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Guest editors

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Disclosure Statement

The guest editor, Vladimir Tesar, declares the following potential conflicts of interests: Receipt of honoraria/consultation fees (Calliditas, Omeros, Travere).

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WELCOME MESSAGE

Dear colleagues,

The 16th International Symposium on IgA nephropathy organized by the International IgA nephropathy Network (IIGANN) will be held virtually in Prague in 21–23 September 2021.

With the recent progress in our understanding of the pathogenesis of IgA nephropathy and so many ongoing clinical trials in patients with IgA nephropathy there is an ever increasing interest in the Symposium among nephrologists, those involved in drug development and most importantly patients with IgA nephropathy. This is reflected in the increased number of registered participants and the number of submitted abstracts. As usual, the Symposium will cover all aspects of IgA nephropathy including genetics, epidemiology, pathogenesis, biomarkers and treatment.

As with the previous Symposium in 2018 in Buenos Aires, we have decided to publish all of the accepted abstracts, along with short summaries of the invited lectures from the Plenary Sessions of the Symposium, in collaboration with Karger in the journal Kidney Diseases. I hope you agree that this publication captures the excellent work being carried out across the globe to better understand IgA nephropathy and provide new treatment options for our patients.

We hope that you will enjoy the Symposium and that this companion publication will provide you with a permanent record of the many presentations you will see over the course of the three days in September.

Jon Barratt and Vladimir Tesar

PLENARY LECTURES

IgAN: A South-Asian perspective

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There are no national glomerulonephritis registries in South-Asia and, therefore, most reports on the epidemiology of glomerular disease are based on single- or multicenter retrospective studies of kidney biopsy registries. IgAN is reported in 10% to 15% of all kidney biopsies in this region[1]. India is the second-most populous country and the Global Burden of Disease study 2015[2] ranks chronic kidney disease (CKD) as the eighth leading cause of death.

Glomerular Research and Clinical Experiments–IgA Nephropathy in Indians (GRACE-IgANI) is the first prospective South Asian IgAN cohort with protocolized follow-up and extensive biosample collection.[3] 201 incident adults with kidney biopsy–proven primary IgAN were recruited into GRACE-IgANI between March 2015 and September 2017. As of September 2020, the cohort had completed 3-year longitudinal follow-up.

The important symptomatic presentation is 'Young Hypertension' with/ without pedal edema. Visual disturbance was an important symptom complex associated with accelerated hypertension. There is no universal screening in South Asia and the health expenses are not borne by the state in most countries of the South Asian region. The vast majority of young hypertensives in South Asia are not screened for underlying CKD. Introduction of a simple screening test such as a urine dipstick for hematuria and proteinuria in this population is likely to be a cost-effective public health strategy. Common symptoms of recurrent visible hematuria associated with a synpharyngitic illness seen in other parts of the world were uncommon in this population. 34% had nephrotic range proteinuria at presentation without significant hypoalbuminaemia. This is unlike other primary nephrotic syndromes like minimal change disease and FSGS.[4]

The median time to nephrologist visits and renal biopsy from onset of any symptom or from detection of abnormal laboratory parameters was <6 months. The overwhelming impression from reviewing the GRACE-IgANI kidney biopsy data was a disproportionate absence of active glomerular lesions and over- representation of segmental sclerosing lesions and tubulointerstitial fibrosis at presentation, often coexistent with relatively well-preserved eGFR and low levels of proteinuria. It was interesting to note that the traditionally benign manifestation of visible hematuria with synpharyngitic illness was frequently accompanied by hypertension (15/20, 75%) and the presence of fibrotic lesions (S1 [13/18, 70.2%] and T1/T2 [11/18, 61.1%]). Even incidentally diagnosed clinically "mild" IgAN was associated with a significant number of fibrotic lesions (S1 [11/16, 68.8%] and T1/T2 [10/16, 62.5%]).[4]

In IgAN patients with proteinuria <1g per day, there were already signs of significant kidney damage in this group, with 21% (9/44) having E1 lesions, 57% (25/44) S1 lesions, 57% (25/44) T1/T2 lesions, and 20% GS (IQR 0–42). Even in the 24 patients with eGFR >60 ml/ min per 1.73 m^2, there was still a significant burden of established kidney damage (24% [5/21] E1 lesions; 38% [8/21] S1 lesions; 19% [25/44] T1/T2 lesions; 0% GS). This may reflect the postulated low nephron number in South Asians, which results in an increased risk of both hypertension and CKD due to glomerular hyperfiltration and hypertrophy, with intraglomerular hypertension resulting in further nephron loss and reduced sodium excretory capacity. Alternatively, there may be a specific fibrotic response to mesangial IgA deposition in South Asians.[4]

Despite optimized supportive care and use of systemic corticosteroids, at 3 years 40% of the GRACE-IgANI cohort displayed rapid CKD progression (≥5 ml/min/1.73m^2/year fall in eGFR) and 37% experienced the composite outcome defined as ≥50% fall in eGFR (CKD EPI) from baseline, eGFR (CKD EPI) <15ml/min/1.73m^2, commencement of kidney replacement therapy or death (manuscript under review and poster ID 33).

When time to kidney survival was stratified by proteinuria over 6 months of follow-up, achieving less than <1g/day significantly increased kidney survival in patients treated with short course of oral steroids along with RASB.

A Cox proportional-hazards model (-2 Log Likelihood= 239.9, Chi square=42.8 (4), p<0.001) identified MEST-T2 score at baseline (HR 4.3, 95% C.I. 2-9.2, p<0.001), Hb ≤ 12 g/dL at 6 months (HR 2.3, 95% C.I. 1.1-4.8, p=0.02), combined 24-hour urine protein ≥1 g/day at 6 months and serum albumin ≤ 4 g/dL at 1 year (HR 2.3, 95% C.I. 1.1-4.6, p=0.02) and fall in eGFR ≥5 ml/min/1.73m^2 at one year (HR 3.8, 95% C.I. 1.9-7.8, p<0.001) as significant predictors for Composite Outcome at 3 years.

Decline in eGFR (CKD-EPI) ≥5ml/min/1.73m^2 in the first year following kidney biopsy had 86% sensitivity and 89% specificity for predicting the CO over a 3-year period and that this measure could in the future be used to better inform patients, plan for renal replacement therapy and select patients for clinical trials or direct more intensive immunosuppression (manuscript under review and poster ID 33).

While this study was not designed to formally validate the risk prediction tool in an Indian population our initial assessment of the performance of the tool in GRACE-IgANI has revealed that the IIGANN risk prediction tool[5] underestimates the actual risk of the CO in an Indian population. It is worth noting that South Asian patients were not included in the development or validation of this model.

Increased mean positivity per glomeruli of C3d deposition was seen with chronicity scores MEST-C (S1, T1/T2) and associated with composite outcome. Unlike C3d, glomerular C4d and glomerular deposition of C5b-9, did not have any significant longitudinal associations in the GRACE-IgANI cohort.

Serum Gd-IgA1 as measured by KM55 ELISA[6] was not a diagnostic marker for IgAN in GRACE-IgANI cohort but serum Gd-IgA1/serum IgA ratio had significant baseline and longitudinal associations in this cohort. There was significant decrease in longitudinally measured serum Gd-IgA1 at one year with short course of oral steroids treatment. Change in serum Gd-IgA1 at one year and two years paralleled the decrease in serum IgA. A Cox proportional-hazards model (-2 Log Likelihood ratio =298.6, Chi square=44.7 (4) p <0.001) identified serum Gd-IgA1/serum IgA ratio >2ug/mg*100 (median cut-off) [HR 2.1, 95% C.I (1.1-4.2), p=0.02], MEST-T2 score [HR 3.5, 95% C.I (1.6-7.3), p=0.001], 24-hour urine protein <1 g/day at 6 months [HR 2.4, 95% C.I (1.1-5.1), p=0.02], rate of eGFR decline at 6 months ≥5 ml/min/1.73m^2 per year [HR 2.9, 95% C.I (1.5-5.5), p=0.001] as significant predictors for Composite Outcome at 3 years (Poster ID 31).

Preliminary analyses in 40 patients at baseline show that there is significant increase in inflammatory makers (hsCRP, TNF RI, CD27, BCMA). There was significant association between baseline inflammatory markers (TNF-R1, CD27 & BCMA) and CO at 3 years whereas baseline elevated hsCRP levels showed a global decrease over time. Placebo-controlled trial in this high-risk population entails ethical dilemmas hence assessing the known impact of IS on surrogate end-points can potentially guide rational therapy (Poster ID 36).

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How do we improve the International IgA Nephropathy Prediction Tool?

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IgA nephropathy (IgAN) has a heterogeneous risk of disease progression to end-stage kidney disease (ESKD) ranging between <10% and over 60%[1]. Until recently, there has been no validated tool to accurately predict the risk of disease progression in individual patients near the time of disease diagnosis at biopsy. Previous efforts to develop prediction models in IgAN have been limited by a lack of large international multi-ethnic databases to support this type of research. The International IgAN Network assembled an international collaboration of researchers to merge existing databases with the goal of developing the first accurate method for individual-patient risk stratification in IgAN[2]. This collaborative effort resulted in the International IgAN Prediction Tool, which can accurately predict the future risk of a 50% decline in eGFR or ESKD in adult patients with IgAN[2]. The Prediction Tool uses predictor variables readily available in clinical practice near the time biopsy, including age, eGFR, proteinuria, blood pressure, MEST histology scores, prior use of immunosuppression and RAS-blockade medications, and ethnicity. The Prediction Tool has undergone several external validation analyses, and is now the recommended method of risk stratification according to the updated KDIGO guidelines[3,4]. Mobile-app or web-based based calculators for the Prediction Tool can be accessed through Calculate by QxMD at www.qxcalc.app.link/igarisk.

The Prediction Tool should not be considered "static" and instead is an open-source model that can be used by any researcher to improve risk stratification in IgAN. Since its development, several questions have been raised about how the Prediction Tool can be further improved. Can the Prediction Tool be applied at later time points in the disease course other than at biopsy? Can the Prediction Tool be updated for use in other populations, such as children with IgAN? Can new predictors of disease progression be added to the Prediction Tool, such as biomarkers?

The Prediction Tool comprises two Cox proportional hazards survival models, one that includes Japanese, Chinese and White ethnicity as a predictor variable and one that does not so that it can be used in other ethnic groups[2]. Each model can be simplified as having two parts: 1) a baseline survival function which provides a baseline level of risk upon which each patient's individual predictor values can sequentially add personalized risk of disease progression; and 2) a group of beta-coefficients that determine how much risk is associated with each predictor value when added to the baseline survival[5]. Both of these parts, the baseline survival function and the beta-coefficients, are provided in detail in the supplementary tables of the original Prediction Tool publication, and can be used by researchers to update the models for use in new settings or populations, or to evaluate the added benefit of new predictor variables[2,6].

The original Prediction Tool was developed to be used in adults at the time of biopsy. However, there are other populations or settings for which we may want to also consider using the Prediction Tool. To investigate this, we need a new dataset that contains the relevant versions of the predictor and outcome variables from the Prediction Tool but that are appropriate for the new population under consideration. The first step is to evaluate the performance of the original Prediction Tool in this dataset without any modification or updating. If this prediction performance proves to be inadequate, then this suggests that the original Prediction Tool may require some form of updating to be used in the new population or setting. This can be accomplished by updating either the baseline survival function, or some or all the beta-coefficients, using the new dataset[6]. There are several methods that can be used to accomplish this depending on the amount of data available[6]. Irrespective of the methods used, the updated Prediction Tool models will be considered "new" models that themselves will require additional external validation in separate datasets.

