a much larger prospective multicenter cohort. By bringing together different adult and pediatric nephrology centers from Switzerland, Italy, France, and Spain, this prospective international cohort will recruit a geographically diverse population of patients with biopsy-proven primary IgAN. The multicenter design is expected to improve recruitment, increase the precision of biomarker estimates, and enhance the external validity of the study findings.

One of the principal strengths of CAIGAN is the standardized prospective collection of blood and urine samples in relation to kidney biopsy and before initiation of immunosuppressive therapy. Immunosuppressive treatment is known to influence complement activation and may substantially modify circulating complement biomarkers. Collecting biological samples before immunosuppressive treatment initiation will minimize the potential influence of immunosuppressive therapy on complement biomarker measurements. To our knowledge, few previous multicenter studies have incorporated this standardized pre-treatment sampling strategy, thereby reducing potential confounding related to immunosuppressive treatment.

Another major strength of CAIGAN is its comprehensive translational design. Rather than investigating complement activation as an isolated biological process, the study aims to characterize complement dysregulation across multiple biological levels, from circulating complement proteins and activation products to functional complement pathway activity, complement-related genetic variants, intra-renal complement deposition, histopathological lesions, and long-term clinical outcomes. An additional innovative aspect is the planned development and validation of assays targeting acquired mechanisms of complement dysregulation, including nephritic factors and complement-directed autoantibodies. Integrating these analyses with complement components, activation products, and functional pathway assessment may help identify distinct biological mechanisms leading to terminal complement activation in individual patients. This mechanistic phenotyping may be particularly relevant in the context of therapies targeting different levels of the complement cascade. This integrated approach will provide a unique opportunity to investigate how systemic complement activation may translate into tissue injury and disease progression.

Importantly, the primary endpoint of the study focuses on the association between circulating sC5b-9 concentrations and the percentage of active glomerular crescents, rather than on conventional clinical markers alone. Whereas proteinuria and reduced kidney function may reflect both active inflammation and irreversible chronic kidney damage, active cellular and fibrocellular crescents represent ongoing immune-mediated glomerular injury and are among the strongest histopathological predictors of adverse kidney outcomes. Relating complement activation directly to active histopathological lesions

may therefore improve the identification of biomarkers that reflect disease activity rather than cumulative kidney damage.

In addition, the establishment of a prospective multicenter biobank, together with standardized clinical, biological, genetic, and histopathological data collection, will provide a valuable resource for future translational research. The availability of these well-characterized samples may facilitate the validation of novel biomarkers, improve understanding of complement-mediated mechanisms of kidney injury, and support future therapeutic studies.

Some limitations should be acknowledged. As an observational cohort study, CAIGAN will identify associations but will not establish definitive causal relationships between complement activation and disease progression. In addition, because participants will be enrolled after histopathological confirmation of IgAN, investigators will be aware of biopsy findings during clinical data collection, introducing a potential for reporting bias. Finally, differences in kidney biopsy sampling and potential interobserver variability among participating centers may influence the detection and classification of focal lesions such as crescents. To mitigate these limitations, histopathological lesions will be classified using standardized definitions of the Oxford MEST-C classification, and a minimum of 10 evaluable glomeruli will be required for study inclusion to ensure a sufficiently reliable assessment of the percentage of active crescents.

In conclusion, CAIGAN is designed as a prospective translational phenotyping study rather than a conventional biomarker study. By integrating standardized pre-treatment biospecimen collection with circulating and tissue complement analyses, complement genetics, detailed histopathology, and prospective clinical follow-up, this study aims to bridge the gap between complement biology and clinical expression of IgAN. The findings are expected to improve our understanding of disease heterogeneity, identify clinically relevant biomarkers of complement-mediated kidney injury, and contribute to the development of precision medicine approaches for patients with IgAN.

List of abbreviations

- Anti-FH: antibodies directed against FH
- AP50: alternative pathway hemolytic activity
- Bb fragment: activation fragment of complement factor B
- C3: complement component C3
- C3a: complement component C3a
- C3NeF: C3 nephritic factor
- C4: complement component C4
- C4d: complement component C4d
- C4NeF: C4 nephritic factor
- C5a: complement component C5a

- C5NeF: C5 nephritic factor
- CFHR: complement factor H-related
- CH50: classical complement pathway hemolytic activity
- CKD: chronic kidney disease
- eGFR: estimated glomerular filtration rate
- ESKF: end-stage kidney failure
- FB: factor B
- FFPE: formalin-fixed paraffin-embedded
- FH: factor H
- FI: factor I
- Gd-IgA1: galactose-deficient IgA1
- IgAN: IgA nephropathy
- IQR: interquartile range
- MAC: membrane attack complex
- MAP: mean arterial pressure
- MASP: mannose-binding lectin-associated serine protease
- MBL: mannose-binding lectin
- MEST-C: Oxford MEST-C classification
- MLPA: multiplex ligation-dependent probe amplification
- RBC/HPF: red blood cells per high-power field
- sC5b-9: C5b-9 complexed with S protein (vitronectin)
- SD: standard deviation
- SGLT2: sodium–glucose cotransporter-2
- TMA: thrombotic microangiopathy
- UACR: urine albumin-to-creatinine ratio
- UPCR: urine protein-to-creatinine ratio
- WES: whole-exome sequencing

Declarations

Ethics approval: The study received ethics approval with conditions from the Commission cantonale d'éthique de la recherche sur l'être humain du Canton de Vaud (CER-VD; Project ID: 2023-00720) on 23 July 2026. The requested amendments have subsequently been submitted to the Ethics Committee and are currently under review. Participant recruitment will not begin until the CER-VD has confirmed that all conditions have been fulfilled.

Ethics approval and consent to participate

The study received ethics approval with conditions from the Commission cantonale d'éthique de la recherche sur l'être humain du Canton de Vaud (CER-VD; Project ID: 2023-00720) on 23 July 2026. The requested amendments have subsequently been submitted to the Ethics Committee and are currently under review. Participant recruitment will not begin until the CER-VD has confirmed that all conditions have been fulfilled. Written informed consent will be obtained from all participants prior to their inclusion in the study. For pediatric participants, written informed consent will be obtained from parents or legal guardians, with assent obtained from children when appropriate according to local regulations.

Consent for publication

Not applicable

Availability of data and materials

No datasets were generated or analysed for the current manuscript, as this article describes the study protocol.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GG conceived the study and led the development of the CAIGAN study protocol and multicenter collaboration. MP, FHG, UHD, AK, RF, DR, HC, FF, and DT contributed to the conceptual development and refinement of the study design and methodology, each bringing complementary expertise to the project. VR supervised and advised on the statistical methodology and sample size calculation. GG drafted the manuscript. All authors critically revised the manuscript for important intellectual content, read, and approved the final manuscript.

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CAIGAN Study Collaborators

The CAIGAN Study Collaborators include the investigators responsible for the scientific and local implementation of the CAIGAN study across the participating centers.