An example of a new population to consider is asking whether the original Prediction Tool could also be used in children with IgAN. This was evaluated using a collaboration of merged cohorts from pediatric centres in Europe, China, Japan and North America[7]. Compared to adults with IgAN who have a linear rate of eGFR decline, children have a unique eGFR trajectory over time that tends to improve until adolescence then decline thereafter. As a result, the original Prediction Tool models did not perform well in children. Consequently, the outcome was modified to a 30% reduction in eGFR or ESKD, and the baseline survival and beta-coefficients were updated using the pediatric dataset. The updated pediatric Prediction Tool models were then able to accurately predict the risk of a 30% decline in eGFR or ESKD in children with IgAN. Another unpublished example is evaluating whether the Prediction Tool can be used in adults at later time points after biopsy. This was investigated using the international collaboration of adult datasets to apply the Prediction Tool one year after biopsy. This work is ongoing but suggests that the original Prediction Tool models require time-updating to be used accurately one year after biopsy.

We may also want to consider whether new predictor variables added to the Prediction Tool can improve risk stratification. This requires a dataset that contains that same predictor and outcome variables as the original Prediction Tool as well as the new predictor variable under consideration. In this situation, the beta-coefficients from the original Prediction Tool models are left unchanged, but the new predictor variable is added to the models (with its associated beta-coefficient) and a new baseline survival function is estimated[6]. The updated Prediction Tool models with the new predictor can then be compared to the original models to see if there is an improvement in prediction performance suggesting benefit from the addition of the new pre-

dictor variable[8]. Similar to above, the updated Prediction Tool models that contain the new predictor variable will be considered "new" models that require additional external validation in separate datasets. An example of this approach is a study that evaluated kidney biopsy tissue expression of microRNA-150-5p, which was added to the original Prediction Tool models and resulted in non-significant increases in C-statistics[9]. This same methodology can be applied to any predictor variable of interest, including biomarkers, pathology lesions, genetics, or clinical parameters.

The International IgAN Prediction Tool can now provide accurate patient-level risk stratification at the time of biopsy. Details of the Prediction Tool are publicly available, and there is a clear framework by which researchers can use this information to build upon and improve the Prediction Tool to further advance personalized risk-prediction in IgAN.

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RNA interference in IgA nephropathy

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For many years most RNA research focused on messenger RNAs (mRNA) as the key intermediaries in the translation of DNA gene sequences into proteins, and apart from ribosomal (rRNA) and transfer RNAs (tRNA) which play central roles in protein synthesis, other 'non-coding' RNAs (ncRNAs) were overlooked as by-products of transcription. Investigations in the late 1970s using single stranded oligonucleotide antisense therapy to manipulate gene expression at best delivered modest results but serendipitously led to the discovery that double-stranded ncRNAs had potent suppressive effects on mRNA transcription. This breakthrough launched a revolutionary new age of RNA interference (RNAi) which has transformed basic biological research as well as translational medicine.

RNAi pathways are naturally found in all living organisms, regulating and fine-tuning gene transcription and post transcriptional processes, as well contributing to genome organisation and stability. But the fact that synthetic double-stranded ncRNAs can be introduced into cultured cells as well as whole living organisms to selectively suppress transcription has made them invaluable tools in basic biology research, biotechnology and medicine.

MicroRNAs (miRs) are small ncRNA which negatively regulate gene expression, regulating physiological processes involved in proliferation, apoptosis, differentiation and development. Dysregulated miR expression has been associated with the pathophysiology of a variety of diseases, including heart disease, cancer, diabetes mellitus and kidney disease. A recognition that miRs influence the function of all segments of the nephron and that dysregulation occurs at all stages of kidney disease from disease onset to the final common pathways of CKD progression explains the intense interest in studying miRs as minimally invasive biomarkers and novel therapeutic targets.

Use of both microarray analysis and high throughput RNA sequencing have reported differential expression of a number of miRs in the kidneys, serum, urine and peripheral blood mononuclear cells (PBMC) of patients with IgAN compared to healthy subjects. We have recently demonstrated in frozen kidney biopsy cores that five miRs -204, 150, 146b, 155 and 135a are differentially expressed in IgAN patients with the progressive form of the disease compared to those with stable IgAN and correlate with clinical parameters associated with progression including eGFR, proteinuria, and MEST-C scores. Moreover, addition of each miR increases the discrimination score of the International IgAN Prediction Tool. Studies in PBMC have separately identified two miRs that are differential expressed in IgAN and target core 1 β1,3 galactosyltransferase (miR-148) and *N*-acetylgalactosyltransferase 2 (miR-let-7b), two key enzymes involved in IgA1 *O*-glycosylation. Urine is one of the least invasive of available biological samples and is therefore a highly convenient tissue to study and not surprisingly, the number of miR analysis studies performed in urine is rising exponentially. While it does appear that urinary miR concentrations increase with severity of kidney disease, with levels generally correlating with proteinuria and glomerulosclerosis, different diseases do exhibit different patterns of miR expression. Data from studies in IgAN are at present inconsistent with urinary miR composition varying between studies depending on the whether the miRs were measured in urinary sediment or exosomes, using microarrays, chips or high throughput RNA sequencing and importantly on the stage of disease being investigated. Furthermore, most of the studies of urine miR excretion have been undertaken in Chinese cohorts, and currently there are still very few studies reporting miR expression profiles in Caucasians.

With the advent of genome-wide association (GWA) studies numerous IgAN disease associated risk loci have been identified, and these have shed light on potential genes, mechanisms and signalling pathways associated with the susceptibility for development of IgAN. Some of these have also been shown to associate with potential miR target sites. For example, a target site for miR-148b has been detected in SNP rs1047763 within the gene *C1GalT1* which encodes for a key enzyme responsible for *O*-galactosylation of IgA1. In addition, a functional SNP, rs2910164, within the miR-146a precursor, which is known to affect the expression of mature miR-146a, is associated with the susceptibility to, and severity of, childhood IgAN. Interestingly, echoing the prevalence of IgAN, the frequency of this SNP differs between subjects of East Asian origin and Europeans.

As might be imagined, with the growing number of studies reporting miR dysregulation in IgAN a number of investigators have evaluated these changes as possible biomarkers in IgAN. However, to date no single/panel of miR biomarkers has demonstrated generalisability across populations, or within a population across different sites. Most critically, none have been independently validated and systematically shown to add value above and beyond biomarkers currently routinely used in IgAN.

Current treatment of IgAN is limited by an incomplete understanding of the mechanisms that drive disease initiation and progression. Therapeutic options are focused on goal-directed supportive care which includes BP control, renin-angiotensin-system inhibition, lifestyle modification and cardiovascular risk management. It is hoped that a more complete understanding of the regulation of gene transcription and post transcriptional processes by miRs may offer novel therapeutic targets. Furthermore, by virtue of their structure ncRNAs are excellent candidates as drugs themselves, capable of delivering a highly targeted approach to therapy. They can be synthesised with relative ease and designed to silence selected transcripts critical to specific

disease-causing biological pathways. RNAi therapeutics offer the ability not only to release transcripts from endogenous suppression, thereby promoting protein synthesis, but also directly disrupt translation of transcripts, thereby causing protein synthesis suppression. RNAi therapeutics, including cemdisiran**,** a short olignonucleotide with a sequence complementary to the transcript of complement component C5, and Ionis-Fb-L_{rx}, a short length antisense olignonucleotide with a sequence complementary to the transcript of complement factor B (CfB), are already being evaluated in IgAN.

It is clear that there is an increasing appreciation of the potential role that miRs and other ncRNAs play in modulating IgAN pathogenesis and that miRs have great potential to deliver both transformative biomarkers to help guide the management of patients with IgAN and offer a new approach to delivery of targeted therapy. However, despite the many publications to date describing miR dysregulation in IgAN the existing data must at present be considered hypothesis generating. Going forward there needs to be a concerted effort to consolidate our understanding with well designed, cross-population validation studies to rigorously evaluate the role of miRs in IgAN pathogenesis and as future clinically meaningful biomarkers.

The use of Artificial Intelligence in kidney biopsy evaluation

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The implementation of artificial intelligence (AI) and particularly deep learning (DL) is expected to transform medical practice. Recent technical improvements in storage, computational power (particularly the graphics processing units - GPUs), and slide scanners (allowing efficient high-throughput digitalization of histological slides) enable digitalization of pathology and application of computation approaches, termed digital pathology. This in turn enables the implementation of AI, and particularly DL, holding an immense potential to improve, advance, and transform diagnostic pathology. AI is readily and broadly implemented in various aspects of our everyday life. In medicine, including pathology, both development and translation are only starting to emerge [1, 2].

AI research can be described as automatization of tasks that would otherwise be performed by humans or augmenting humans performing such tasks (the so-called augmented intelligence). Machine learning (ML) is a branch of AI where Data and conclusions/answers are given to a computer that finds statistical structure within the data to generate the respective conclusion/answer. DL is a type of ML, in which rules are derived from the data by deriving meaningful representations within multiple successive processing layers (so-called neural networks). The depth of a DL model is given by its number of layers. DL, particularly the convolutional neural networks (CNN), are particularly efficient in visual tasks and were shown their medical applicability in several specialties, including ophthalmology, radiology, and pathology (mostly in the cancer field of onco-pathology) [1, 2]. Modern hospitals using digital records produce enormous amounts of data every day, making the integration of AI into regular practice not only possible but inevitable. As the technology is changing, the role of physicians and physician-scientists needs to be modulated to acquire the expertise to ensure clinical relevance, applicability, as well as the ethical use of AI in the future.

In digital pathology, AI can support in various ways. It allows for reproducible quantitative analysis of features that pathologists a) can visualize and count (support system), b) can visualize but cannot quantify (augmentation), or even c) currently cannot detect e.g. based on subvisual features (novel data mining).

One example of how DL can advance precision medicine was shown in the field of colorectal cancer. A CNN algorithm, just using a single H&E stained histology slide, was able to predict microsatellite instability, a therapeutically relevant molecular alteration in colorectal cancer [3]. This has potentially important clinical implications since microsatellite instability analyses require molecular pathology methods which are much more costly and time-consuming compared to such a digital DL algorithm. The performance of DL strongly depends on the amount of data. This was nicely documented in a follow-up study on microsatellite instability detection in colorectal cancer, in which a larger training dataset resulted in further improvement of diagnostic accuracy of the CNN algorithm [4]. Compared to cancer, there are several important challenges in renal pathology: it is morphologically more demanding than the majority of cancers with at the same time virtually no (or rather small) available histology repositories and registries. Another study showed, that the approach of deriving therapeutically relevant molecular alterations from cancer histology is potentially possible in the majority of main cancer types and most druggable genetic alterations [5].

Examples of how DL can augment histopathology diagnostic in kidney biopsies were recently shown for quantification of kidney fibrosis. A CNN was able to mimic and reproduce quantitative assessment of kidney fibrosis on whole slide images of renal biopsies [6]. In another example, a DL pipeline was shown to automatically, precisely, and in high-throughput segment main renal histological compartments in kidney tissues from various species. This included glomeruli (tuft and capsule separately), arteries (lumen and media separately), veins, tubules, and interstitium. This approach not only strongly facilitates compartment-specific analyses but also allows a previously unprecedented quantitative approach, e.g. analyzing (hundreds of) thousands of kidney structures like tubules or glomeruli [7].

To understand the potential of AI in histopathological analyses of IgA nephropathy (IgAN), we propose a joined approach and project, the „AI.IgAN“. The AI.IgAN will apply our established pipeline and experience in DL in renal biopsies to develop, test, and validate DL models for the prediction of relevant clinical outcome parameters from kidney biopsy histology in IgA Nephropathy. We hypothesize that DL models of IgAN biopsies might be helpful to further improve precision medicine and nephropathology diagnostics. This project not only aims to understand the potentials but also pitfalls of implementation of DL in biopsy diagnostic and to analyze the potential of DL in deriving novel, reproducible and improved-personalized patient care in IgAN. Several centers are already involved and the participation is opened for all interested centers.

In conclusion, AI-augmented digital (nephro)pathology will expectedly transform this diagnostic field enabling more reproducible, quantitative, computational, and thereby “precision” histology analyses and data mining.

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Is there a link between collagen IV mutations and IgAN?

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IgA nephropathy (IgAN) is a primary glomerulonephritis clinically characterized

by microscopic hematuria and proteinuria. The diagnosis is based on kidney biopsy with IgA deposits with a wide spectrum of glomerular inflammation from mesangial proliferation to crescents. Also, diverse findings have been reported on light and electron microscopy. [1]

Although familial related cases have been described and predisposing genetic factors have been suggested, [2] IgAN usually develops in a sporadic form. Three loci could have genetic susceptibility for familial IgAN with IgAN 1 locus on chromosome 6q22-23, IgAN 2 locus on chromosome 4q26-31 and IgAN 3 locus on chromosome 17q12-22. [3, 4] Curiously, another locus has been observed in a Canadian cohort 2q36 [5] coding for COLA4 and COL4A3 described in thin membrane or Alport syndrome. Alport syndrome is a hereditary genetic disorder nephritis due to mutations in the genes encoding alpha-3, alpha-4, and alpha-5 of type 4 collagen (COL4A3, COL4A4, COL4A5).

Since IgAN and Alport syndrome could have the same clinical presentation (hematuria, proteinuria, end stage renal disease), an association has been suggested between these diseases. [6] In IgAN, thin basement membrane [7 8] and glomerular basement membrane injuries have been observed (double contours, gaps, thinning with a lamellar and reticular structure, rupture). [9]

Twenty six years ago, Bertoux at al reported 23 IgAN patients with thin glomerular membrane in a cohort of 58 IgAN patients indicating either a specific subgroup (with an unfavorable prognosis) or an association with thin membrane nephropathy.[10] Recently, in familial IgAN, type 4 collagen mutation has been reported underlying the possibility of Alport family disease with IgA deposits. [11] Pathogenic or Likely Pathogenic variants COL4A3-5 segregating

in 9 families were identified in 46 familial IgAN. COL4A3-5 variants autosomal dominant inheritance have been found in 6 (67%) out of 9 families, with 2 harboring heterozygous variants in COL4A3 and 4 in COL4A4. X-linked inheritance have been observed in the remaining 3, with variants in COL4A5. Substitution or missense mutations in highly conserved glycine residues in the triple helical collagenous domain were noted in 7 of the 9 families (78%); the other 2 families (22%) had frameshift variants. Unfortunately, detailed histopathology was not available in these families. In pediatrics, only case reports described collagen variants in supposed IgAN family. Hemizygous mutation of COL4A5 has been described in 9 years-old Asiatic cIgAN and his parents too. [12] Also a single heterozygous mutation affecting a splice acceptor site in the COL4A3 gene has been described also in 9 years old Caucasian cIgAN. [13]

Recently, we have reported retrospectively collagen variant in 36 children IgAN, by sequencing COL4A3, COL4A4, COL4A5 coding regions. We identified pathogenic or likely pathogenic inherited COL4A3 variants in 4 patients (COL4-cIgAN) out of 36 (11.1%). Three patients presented heterozygous carrier of variants in COL4A3 gene and one patient had two variants in trans corresponding to heterozygous compound variants affecting COL4A3. Every COL4-cIgAN had missense variant affecting COL4A3 gene. COl4A3 protein was affected a on glycine residue (p.G1277S) located in the collagenous domain of a3 of collagen IV or located in the NC1 domain of a3 collagen (IV) (p.F1504L and p.L1474P). In this cohort, most cases presented an autosomal inheritance with heterozygous COL4A3 variants, as described in familial focal segmental glomerulosclerosis.[14]

At the onset of the disease, all patient presented macroscopic hematuria and 3 patients (75 %) presented nephrotic syndrome including 2 patients with a kidney renal failure. Nephrotic cIgAN presented the full spectrum of proliferative lesions according Oxford classification (M1, E1 and C1 lesion). Moreover, these 3 patients presented also high levels of Gd–IgA1 level, and plasma immune complexes. These immune complexes are specific to IgAN [15-17] These 3 patients got an intense immunosuppressive treatment with an improvement of histological lesions during the follow up.

One patient with p.G1277S potentially had a presentation compatible with Alport syndrome with macroscopic hematuria and small proteinuria. Absence of immune complexes with a low Gd-IgA1 associated with a low grade of proliferation were observed for this patient. At the last follow-up, this, patient had only microscopic hematuria, with a normal eGFR.

For the 3 other patients, the clinical and histological evolution together with the immunosuppression response and detection of a high level of specific immune complexes (Gd-IgA1, IgG-IgA, sCD89-IgA, and free sCD89) may support a superimposed Alport syndrome in patients with IgAN. One patient got a kidney transplantation with an IgAN recurrence. The detection of COL4A3 variants usually correlated with Alport syndrome in adults should not be a reason for avoiding an immunosuppressive regimen in cIgAN.

The observation of COL4A3 heterozygous variants seem to become predisposed to serious IgAN presentation. The clinical presentation of COL4A3-cIgAN patients was more severe at onset compared with the non–COL4A3-cIgAN patients, with a more

profound nephrotic syndrome. COL4A3-cIgAN patients performed repeat kidney biopsies with many relapses, with the development of a high proportion of glomerulosclerosis at the last follow-up associated with a lower eGFR compared with non–COL4A3- cIgAN patients. It would be of interest to screen patients diagnosed with severe IgAN at the onset for collagenous variants and risk stratification could be based on the presence of a rare genetic variant of the COL4A3 gene.

Histopathologic features provide important and reliable information for IgAN diagnosis, staging, and prognosis used extensively by clinicians in clinical practice. Nevertheless, the integration of collagenous features could lead to more accurate prediction of patient outcome. Despite being classified as potentially pathogenic, the role of these collagenous variants affecting COL4A3 is still unknown but confirms the heterogeneous nature of IgAN. Sequencing the COL4A3 variant at the cIgAN onset could stratify the risk for severe flare-up, especially with nephrotic syndrome presentation. It could help us to better understand the wide spectrum of IgAN presentation and prognosis. Similarly to newly discovered immune biomarkers based on IgA complexes, such as sCD89-IgA, free sCD89, Gd-IgA1, and IgG-IgA, and based on our findings and to be confirmed in a larger study, predictive and diagnostic factors could be uncovered using DNA sequencing during initial management of IgAN as an indicative prognostic biomarker of IgAN suitable for risk stratification.

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The importance of reporting patient reported outcomes in trials in IgA nephropathy

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When making treatment decisions, patients, care partners and health professionals need relevant and precise information about a particular set of outcomes. In trials in patients with IgA nephropathy, surrogate outcomes are overwhelmingly reported and patient-reported outcomes have not been prioritized for inclusion. Treatment-related adverse events have not fared much better, though with recent exceptions.(1-4) Yet it is often those patient-reported aspects and treatment harms that are more relevant for patients and care partners when it comes to shared treatment decision-making for patients with IgA nephropathy.

IgA nephropathy and its treatment(s) have outcomes whose impacts are best assessed by direct patient report and several of these are critically important to treatment decisions. Patients may experience swelling, fatigue and symptoms from chronic kidney disease but also distressing impacts of immuosuppressive therapies (e.g. corticosteroids).(5) In the short term, treatment harms can have greater impact on the patient than their glomerular disease, and these may include nausea, mood changes, altered physical appearance, and reduced mobility, strength or self-esteem.(6) Other more distal impacts of IgAN and its treatment include anxiety, depression and impaired quality of life.(7) When weighing treatment decisions, the net benefit and harm for each therapy must be compared as both disease and treatment may change symptom burden, day-to-day functioning and quality of life.

Patients with glomerular disease and their care partners have identified patient-reported outcomes as critically important in treatment decisions.(8) Using focus groups with nominal group technique, 134 patients and care partners from Australia, United States, United Kingdom and Hong Kong identified and ranked candidate trial outcomes.(8) Overall, kidney function, need for dialysis or transplant and mortality were highly prioritized. Life participation, defined as being able to participate in meaningful life activities, was the most important patient-reported outcome. Patients and care partners also highly prioritized mobility, cognition, fatigue, physical functioning/strength and depression as trial outcomes.(8, 9) However life participation was prioritized above these other patient-reported outcomes because it was the underlying major impact and the most important one for decision-making. Notably, these outcomes were more important to patients than other commonly measured outcomes including blood pressure, proteinuria and hospitalization.

Health professionals recognize the necessity of capturing patient reported outcomes, though there are some differences in their priorities for outcomes when compared to patients (e.g. death, hospitalization). An international, two-round Delphi survey that included 412 patients with glomerular disease (253 with IgA nephropathy) and 288 health professionals showed kidney function (including need for dialysis or transplant), cardiovascular disease, relapse/remission, death and life participation were shared top priorities as trial outcomes.(9) Therefore, life participation appears to be a fundamentally important construct for patients with glomerular disease including IgAN, which comprises those negative impacts from disease and treatment(s) but also protective or positive aspects. It is frequently and significantly impaired, but can give patients' purpose and self-worth, preserve a sense of self and strengthen mental health.(8, 9) The impacts on this domain are also under-appreciated by health professionals and difficult to address, which increases the importance of knowing how it might change post treatment.

Implementing routine reporting of patient-reported outcomes such as life participation in IgA trials is one of the aims of the Standardized Outcomes in Nephrology – Glomerular Disease (SONG-GD) initiative.(10) SONG-GD is establishing a core outcome set for trials in glomerular disease, which is a standardized set of outcomes to be routinely reported in all trials in this field.(11) They can help ensure that critically important outcomes common to all stakeholders are consistently reported using the same measures. Challenges to the implementation of core outcomes include their feasibility and usability, and this may entail the development or validation of a new measure - as would be the case for life participation. Strategies to achieve their uptake include partnering with key stakeholders such as funders, regulators and engaging all end users of the data in order to increase their willingness to be included in trials.(12) Once implemented, a core outcome set for glomerular disease would ensure the evidence base for treatment decisions is more relevant and comparable across therapies for both patients and health professionals.

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What progress has been made in understanding IgA Nephropathy in children in the last decade?

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Almost a decade ago, on May 26–28, 2009, an international symposium on IgA nephropathy (IgAN) was convened in Stresa, Italy, as a Satellite Symposium of the World Congress of Nephrology held in Milan (1). The organizers reported the advancement in defining pathogenesis - defective galactosylation of IgA molecules (Gd- IgA1) with production and renal deposition of circulating immune complexes (2) - and systematizing the approach to morphology of the disease with the Oxford Classification for IgAN (3). Ten years ago, little attention was paid to IgAN in children, since the general opinion was that Pediatricians mostly face a benign disease, with bouts of gross hematuria rapidly vanishing or persistent mild microscopic hematuria, which rarely progresses to chronic kidney failure. In a well-studied Swedish cohort of 72 children with IgAN followed for a median of 8 years, half of the children did not show any clinical signs of the disease, indicating low disease activity or, possibly, recovery (4). Some decline in GFR was detected in 3% only, persistent proteinuria in 35% and hypertension in 9%, favoring the impression of a rather benign disease. However, long-term follow-up studies had reported a Kaplan-Meier actuarial survival from ESKF at 20 years of 82% in children with IgAN in Japan and 70% in USA (5,6). Hence, there was the clear perception that further studies were needed to identify the subgroup of children at risk of late progression.