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Figures

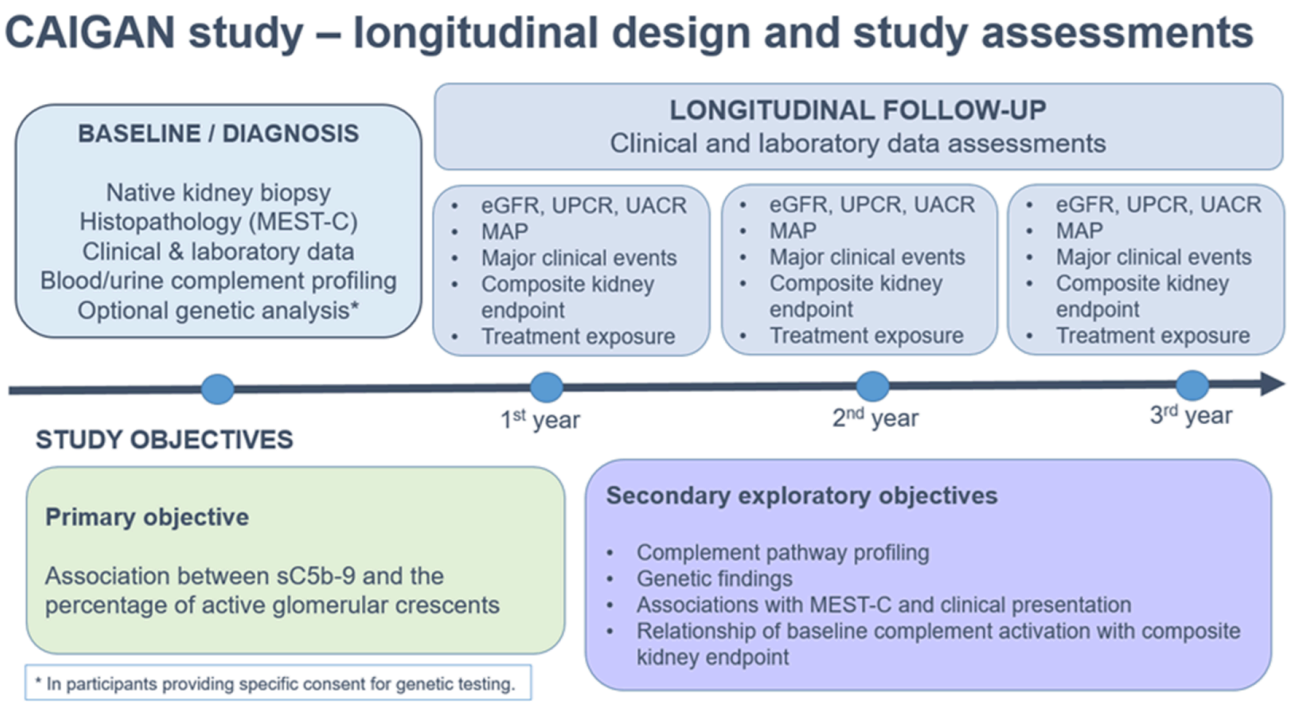


Figure 1

Overview of the CAIGAN study design and longitudinal follow-up.

Participants with biopsy-proven primary IgA nephropathy (IgAN) are enrolled at the time of diagnosis and undergo baseline clinical and laboratory assessment, complement profiling in blood and urine, and histopathological evaluation of the diagnostic native kidney biopsy. Genetic analyses are performed in consenting participants according to the study protocol. Clinical and laboratory data are collected

longitudinally during the 3-year follow-up period to assess kidney outcomes and their association with baseline complement activation, clinical characteristics, and histopathological findings. No protocol-mandated repeat kidney biopsy is planned during follow-up.

Abbreviations:eGFR, estimated glomerular filtration rate; IgAN, IgA nephropathy; MEST-C, mesangial hypercellularity, endocapillary hypercellularity, segmental sclerosis, tubular atrophy/interstitial fibrosis, and crescents.