The most relevant advancement in understanding IgAN in children in the last decade is that the simple application of the general concepts of pathogenesis and risk factors detected in adults do not perfectly fit in children. Therapeutic approaches should be personalized to the pediatric risk.

Genetic and pathogenetic factors

A genetic signature is stronger than in adults is detected in children with IgAN. Gd-IgA1 levels were found highly inherited (7). Genome-wide association studies (GWAS) showed a particularly strong association in children with IgAN with 14 of 15 risk alleles related to intestinal mucosal immunity (GALT) (8). Moreover, there are new data on association between IgAN and COL4A5 and COL4A3 variants (9,10), usually associated with autosomal recessive Alport syndrome. About 40% families with thin membrane disease (TBMN) segregates with the COL4A3/COL4A4 locus and several studies reported an association between TBMD and IgAN particularly in children (11,12). These genes may favor early manifestation of hematuria in children with IgAN.

Mucosal immunity environmental challenges are common in children with tonsillitis and gastroenteritis. There is an age-related capacity of control of inflammation and tissue repair which is reduced in the transition from childhood to adult life. This event is regulated by the CD4+ regulatory T lymphocytes (Treg). Treg cells are originated and orchestrated by complex mechanisms which have the first origin in the tymus, a lymphoid organ which undergoes involution during transition from childhood to adulthood (13). The thymus as well as tonsils and adenoids attain approximately 200% growth by late childhood and then involute (14). Peyer's patches, which are the GALT mayor site of IgA-producing cells, show similar age-related changes (15).

Clinical presentation and progression

The variability of progression of IgAN in children is mostly depending on the indications to perform diagnostic renal biopsy, after chance detection of microscopic hematuria or shortly after acute nephritic syndrome episodes or after a relentless slow increase in proteinuria. Asymptomatic IgAN, detected by school programs in Japan and Korea (13,14), do not simply represent an early phase of the disease, since it does not implicate a more rapid progression, and spontaneous remissions occur, though with mild relapses (15). In Europe as in many continents the diagnosis of IgAN in children follows the detection of persistent microscopic hematuria with proteinuria (16). An onset with acute nephritic syndrome is common, with inflammatory glomerular lesions and, over the short-term, possible improvement either spontaneous or favored by corticosteroid/immunosuppressive treatment. Remission is more common in children due to the age-related capacity of tissue repair and control of inflammation (17).

Clinical risk factors

The value of proteinuria, hypertension and reduced GFR at renal biopsy as prognostic markers of progression, assessed in adults more than 20 years ago were rarely confirmed in children (18). The European Validation Study of the Oxford classification (VALIGA) cohort enrolled 174 children and 973 adults with IgAN (19,20) with median proteinuria of 0.8 (0.3-2.2) g/1.73m^2/day in children and 1.4 (0.7-2.6) g/day in adults. Over a similar follow up of a median of 4.6 years, the rate of eGFR loss was significantly faster in adults versus children, being -1.8 (-3.4+0.5) ml/min/1.73m^2/year and 0 (-1.7+0.76) ml/min/1.73m^2/year respectively). The combined end point of 50% decrease in eGFR or ESKD was reached in 16% adults and 6% children, and ESKD alone in 12% and 4.6% respectively. The median value of follow-up proteinuria in children was 0.56 (0.2 -1.0) g/day/1.73m^2, representing a persistent risk of progression (19). Notably, eGFR and proteinuria at renal biopsy were

not predictive of outcome in children while they were significant predictors in adults. Multivariate models based on clinical data were significant in children only when including follow-up data.

In VALIGA patients the hazard of the combined end point increased with age at renal biopsy until a plateau, which was reached at 23 years (21). An improvement in eGFR mostly after corticosteroid-immunosuppressive treatment rendered not significant the prediction value of the clinical data at renal biopsy also in the French cohorts, 82 children with IgAN in whom biopsy was performed shortly after the acute clinical onset (22).

Pathology risk factors

More than 10 years ago Haas et al reported for the first time a comparison of histological features (I-V classes) and outcome in adults and children with IgAN (24). The 10-year survival to ESKD was significantly different (children 80%, adults 35%), however, at multivariate analysis only interstitial fibrosis was a significant independent predictor.

The Oxford Classification of IgAN (3) detected the prognostic value of mesangial (M) or endocapillary (E) hypercellularity, segmental glomerulosclerosis (S) and tubular atrophy-interstitial fibrosis (T) score that was similar in children and in adults (25). National or single center studies in pediatric cohorts from Sweden (26), Japan (27), China (28), and Brazil (29) confirmed the value of some MEST score by univariate analysis. However, in multivariate models no individual feature maintained an independent value, apart from T lesions in Chinese children (28). In the European Validation study the prognostic predictive value of MST lesions was confirmed only in 261 young subjects <23 years of age, not in the cohort aged less than 18 years (22).

Prediction tool in children using clinical and pathology data

The above observation clearly indicated the need of risk prediction assessed on the combination of clinical and pathology features. To this aim, the International IgAN Network updated the model set in adults (29,30). The analytic cohort included 1,060 children with IgAN. The median eGFR at biopsy was 98 ml/min/1.73m^2 (79-118) with proteinuria 1.2g/day/1.73m^2 (0.5–3.0). The median follow-up was 3.9 years, and 48% were followed into adulthood. The primary outcome (50% decline in eGFR or ESKD) occurred in 52 children whereas the secondary outcome (30% decline in eGFR or ESKD) occurred in 117 children. Un updated model using the secondary outcome the predictors selected were the M and S scores, MAP for the model without race/ethnicity and eGFR. For the model with race/ethnicity, the same predictors were selected in addition to the T-score and Japanese race/ethnicity. Crescents were not associated with 30% reduction in eGFR or ESRF even after adjusting for CS/IS use.

Calibration showed good agreement between predicted and observed risk, with satisfying improved ability to differentiate various risk groups.

Disease trajectory over time

The eGFR trajectories in children were non-linear with an initial increase in eGFR followed by a subsequent decline. A higher predicted risk of the 30% decline in eGFR or ESRF was associated with a smaller initial increase in eGFR followed by a more rapid decline. Children with IgAN tend to have an initial increase in eGFR up to 15-18 years of age and then start a linear decline like that seen in adults. The eGFR trajectory in children may relate to more active lesions and great capacity for repair and control inflammation (19).

Among the progresses made in understanding IgAN in children, the detection of specific age-related risk factors for progression represents a major step to allow the identification of different risk levels and select cases with similar risk factors to receive treatment or placebo .

Conclusion

New advancements in IgAN in children have provided insight into the value of approach the disease individualizing each case on the basis of onset as history of onset, reason for performing renal biopsy, family history, time elapsed before performing renal biopsy and MEST-C score. When available, genetic data would be useful. All these data are helpful in classify children with different progression risk, mostly based on the Pediatric predictor tool. Modifications in proteinuria and eGFR over a short time (6 months -one years, times which are usually considered sufficient to establish a potential benefit of a treatment) may we incorrect in some cases, that will display stable clinical features only 2-5-years later. The best prognosis is for those with initial greater increase in eGFR , but the interest is focused on long-term prognosis , when the trajectory decreases like in adults. If we wish to compare the effect of treatments in children, we have to consider to plan the evaluation on eGFR decline after some years, when the potential of fully respond to therapy of recovery - largely dependent on the age of the children affected by IgAN - will go to physyiological exhaustion.

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Is IgAN a disease of mistrafficking B cells?

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The clinical observation of episodes of macrohaematuria concomitant with infections of the upper respiratory tract (tonsillitis) or intestinal mucous membranes, as well as the fact that IgA is the most represented immunoglobulin in mucosal secretions, has increased the importance of mucosal immunity in IgAN in recent years. The link between IgAN and mucous membranes is also supported by associations with celiac disease and herpetiform dermatitis, possibly due to increased intestinal permeability and high levels of IgA against gliadin [1].

The gut microbiota influences both locally and systemically the recruitment, development, and activity of innate and adaptive immune cells in mucosa associated lymphoid tissue (MALT) [2]. The gut immune system is unique and defends against infections by producing IgA, likely contributing to dietary protein and commensal microbiota tolerance [1]. In the gastrointestinal system, IgA is the most common antibody isotype. The isotype switch to IgA, occurring in gut-associated lymphoid tissues (GALTs) and mesenteric lymphnodes, contributes to the prevalence of IgA production by intestinal plasma cells [1,3].

T-cell-dependent (TD) or -independent (TI) mechanisms can both stimulate IgA synthesis in MALT [4]. TI-IgA class switching of B cells can occur in the lamina propria and is induced by many cytokines, but mostly by tumour necrosis factor (TNF) ligand superfamily member 13 (APRIL) and by the proliferation-inducing ligand and B-cell activating factor (also known as BAFF).

Because secretory IgA activity occurs in the intestinal mucosa, the function of microbiota and intestinal immunity in IgAN is particularly intriguing. Increased blood levels of galactose-deficient IgA (Gd-IgA) and mesangial deposits of IgA were detected in a transgenic mouse model overexpressing BAFF, but the presence of intestinal microbiota was required for the development of this phenotype [5]. Moreover, between patients with IgAN (progressor versus non-progressor) and healthy subjects (HSs), a distinct profile was detected in both the make-up of the faecal microbiota and the metabolomic profile [6]. Furthermore, to demonstrate the role of microbiota in disease progression, we were recently able to show that fecal microbiota transplantation may modulate renal phenotype in the humanized mouse model of IgAN (Lauriero G. et al., data under review).

As well as, recently, the α1KICD9Tg mice were also challenged by rifaximin (NORMIX®) treatment, a non-absorbable oral antibiotic, that induces positive modulation of the gut microbiota, favoring the growth of bacteria beneficial to the host. Rifaximin treatment decreased the hIgA1 mesangial deposition, CD11b+ cell infiltration and urinary protein-to-creatinine ratio, serum levels of hIgA1–sCD89 and mIgG–hIgA1 complexes. Moreover, under gene expression profiling, our findings support reduced gut inflammation following rifaximin treatment, showed by a downregulation of TNF-α and BAFF gene transcription [7].

Based on these studies, it can be hypothesized a possible link between commensal bacteria, higher BAFF levels, disrupted homoeostasis of intestinal-activated B cells and intestinal IgA class switch in IgAN patients [8,9]. The role of the complex intestinal immune network and intestinal mucosal hyper-responsiveness in the pathogenesis of IgAN has been recently supported, for the first time, by our findings showing a significant difference in the number of intestinally activated B lymphocytes in IgAN patients compared to HCs [8]. The intestinal–renal axis is important in Berger's glomerulonephritis, where several factors (e.g. genetics [10,11], infections [12], and food antigens [13,14]) may play a role in the disease complex pathogenesis and provide novel therapeutic targets to slow down disease progression.

Interestingly, IL-6 pathway seems to be involved in this complex intestinal immune network particularly in mediating the production of aberrantly glycosylated IgA (15, 16). Recent studies have demonstrated that the expression of the CD37 protein on the B cells of IgAN patients is reduced compared to healthy donors. In CD37 knock out mice, IL6 levels were elevated along with increased deposited complexes and low kidney function. In contrast, mice with double knock out for IL6 and CD37 showed no glomerular IgA deposits and were protected from exacerbated renal failure after treatment with lipopolysaccharides [15]. These data suggest that IL-6 mediates renal pathology in CD37 knockout mice, and that CD37 may protect against IgA nephropathy by inhibiting the IL-6 pathway itself. However, the reason and mechanism why elevated levels of IL-6 are present in IgAN patients are still unknown.

One possible explanation comes out from our whole genome screening for DNA methylation in IgAN patients, that identified, among others, three regions with altered DNA methylation capable of influencing the expression of genes involved in the response and proliferation of T and B cells. In particular, a hypermethylated region was identified comprising Vault RNA 2-1 (VTRNA2-1), a non-coding RNA also known as precursor of miR-886 (Pre-mi-RNA). Consistently, the VTRNA2-1 expression was found down-regulated in IgAN patients [17].

We are currently investigating whether and how, in IgAN patients, the down-regulation of VTRNA2-1 induces the PKR / CREB pathway that stimulates the secretion of IL-6, the cytokine with a key role in mediating the production of deglycosylated IgA. VTRNA2-1 is known as a specific inhibitor of PKR (Protein Kinase RNA-enabled), an interferon-inducible double-stranded RNA-dependent kinase. Consequently, it can inhibit also the cAMP-response element binding protein (CREB). Suppression of

VTRNA2-1 activates PKR/CREB pathways, such as eIF2a phosphorylation and the NF-kB pathway, leading to impaired cell proliferation and IL-6 production[18].

Normally, in healthy subjects (HS), the VTRNA2-1, by inhibiting the PKR/CREB pathway, also inhibits the expression and production of IL-6. Therefore, in IgAN patients, the reduced expression of VTRNA2-1 explains the regulatory mechanism that induces high levels of IL-6 and mediates the aberrant production of degalactosylated IgA. This mechanism is based on the epigenetic down-regulation of VTRNA-2-1 RNA and on the activation of the PKR / CREB pathway that in IgAN may be induced by bacterial or viral RNA deriving from commensal bacteria or viral infections.

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How has COVID-19 changed our views on delivering clinical trials?

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"Never let a good crisis go to waste" is a statement attributed to Winston Churchill, and one that the clinical trial community needs to strongly embrace in order to leverage some of the silver linings of the COVID 19 pandemic.

The pandemic clearly disrupted clinical care, forcing nephrologists to be creative in caring for one of our most vulnerable populations, dialysis patients, who could not simply just stay home during quarantines. Cohorting dialysis shifts and re-visiting old treatments such as initiating acute peritoneal dialysis became essential tools. The conduct of clinical trials needed to pause for the most part to focus on clinical care. But this medical and public health crisis sparked an urgency recognizing that clinical trials were the path towards treatment and vaccines to lift us out of the pandemic.

One particular recent innovation is the master protocol (MP) which had previously been used mostly in oncology. However, quickly implementing one type of MP, the platform design, for COVID-19 treatments truly maximized the speed and efficiency towards understanding which current therapies could be used safely and effectively. The RECOVERY Trial (Randomized Evaluation of COVID-19 Therapy) enrolled its first patient in March 2020, and by June had randomized over 11,800 patients. Within 3 months of that first enrolled patient, data had quickly accrued to demonstrate the effects of hydroxychloroquine, dexamethasone and lopinavir-ritonavir. Trial implementation generated fast results that shifted clinical care, underscoring 2 important principles: trial participation can be an option for care, and innovative trial design was essential in obtaining data at an unprecedented yet critical speed during this unsettling pandemic. COVID-19 trials, including those investigating novel vaccines, required many in the medical community to embrace a research ready culture. Medical staff at one particular center had morning huddles, evaluating which, not if, COVID-19 trials a newly admitted patient would enter. Hopefully such concepts (master protocols and an on-study culture) will be picked up with enthusiasm in nephrology.

As the COVID-19 investigations continued, most nephrology trials had to pause trial activity to focus on keeping patients safe and so that staff could be allocated to more pressing needs related to the pandemic. As principal investigators (PI) started to slowly resume nephrology clinical trials, another innovation was embraced: applying telehealth to both clinical care and trial conduct. Telehealth (also known as telemedicine) has been used in clinical medicine for several years in areas such as burn units, stroke/TIA and mental health management. But as patients were quarantined at home, nephrology clinical care pivoted to telehealth, and the concept of bringing care directly to the patient at home inspired creative solutions for also bringing more trial activity closer to home for patients.

Decentralized trials intend to accomplish what is sounds like…the investigator site no longer is the central hub for trial procedures, which get shifter closer to the patient, whether that is at Patient Service Centers in retail pharmacies or services right at the patient's home (e.g. home health nurses and phlebotomists, home delivery of study drug and trial tools such as blood pressure cuffs or scales, or courier pick up of lab samples). Schedule of assessments were re-tooled so that not every procedure had to be performed at the PI site, and remote and video technology also enabled this shift. Although many stakeholders had been preaching for more patient-centric protocols for years, the pandemic fortunately left no choice but to inspire procedures that would be less burdensome to patients, such as video training/observation of SC injections instead of wasting time and money traveling to the site. This necessary evolution towards trials with more built-in virtual and hybrid elements also opened the door to increased patient choice; with more options patients are now empowered to select how they prefer to engage in trial activities.

The crisis of this pandemic also shined an unfortunate but real spotlight on health care disparities, which must inspire our trial community to also examine how to engage and enroll more diverse communities of color. The FDA endorses this call to action in their related FDA guidance document finalized in Nov. 2020. Many of the elements of virtual trials may also support how we better reach communities of color with challenges to access of care. We must promote the goal of studying more diverse populations so that we generate direct data (rather than extrapolate) on the very people we need to treat with novel therapies.

This session will delve into the above topics, and also provide feedback from patients, PIs and industry who were informally surveyed for their related perspectives. We must leverage the valuable insight gained from a difficult and disruptive year as we press forward in improving the design and conduct of future clinical trials. The unmasking of disparities in both clinical care and trials, together with the aforementioned innovations in trial design and conduct, proves that this crisis has certainly not been a waste.

New insights into mucosal IgA-producing plasma cells – Implications for IgA nephropathy

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IgA antibodies are produced at mucosal surfaces, notably the gut and the respiratory tract, by plasma cells. Plasma cells are derived from B cells that are activated in the germinal center or in extrafollicular reactions. After this activation step, some B cells can differentiate into short lived plasmablasts and long-lived plasma cells. In humans, IgA plasma cells can live for decades in the gut lamina propria[1] where their specificity and number are dictated by the commensal microbiota that reside in the adjacent lumen.

Continual transport of IgA occurs across the single epithelial layer that separates the host from the outside world. Specifically, IgA dimers bind to the polymeric IgA receptor expressed on the epithelium, followed by trans-epithelial transport and release of the dimerized IgA in association with the secretory component. This secretory component associated IgA is critical for shielding the host from potentially invasive microbes[2].

Our classical understanding of IgA-producing plasma cells is that they remain in their mucosal tissues where they produce their antibodies in order to maintain homeostasis between the host and its microbiota. The microbiota at these sites is also very important for dictating the numbers and specificity of IgA-producing plasma cells[3,4]. However, multiple studies have now shown that IgA-producing plasma cells can leave the gut and travel to other mucosal and non-mucosal tissues[5-10]. In addition, although the main role for IgA-producing plasma cells is to produce large amounts of IgA to shield the host against microbes, there is an increasing appreciation of plasma cells, including IgA-producing plasma cells, as producers of cytokines that can potentially regulate the immune response[11,12]. For example, IgA-producing plasma cells can produce IL-10 that suppresses the anti-cancer T cell response in the liver and prostate[13,14], and inflammation in the brain[5]. These new insights on IgA-producing plasma cells have tremendous implications for a number of disease states, including IgA nephropathy.

In my presentation, I will provide some insights into how IgA producing cells are generated in the gut using rotavirus as a model of intestinal infection[15]. I will also provide an example of IgA-producing plasma cell migration from the gut to the inflamed brain, and provide present evidence they can produce IL10 at this location[5]. In addition, I will present new data showing evidence of a mucosal IgA response in the oral cavity of subjects immunized with mRNA vaccines to SARS-CoV-2 via the parenteral route[16]. Lastly, I will provide a perspective on how the evolutionary pressure to produce IgA by mucosal plasma cells[17] may be a maladaptive feature in the context of IgA nephropathy.

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Modulating non-immune pathways-how might this help?

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Immunoglobulin A (IgA) nephropathy is the most common primary glomerular disease worldwide. The established mainstay of therapy for the management of patients with IgA nepropahty is inhibition of the renin-angiotensin-aldosteron-system in patients with proteinuria > 1 g/dah. However, there has been little progress in the treatment strategies over the last two or three decades. Approximately 30% of patients with IgA nephropathy progress to kidney failure, and risk factors for deterioration of kidney function include decreased estimated glomerular filtration rate (eGFR), persistent proteinuria and hypertension.

Various new therapies are currently in development for the treatment of IgA nephropathy. Two drug classes, targeting non-immune pathways may be of particular interest. These are sodium-glucose cotransporter-2 (SGLT2) inhibitors and endothelin receptor A antagonists. The former drug class reduces glucose reabsorption in the proximal convoluted tubule of the kidney, thereby enhancing urinary glucose excretion. Because these agents improve glycaemic control, they were initially developed for the treatment of type 2 diabetes. Clinical studies have also shown that SGLT2 inhibitors cause an early and reversible reductions in eGFR suggesting that they reduce intraglomerular pressure and glomerular hyperfiltration, which may preserve long-term kidney function. As glomerular hyperfiltration is a pathological pathway leading to kidney damage in multiple types of kidney disease, SGLT2 inhibitors may well be efficacious in patients with IgA nephropathy.

Endothelin receptor antagonists are the second drug class of interest. Endothelin-1 is a potent vasoconstrictor and has been implicated in the progression of chronic kidney disease. Endothelin-1 can be released in the setting of hypertension, obesity, diabetes and activates pro-inflammatory pathways, causes extracellular matrix expansion, podocyte damage, and proteinuria. Endothelin-1 seems to be a logical target for treatment in patients with IgA nephropathy. Endothelin-1 is elevated in IgA nephropathy monocytes and kidneys, and neutrophils from IgA nephropathy patients, compared to controls, hyper-stimulate mesangial cell ET-1 production. In addition, renal ET-1 levels measured in biopsies from patients with IgA nephropathy directly correlates with albuminuria and 1-year progression. A clinical study in patients with CKD, including patients with IgA nephropathy, demonstrated profound reductions in albuminuria suggesting that these agents may also reduce the risk of kidney failure. More recently, top-line data from the PROTECT trial demonstrated that 36 weeks of treatment with the dual Angiotensin Receptor Blocker – endothelin receptor antagonist sparsentan resulted in a greater than threefold reduction of proteinuria (49.8% reduction in proteinuria from baseline) compared to the active control irbesartan (15.1%; p for difference <0.0001). Sparsentan was generally well-tolerated. These promising data support a future role of endothelin receptor antagonist for the treatment of IgA Nephropathy.