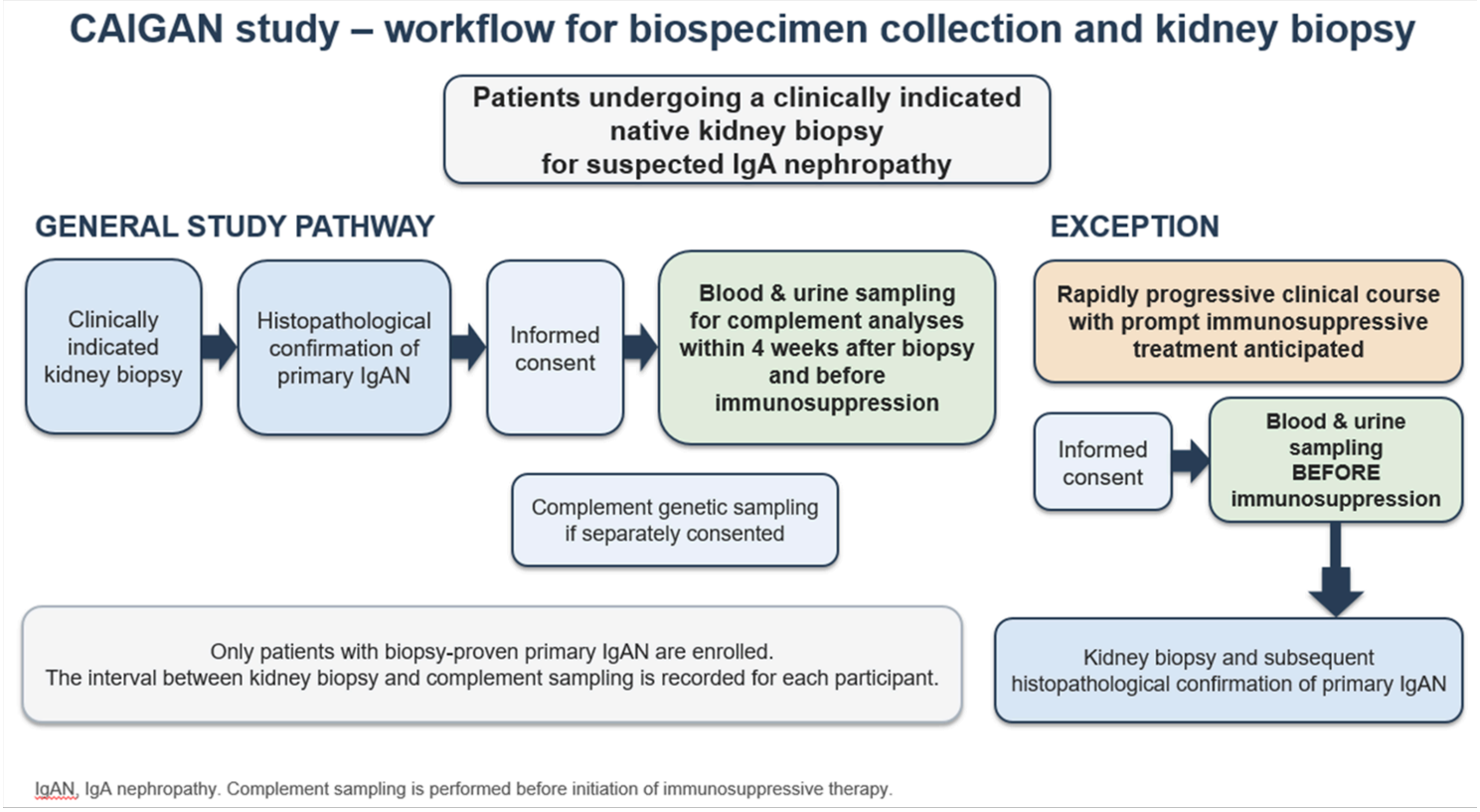


Figure 2

Study workflow for biospecimen collection and kidney biopsy in patients with suspected IgA nephropathy.

Study workflow for the collection of blood and urine samples for complement analyses in patients undergoing a clinically indicated native kidney biopsy for suspected IgA nephropathy (IgAN). As a general rule, histopathological confirmation of primary IgAN is established before study-specific biospecimen collection. Following informed consent, blood and urine samples for complement analyses are collected within a maximum of 4 weeks after kidney biopsy and before initiation of immunosuppressive therapy; samples for complement genetic analyses are collected in consenting participants. In patients presenting with a rapidly progressive clinical course for which prompt immunosuppressive treatment is anticipated, blood and urine samples for complement analyses may be collected after informed consent and before histopathological confirmation, provided that sampling

occurs before administration of immunosuppressive therapy. Only patients with subsequently confirmed primary IgAN are enrolled in the study.