Use of Surrogate Endpoints in IgA Nephropathy: Results from CKD-EPI

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IgA nephropathy (IgAN) is rare, but the most common cause of glomerulonephritis, with few proven therapies. Trials early in the disease course are challenging to undertake because of low event rate for clinical endpoints, generally defined as kidney failure (treated end stage kidney disease (ESKD) or glomerular filtration rate (GFR) < 15 mL/min/1.73 m^2) or doubling of serum creatinine (S_{cr}). In many chronic kidney diseases (CKD), a large decline in GFR, assessed as a doubling of S_{cr} from baseline, and more recently 30 or 40% decline in GFR, has often been used as a surrogate endpoint for kidney failure in randomized clinical trials (RCTs) of patients with low levels of GFR or rapidly progressive disease RCTs.[1-3]

In a rare disease such as IgAN, these endpoints may not be feasible because of the long duration of the disease, leading to large expense and complexity of trials that would be required to detect treatment effects on a large decline in GFR. In addition, the goal of most therapeutic strategies is to treat the disease early, prior to development of irreversible changes. These issues have likely contributed to the paucity of therapies. Evidence supports early changes in urine protein as a reasonably likely surrogate in IgAN.[4,5] First, pathological data shows that the degree of urine protein correlates with greater evidence of disease.[6-8] Second, baseline levels of urine protein are prognostic for long-term disease progression,[9-16] and attenuation of urine protein after steroid therapy is associated with improved prognosis.[17,18]

In the US, reasonably likely surrogate endpoints can be used as a basis for accelerated approval of therapies intended to treat serious or life-threating conditions, such as IgAN.[19,20] The clinical benefit of products approved under this program would need to be verified in a post-marketing confirmatory trial.[21] Recent empirical data demonstrated the validity of GFR slope as a surrogate endpoint for clinical benefit in general CKD progression studies.[22-26] For IgAN, the slope of GFR decline would be a more viable endpoint for verification in post-marketing confirmatory trials given the low likelihood of sufficient clinical events.

The Chronic Kidney Disease Epidemiology collaboration (CKD-EPI) is a research group with the goal to provide evidence for the use of surrogate endpoints in CKD progression[27]. Over the past nearly two decades, we have assembled the available randomized control trials evaluating therapies in progression of CKD. Twelve of those were in IgAN. These twelve studies were included that investigated four intervention types [renin angiotensin system blockade (RASB), fish oil, steroids, or other immunosuppression agents. Using the individual patient data, we performed a Bayesian mixed effects analysis to relate the treatment effects on the surrogate endpoint to treatment effects on the established endpoint across these studies. The comparisons provide information on the magnitude and strength of the association between the two treatment effects and predicted treatment effects that can be used for use in the design of future trials.

We have performed the following three evaluations. First, we compared treatment effects on changes in urine protein evaluated over 6, 9 and 12 months to treatment effects on the clinical endpoint. Clinical endpoint is defined as doubling of serum creatinine, GFR < 15 ml/min per 1.73 m^2 or initiation of kidney replacement therapy[5,16]. Second, we compared treatment effects on GFR slope to treatment effects on the clinical endpoint. These associations were stronger than observed for changes in urine protein[23,24]. More recently, we compared treatment effects on urine protein to those of GFR slope[28]. In this set of analyses, across all studies, treatment effects on proteinuria accurately predicted treatment effects on the total slope at 3 years (median R^2=0.88 [95% Bayesian credible Interval (BCI) 0.06-1) and on the chronic slope (R^2 0.98 [95% BCI 0.29-1]). For future trials, an observed treatment effect of approximately 30% reduction in proteinuria would confer probabilities of at least 90% for nonzero treatment benefits on the total and chronic slope of eGFR.

A key limitations of these analyses in IgAN are that the study population was restricted to 12 trials of small sample size, leading to imprecise results, and therefore to uncertainty in the design of future trials. The imprecision is the result of the limited number of studies, all with small samples sizes, rather than an inherent limitation of urine protein as an endpoint in IgAN. Future analyses relating treatment effects on change in urine protein to treatment effects on GFR slope in the overall set of CKD studies, are expected to achieve higher level of precision.

All together, our results provide data that facilitate drug development for new treatments for IgAN. In particular, the data can be used to support the use of change in urine protein as an initial surrogate endpoint followed by GFR slope for subsequent confirmatory studies and accumulation of safety data Other applications are for early phase studies for proof-of-concept or dose finding.

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Complement in IgA nephropathy, what we know and what we don't

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Complement components are clearly activated in the kidneys of patients with IgA nephropathy (1). This disease follows a complex pathophysiology whereby galactose deficient IgA production is increased and maintained at significant levels, autoantibodies form to this IgA leading to immune complex formation, these complexes deposit in the glomerular mesangium, and this can lead to localized inflammation, injury and downstream renal fibrosis (2). Evidence continues to mount that the complement system is involved in this process. The lines of evidence include genetic predisposition, changes in serum biomarkers, evidence of complement products in the kidney, as well as increases in urinary markers of complement activation among patients with IgA nephropathy. These changes are often associated with kidney disease prognosis, where greater amounts or activity of the complements system correlate with worse disease and/or faster disease progression (3). Furthermore, initial experience with agents that alter complement activity suggest that these agents may be disease modifying.

Patients with various genetic alterations in complement Factor H have been found to have an increased risk of developing IgA nephropathy (4). Serum markers of alternative complement pathway activation or of lectin pathway activation have been variably reported to be increased among patients with IgA nephropathy. Serum levels of C3 have correlated with kidney outcomes in this disease as well (5). Urinary excretion of Factor H and of the terminal complement pathway (C5b-9) was found to be increased and to correlate with progression risk as well. Multiple components of complement pathways (ie: C3, C5b-9), particularly the alternate (Factor H, MASP-3, properdin) and lectin pathways (ficolin, MASP 2, C4D) have been found in kidney biopsy tissue of patients with IgA nephropathy and often correlate with the disease activity determined from the biopsy (6). Furthermore, evidence from experimental models of kidney disease suggest that the lectin pathway may play a pivotal role in tubulointerstitial injury and scar formation. This has derived from models of ischemia perfusion injury and from protein overload models of interstitial fibrosis (7). Thus, activation of the complement system is closely associated with glomerular inflammation and sclerosis marked by proteinuria as well as having the potential to augment tubule-interstitial sclerosis leading to decrements in renal function.

However, we do not yet know whether the association of complement activation with kidney injury in IgA nephropathy means that this process is causal. However, very early experience suggests that targeting the complement system in IgA nephropathy may indeed be able to improve proteinuria and stabilize the decline in kidney function. Results from phase II studies of narsoplimab have suggested an anti-proteinuric and GFR stablilizing effect, even among patients with advancing kidney dysfunction and heavy proteinuria (8). Scattered reports of eculizumab reducing proteinuria and improving kidney function have also been published (9). Abstract presentations for C5a inhibition and for Factor B inhibition also suggest some promise for improved outcomes, with reduced proteinuria and improved rates of eGFR loss, at least in some study participants. Of course, more complete, truly pivotal studies are required to ultimately prove that targeting complement activation can benefit patients with IgA nephropathy. It will also be essential to prove that this can be done safely and in a well-tolerated manner.

We are entering an exciting time for the understanding and treatment of IgA nephropathy. There is an abundance of data suggesting strong involvement of complement in the pathogenesis of this common glomerular disease. We now have many tools to interrupt complement activation. Hopefully, these agents will be able to improve outcomes in our patients.

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Novel clinical designs and what they can deliver in IgAN

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Over the last decade, there has been an increase in understanding of mechanisms of IGAN, an acknowledgement that there is heterogeneity in presentation, progression and outcomes, prediction models to facilitate prognostication, and newer therapeutic targets and agents being trialed and tested in phase 2 and 3 studies. IgAN is both a common and a rare condition, has variable prevalence around the globe, and in different ethnic groups, and can be part of a systemic disorder, or solely affect the kidneys. The increase interest in complement mediated pathways, inflammation and fibrosis pathways, and improved standardized evaluation of clinical biopsy specimens have moved the field forward rapidly in a relatively short period of time.

The classic randomized control trials have long been touted as the gold standard to evaluate novel therapies, bringing rigor to comparing placebo to new therapies or standard of care to new therapies. Inclusion and exclusion criteria often limit the generalizability of the studies, and in the traditional RCT, the expense and time to conduct the study limit sample size, and thus often the conclusions. There is increasing recognition that traditional RCTs are inefficient, inflexible and expensive and non-generalizable to clinical practice. Furthermore, within nephrology there has been a history of complacency, negative trials, and some inertia to enter people into clinical trials. While the recent experience with SGLT2i, nonsteroidal MRA's and endothelin antagonists has begun to change the tide in nephrology, there remains a continued paucity of clinical trials, and only a fledging culture of integrating research into clinical practice in a functional manner.

Novel strategies and approaches are being proposed to facilitate change in the nephrology culture, and more importantly, change in the outcomes of patients. Patient engagement is driving priority setting, and facilitating identification of patient relevant outcomes for different conditions; standardized outcome definitions will permit comparison of outcomes across different trials, novel trial designs, the development of minimal data sets, and efficient data capture with electronic databases, all are changing the way in which clinical trials can and will be designed.

IGAN might be considered an important prototype condition in nephrology, which would lend itself well to novel study designs including adaptive and platform studies. The heterogeneity of the condition, diversity of biopsy findings, increasing use of prediction tools, biomarkers and molecular targets being discovered limit the ability to answer important questions with large scale RCTs designed according to conventional standards. Appreciating the concepts of responders and non-responders, deep phenotyping currently ongoing, and 'precision medicine', the nephrology community should be able to embrace the concept of novel study designs in order to advance the field of IGAN and improve patient outcomes, in a more timely manner than has been the case to date.

Consideration of pragmatic trials, adaptive clinical trials, and platform trials and the pros and cons of each of these depend on right research questions being asked in a design appropriate to the populations of interest, and possible therapeutic options. Adaptive *platform* designs offer flexible features such as dropping treatments for futility, declaring one or more treatments superior, or adding new treatments to be tested during the course of a trial.

In the oncology world the development of robust platforms in which to study new drug therapies in an efficient and effective manner, whilst characterizing individuals based on biomarkers, imaging and molecular signatures found in biopsy tissue (iSPY-2 platform) has been widely successful and serves as an interesting paradigm for the study of IGAN. Imagine the reconstruction of Phase 2 clinical enterprise that "identifies early surrogate efficacy endpoints, adaptive randomization to make the most over every pt enrolled, real world controls, multiple agents can be independently evaluated in parallel, randomization targets agents addressing molecular subtypes, a master protocol which compressing start up times, an integrated platform for companion biomarker discovery. The iSPY2 trial is successful not simply the result of a single innovation in trial design, but because they have pain-staking deconstructed and re-engineered the entire clinical trial enterprise, from protocol development through registration" (www.ispytrials.org). National and international collaboration amongst IgAN investigators and patients could create a similar enterprise, which would advance the field in exciting ways.

A role for the alternative pathway?

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The complement system is a network of activating and regulating proteins that marks damaged and non-self cells and tissue and is essential to innate and adaptive immunity. The complement system is characterised by multiple variants that influence protein structure, abundance, and activation. The alternative pathway arm of complement activation has two important roles. First, it can trigger complement system activity via constant tick-over hydrolysis and activation of C3. Second, it is responsible for amplification of complement system activity and subsequent cascade to terminal pathway activation regardless of the complement trigger. Alternative pathway activity can be detected by the presence of activated C3 in kidney biopsies (referred to as C3). The antibodies used are typically raised against C3c and also react with C3b and iC3b. Complement activation fragments and reduced circulating levels of intact C3 are also evidence of alternative pathway activity. There is long-standing and established evidence of alternative pathway activity in IgAN. Mesangial co-deposition of C3 and IgA is characteristic of IgAN, being present in at least 90% of biopsies and noted in the earliest descriptions of IgAN(1). Properdin, that binds and stabilises the alternative pathway C3 convertase, can be detected in more than 75% of IgAN cases(2). Also, decreased plasma C3 levels with increased C3 activation products (iC3b and C3d) were observed in some IgAN patients(3, 4).

Biomarkers of alternative pathway activity do not identify the triggers of complement activity nor demonstrate a pathogenic role for complement activation in IgAN. However recent data have demonstrated associations between markers of alternative pathway activity and IgAN severity. These suggest alternative pathway activity contributes to glomerular injury in IgAN. In a study of 343 IgAN patients from Korea, the abundance of mesangial and capillary wall C3 deposition correlated with morphology features of glomerular inflammation and IgAN severity(5). In the same cohort, lower serum C3 at diagnosis associated with mesangial C3 deposition and worse prognosis, demonstrating a link between serology and glomerular alternative pathway activity and glomerular inflammation and injury. In a large cohort of IgAN patients from China, the combination of increased circulating galactose deficient IgA1 and C3 cleavage independently associated with kidney disease progression or failure (6).

A growing body of evidence is beginning to reveal how complement activity could influence IgAN severity. Analysis of IgA1-containing immune complexes precipitated from patient serum detected C3 in complex with IgA1 in 48% of IgAN patients(7). This suggests the essential effector molecule of IgAN, IgA1, could act as a complement activating surface that carries activated complement fragments into mesangial deposits. Consistent with this, *In vitro* assays using plate-bound or aggregated IgA1 demonstrate complement activation via the alternative pathway(8) and C3 breakdown products like iC3b, C3c, and C3dg are detected by proteomic analysis of circulating IgA1-containing immune complexes(9).

Recent evidence suggests imbalanced alternative pathway regulation influences IgAN pathogenesis. Specifically, variants of the essential complement regulator, factor H (FH), and the factor H related proteins 1 (FHR1) and 5 (FHR5), which compete and interfere with FH regulation, associate with IgAN risk and severity. Large, replicated genome wide association studies have demonstrated FHR1 gene deletion (del*CFHR3-R1* polymorphism) reduces the risk of developing IgAN (10). In Chinese cohorts, del*CFHR3-R1* associated with higher circulating C3 and FH levels and reduced mesangial C3 deposition(11). Two independent studies showed the plasma FHR1/FH ratio associated with IgAN disease severity(12, 13). In large British and Chinese cohorts, higher FHR5 plasma levels associated with IGAN risk (13, 14) and, in the larger, Chinese, cohort proteinuria and reduced eGFR(14). In an IgAN patient cohort from the UK, glomerular FHR5 deposition associated with IgAN severity and glomerular deposition of C3b/iC3b/C3c, C3dg and C5b9 (complement terminal pathway marker)(15). A cohort of 56 IgAN patients from China replicated associations between glomerular FHR5 deposition and histology markers of IgAN severity and, importantly, demonstrated glomerular FHR5 co-localisation with IgA and C3c, indicating its role in influencing complement activation at points of IgA deposition(16). Evidence that FHR variants can drive glomerular injury is provided by rare familial cases of C3 glomerulopathy, such as CFHR5 nephropathy(17-19). The ability of FHR1 and FHR5 homodimers to interfere with the physiological actions of FH through binding to C3 activation fragments has been demonstrated *in vitro* (19, 20). Thereby, imbalances in FHR1, FHR5 and FH might increase alternative pathway activation and C3 cleavage in response to mesangial IgA1 deposition, leading to amplified complement-dependent inflammation and injury.

In summary, the alternative pathway likely contributes to IgAN pathogenesis and severity. However, the extent to which the alternative pathway determines kidney injury is variable. Understanding the impact of alternative pathway activity on IgAN pathogenesis will depend on elucidating the mechanisms that link complement activity and glomerular injury in IgAN.

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25 years since IgA1 glycosylation changes were first described – what will the next 25 years bring?

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IgA nephropathy (IgAN), the most common primary glomerulonephritis in the world, is a chronic progressive kidney disease with significant morbidity and mortality and reduced life span.[1,2] The disease, described for the first time in 1968,[3] is characterized by glomerular immunodeposits enriched for IgA1 glycoforms with some *O*-glycans deficient in galactose (Gal)[4,5] and for IgG autoantibodies with specificity for such Gal-deficient IgA1.[6] Patients with IgAN exhibit heterogeneous clinical and pathological features with variable rates of progression.[7,8] Only a fraction of patients with IgAN enters a sustained clinical remission. Without any disease-specific treatment available, up to 40% of patients with IgAN progress to end-stage kidney disease within 20 years of diagnosis.[7] Furthermore, IgAN recurs in >50% of patients with kidney transplantation within 5 years.[1]

Discovery of abnormal glycosylation of IgA1 in patients with IgAN was based on using lectins specific for *O*-glycans, such as the plant lectin jacalin or lectins from snails, *Helix aspersa* and *Helix pomatia*.[9-12] Jacalin is specific for core 1 *O*-glycans, i.e., *N*-acetylgalactosamine (GalNAc) with β1,3-linked Gal, whereas the snail lectins are specific for terminal GalNAc. The snail lectins were used in quantitative assays that revealed that most patients with IgAN have elevated levels of serum IgA1 with some *O*-glycans deficient in Gal.[13] This quantitative lectin-ELISA enabled genome-wide association studies (GWAS) that identified single-nucleotide polymorphisms that control expression of genes involved in *O*-glycosylation of IgA1.[14,15]

Additional gas-chromatographic and mass-spectrometric techniques have been used for targeted analyses of *O*-glycosylation of total serum IgA1 (for review see[16-19]). These and other techniques enable studies of IgA1 glycoforms bound in the pathogenic immune complexes that deposit in the kidneys to activate mesangial cells and induce glomerular injury.[20,21] It is hoped that progress in glycome-profiling technologies of IgA1 will catch up with that of IgG (see e.g.,[22,23]) to enable high-throughput quantitative phenotyping for GWAS of IgA1 *O*-glycosylation. These approaches can be complemented by functional studies using IgA1-producing cells and biochemical enzymatic studies[24,25] as well as by single-cell-based -omics studies (e.g.,[26]).

Gal-deficient IgA1 is recognized by IgG autoantibodies,[27] resulting in the formation of immune complexes. These IgG autoantibodies exhibit somatic mutations suggestive of immune responses to an antigen[28] and their serum levels correlate with those of the autoantigen, Gal-deficient IgA1.[29] Serum levels of Gal-deficient IgA1 and the corresponding IgG autoantibodies predict disease progression[30-32] and recurrence after kidney transplantation.[33,34]

Glomerular immunodeposits of patients with IgAN are enriched for IgG autoantibodies specific for Gal-deficient IgA1,[6] consistent with the multi-hit hypothesis for pathogenesis of IgAN. Recently, experimental evidence of the pathogenic potential of these IgG autoantibodies came from experiments based on injections of pre-formed immune complexes consisting of human Gal-deficient IgA1 and human IgG autoantibodies into immunodeficient mice.[35] These immune complexes, but not Gal-deficient IgA1 or IgG autoantibodies alone, induced glomerular injury with histologic features mimicking IgAN, confirming pathogenic role of IgG autoantibodies specific for Gal-deficient IgA1.

It is hoped that during the next 25 years, we will develop a better understanding of the mechanisms of the formation and activities of the pathogenic IgA1-containing immune complexes in IgAN. The areas of high interest in this regard include: i) defining specific epitopes and glycoforms of Gal-deficient IgA1 autoantigen, ii) characterizing heterogeneity of IgG autoantibodies and defining their epitopes, iii) defining Gal-deficient IgA1 glycoforms and the corresponding IgG autoantibodies that are associated with disease progression, iv) determining why some individuals with elevated circulating levels of Gal-deficient IgA1 (e.g., some blood relatives of IgAN patients) do not develop clinically apparent IgAN, and v) defining other biologically relevant components of IgA1-IgG immune complexes in IgAN. Together, defining the origins and mechanisms involved in the production of Gal-deficient IgA1 and the corresponding IgG autoantibodies and their pathogenic complexes will provide information for design and pre-clinical testing of disease-specific therapies. Such therapies are needed to improve the standard of care for patients with IgAN.

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Challenges of designing a clinical trial in paediatric kidney disease

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Designing a clinical trial in children with chronic kidney disease (CKD) comes with unique challenges for investigators but unmet opportunities for the benefit of children's health. Despite a large increase in the last decade to develop paediatric-specific drug trials, there remains a substantial gap between the number of paediatric and adult randomised controlled trials that are being conducted throughout the world (1). New treatments reach adults many years before they can be used in children. Children with kidney disease deserve to be treated in a similar manner to adults, with therapeutic agents available once they have undergone the same rigorous drug development as they do for adult disease; allowing fair and equal opportunities for this vulnerable group (2).

There are many factors that present challenges to investigators when designing clinical trials for children with kidney disease and these include factors associated with the drug itself such as pharmacodynamics and the drug formulation, more complex ethical and regulatory considerations, together with the trial design and significant financial implications. These create barriers that are generally not due to a reluctance for patients to take part in research (3). With regards to the drug itself, it has long been recognised that the specific handling of drugs is different in children when compared to adults and even differs in children as they develop from the neonatal period to adolescence. The pharmacokinetics and pharmacodynamics of many drugs in children differ due to the physiological difference in body size and composition. For example, a neonate has a total body water content of up to 83% in contrast to 45-60% in an adult (4) and extremely premature infants who are small for gestational age have negligible body fat content (5); thus drugs will be handled differently demanding specific evaluation in target age groups (6). The formulation of the drug suitable for children is also incredibly important as young children are generally unable to swallow large tablets. Liquid formulations then need to be evaluated for their palatability and consideration of the strength to allow differing doses in an appropriate volume for safe administration. Excipients used also need to be safe and suitable for use in children.

Ethical considerations in conducting research in children are more complex when compared to adults, with informed consent relying on parental consent in partnership with child assent. This requires age specific documentation such as patient information leaflets. Blood sample volumes to be collected for research are determined by the child's age and samples may only be permitted if the patient is having blood collected for clinical purposes too. There are data protection considerations for research in minors especially around when the child reaches the age of an adult and acquires the ability to consent for themselves and logistical issues once the child transfers to a different centre under the care of an adult Nephrologist. These circumstances can make longitudinal studies in children more complex. Additionally, insurance and regulations for studying children can be more expensive and may differ in different countries.

A clinical trial in children can be complex to design statistically due to a smaller population spread across many centres needing multicentre engagement. There may also be a lack of validated clinical end points in children and scoring tools need to be developed to suit children of differing ages/abilities and incorporate caregivers. The financial implications of drug development through clinical trials are huge as multicentre/international sites may be needed and there is a relatively small market size once a drug has been successfully developed. Trials may also require additional staff training and insurance to deal with potential for long term consequences in this group of patients. These physiological, ethical, regulatory and statistical differences of studying medications in children present barriers that do not provide an incentive for industry to study new agents in children thus creating inequalities.

The introduction of data exclusivity, market protection, orphan and paediatric rewards for industry, have positively incentivised efforts (7). Further developments include empowering professionals and patients to request and assist with paediatric investigation plans and explore use of innovative trial designs that can adapt to meet the needs of diverse patient groups. Continued efforts to improve liaison between industry and nephrology to support the inclusion of children with kidney disease to clinical trials will improve outcomes for our future generations.

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The revised KDIGO guidelines for the treatment of IgA nephropathy

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Updating the KDIGO guidelines for treatment of IgAN

The Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guidelines for the treatment of glomerulonephritis are due to be published in 2021, and represent an update of the seminal guidelines published in 2012[1]. These guidelines represent a methodologically rigorous international effort to provide a transparent assessment of peer-reviewed published data describing the effectiveness of a broad spectrum of interventions to treat glomerulonephritis and prevent complications of this important cause of kidney failure and premature mortality. A draft of the guideline has been circulated for public review in 2020, and the final draft is in preparation for publication (as of August 2021).

While IgA nephropathy (IgAN) remains a leading cause of kidney failure globally there remain important areas of clinical equipoise and variability in practice patterns in the treatment of IgAN. In addition to evidence-based recommendations based upon systematic review of the literature, the revised guidelines provide expert "practice points" in areas where there is insufficient or inconclusive evidence to guide care. The guidelines are expected to provide clinicians and patients with the information needed to balance the risks and benefits of various treatment options, to guide shared decision-making with patients, and to help mitigate toxicity of immunosuppression. Three themes emerge from revised guidelines. First, there is an emphasis on individualization of treatment for each patient, including identifying patients at highest risk of progression. Second, careful selection of patients who may benefit from immunosuppression is emphasized, in an effort to spare treatment emergent toxicity. Finally, the need for additional research is highlighted throughout the guidelines.

The clinical outcome of patients with IgAN is highly variable. A key first step in the management of patients with IgAN is identifying individuals at highest risk of progressive disease and kidney failure. Since the 2012 publication, the International IgA Nephropathy prediction[2] tool has been derived to provide prognostic information for individual patients. This algorithm was derived and validated in a cohort of close to 4000 subjects. The prediction tool (www.qxmd.com) incorporates patient age, race, GFR at biopsy, proteinuria, medications and blood pressure at the time of biopsy and histologic score. The risk of halving of kidney function or kidney failure can be estimated for a horizon of up to 6.7 years following biopsy. While the prediction tool does not guide the selection of therapy, the prognostic information is important to help inform patients about their disease trajectory, and to help inform discussions with patients about treatment options.

Since the publication of the 2012 guidelines, important clinical trials investigating the potential role for corticosteroids have been published including the STOP-IgAN and TESTING studies[3, 4]. The results of these trials are difficult to reconcile; while the STOP-IgAN trial did not show long term benefit of corticosteroids, the early TESTING results suggest efficacy to reduce proteinuria and prevent kidney failure, however this came at the expense of significant potential for toxicity including two deaths in the initial recruitment phase. The new guidelines therefore must present the data supporting efficacy, but emphasize identification of patients who are at risk of treatment toxicity and strategies to mitigate the toxicity observed in clinical trials.

The upcoming guidelines highlight challenges in identifying patients at risk of progressive IgAN and the risks associated with corticosteroid use – the only evidence-based treatment option in this disease. This emphasizes the critical need for research to advance the care and improve outcomes of this patient population. Therefor throughout the IgAN guidelines, there are references to the need for ongoing research. At the time of diagnosis, it is recommended to try to enroll patients in a disease or clinical trial registry to provide natural history insights, refine prognostic tools, and to facilitate clinical trial recruitment. The paucity of evidence-based recommendations underlines the need for international multi-centre clinical trials. Given the complications observed in the TESTING study, development and evaluation of less toxic treatments for IgAN is imperative. Fortunately, exciting emerging treatment options are on the horizon, including nephroprotective strategies (ex. SGLT2 inhibitors, endothelin inhibitors) and strategies targeting disease immunopathogenesis (ex. Complement modulation, local-acting corticosteroids, cytokine inhibitors). It is anticipated that the revised KDIGO clinical guidelines for the treatment of IgAN will not only help individualize therapy and improve safety of available treatments but will also highlight areas of clinical need and promising new approaches to improve the outcome of patients with IgAN.

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What can CureGN tell us about IgAN in children and young adults?

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Observational cohort studies in nephrology such as CRIC (Chronic Renal Insufficiency Cohort study), DOPPS (Dialysis Outcomes and Practice Patterns), and CKiD (Chronic Kidney Disease in Children study) have been rich sources of information about risk factors for progression of kidney disease, complications, and response to treatment. Until recently, there was not a similar cohort study for patients with glomerular disease. CureGN is an observational cohort study of children and adults with glomerular diseases including minimal change disease, focal segmental glomerulosclerosis, membranous nephropathy, and IgA nephropathy (IgAN) or IgA vasculitis (IgAV) that started enrollment in 2015.[1] This study is sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and Nephcure Kidney International. A major goal of this study is to establish an infrastructure to advance knowledge and foster research in these disorders.

Over 2400 CureGN participants including 667 patients with IgAN or IgAV were recruited from over 60 sites within the United States, Canada, Italy, and Poland and will be followed as long as the study is ongoing. Participants must have had a diagnostic kidney biopsy within 5 years of enrollment. Biopsy reports are reviewed by a central study kidney pathologist and must demonstrate at least 5 glomeruli on light microscopy and ≥ 1+ IgA dominant or co-dominant diffuse mesangial staining to be included in the IgA cohort. In addition, diagnosis of IgAV was made clinically by the enrolling nephrologist based on the presence of both renal and extra-renal manifestations (e.g. palpable purpuric rash, gastrointestinal involvement, arthalgias/arthritis). Importantly, biopsy slides are scanned centrally into a digital pathology repository and scored by a study pathologist. This pathology resource will be invaluable for future studies of this cohort.

After enrollment, participants are seen yearly for in person study visits that include biosample collection (24 hour urine, first morning urine, plasma, serum, DNA, RNA), administration of patient reported outcome questionnaires, recording of medical history including hospitalizations and treatment, and input of standard of care lab values. One or two additional in-person or virtual study visits are completed yearly. Central measurements of serum creatinine and urine protein to creatinine ratio are obtained and the remainder of the biosamples are stored in the NIDDK biorepository and are available for use by researchers.

The CureGN cohort has enrolled 506 participants with IgAN (173 children and 333 adults) as well as 161 participants with IgAV (112 children and 49 adults).[2] Characteristics of children in the IgAN cohort at time of biopsy include median age 12.5 years (IQR 9.9-15.2), eGFR 98.6 ml/min/1.73m^2 (IQR 75.9-122), and urine protein to creatinine ratio (UPCR) of 1.1 mg/mg (IQR 0.3-2.3).[2] There is a wide range of disease severity demonstrated by 18% of children having a UPCR of ≥ 3 and 23% with UPCR < 0.3 at time of biopsy. Compared to adults with IgAN, children had lower UPCR and higher eGFR at the time of biopsy. Characteristics of children in the IgAV cohort at time of biopsy include median age 9.5 years (IQR 6.9-13.6), eGFR 109.5 ml/min/1.73m^2 (IQR 82.4-127.7), and urine protein to creatinine ratio (UPCR) of 2.1 mg/mg (IQR 0.7-5.0).[2] Longitudinal follow up is ongoing to determine how these parameters change over time.

Children with IgAN were enrolled a median of 1.3 years (IQR 0.4-3.0) after diagnosis and children with IgAV were enrolled a median of 0.6 years (IQR 0.2-1.5) after diagnosis.[2] A majority of children had been treated with immunosuppression prior to enrollment including 50.3% of children with IgAN and 75.9% of children with IgAV.[2] Reflecting the lack of data and consensus for treatment of these disorders in childhood, there was wide variability in treatments received. Children with IgAN had been treated with corticosteroids (46.2%), mycophenolate mofetil (14.5%), azathioprine (5.2%) and cyclophosphamide (4.0%) prior to enrollment. Similarly, children with IgAV had been treated with corticosteroids (73.2%), mycophenolate mofetil (20.5%), azathioprine (8.9%) and cyclophosphamide (8.0%) prior to enrollment. Surprisingly, only 67.1% of children with IgAN and 62.5% of children with IgAV had been treated with renin angiotensin aldosterone system (RAAS) blockade despite the widely recognized benefits of these drugs in patients with glomerular disease.

Children with glomerular disease including IgAN and IgAV are at increased risk for cardiovascular disease, thus careful attention to and treatment of modifiable risk factors is important to minimize their risk of cardiovascular events. At the time of enrollment in CureGN, there was a significant burden of cardiovascular risk factors in children in the IgA cohort including 27% with hypertension, 29% with obesity, 55% with dyslipidemia, 13% with prematurity, and 23% with exposure to secondhand smoke.[3] Only 70% of children in the IgA cohort with hypertension at enrollment were receiving treatment.[3] Only 32% of children in the IgA cohort had had lipid screening performed prior to enrollment and only 13% of children with dyslipidemia were treated.[3] Cardiovascular outcomes are being monitored in CureGN during the follow up period.

The children and adults in the IgAN and IgAV cohorts of the CureGN study provide a rich source of data and biosamples for researchers to utilize to advance our understanding of and care for patients with these disorders. Researchers do not need to be investigators to access the data or samples, but must collaborate with a CureGN investigator and submit an ancillary study proposal for approval by the steering committee. More information and application documents can be found at CureGN.org.

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New Targets for New Therapies in IgAN & IgAV: Inhibiting Complement Activation in IgAN

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IgA nephropathy (IgAN) is the most common primary glomerulonephritis in the world and is a significant cause of morbidity. About 20 to 40% of patients reach end-stage kidney disease (ESKD) within 20 years of diagnosis which, in turn, increases their mortality risk as evidenced by shortening patients' lifespan by a decade[1]. To date, there is no approved disease-specific treatment for IgAN. Currently, the mainstay of treatment is aggressive supportive care that includes renin-angiotensin-aldosterone system (RAAS) blockade, blood-pressure control, dietary modifications, weight loss, exercise, and smoking cessation[2]. Despite these interventions, a significant subset of patients continue to have proteinuria >1 g/day that translates into a loss of estimated glomerular filtration rate (eGFR) over time[3]. For those high-risk patients, aggressive treatments that target disease-specific pathways are essential. Scientific advances in the last few decades have increased our understanding of the disease pathogenesis and shed light on potential therapeutic targets. Mounting clinical, biochemical, and genetic evidence highlight the pivotal role of complement activation in the onset and progression of IgAN[4-10]. In particular, the lectin and alternative complement pathways are involved and evidence of complement activation in kidney biopsies portends a more severe renal prognosis[11-13]. The availability of complement inhibitors, along with these facts, have sparked interest in testing their efficacy in the treatment of IgAN. These inhibitors span a gamut of compounds at various stages of development and include monoclonal antibodies, small molecules, and short peptides that hinder formation of protein complexes and/or enzymatic reactions. Initial promising clinical data came from case reports. Eculizumab (a humanized recombinant monoclonal antibody that selectively inhibits cleavage of C5 by C5 convertase thereby preventing formation of soluble membrane attack complex) was used as a rescue treatment in native-kidney as well as post-transplant IgAN cases, leading to partial and short-lived improvement[14-16]. Several clinical trials have since been initiated to test the effectiveness of blocking the complement cascade. Ravulizumab (a long-acting, humanized, recombinant monoclonal antibody against C5) is being tested in a phase II clinical trial (ClinicalTrials.gov Identifier: NCT04564339).

Avacopan (CCX168; a small molecule blocking C5a receptor) was evaluated in a phase II open-label trial that enrolled 7 IgAN patients with preserved eGFR (>60 ml/min/1.73 m^2) and urinary protein/creatinine ratio (UPCR) >1 g/g. After 12 weeks, 3 patients had a 50% reduction in UPCR. For the duration of the trial, Avacopan was safe and well tolerated[17].

Cemdisiran (ALN-CC5; a synthetic small interfering RNA that suppresses C5 production in the liver) is in a phase II, randomized placebo-controlled clinical trial). The trial has completed enrollment with 31 IgAN patients with 24-h urinary protein >1 g (ClinicalTrials.gov Identifier: NCT03841448).

The cleavage of C3 into C3a and C3b by C3 convertases is a crucial step in the amplification of the classical, alternative, and lectin pathways. Compstatin and its pegylated derivative, pegcetacopan (APL-2), bind to C3 and prevent its cleavage. APL-2 is currently being evaluated in a phase II open-label basket clinical trial that includes IgAN patients with UPCR >750 mg/g on 24-h urine collection and eGFR ≥30 ml/min/1.73 m^2 (ClinicalTrials.gov Identifier: NCT03453619).

The alternative complement pathway plays a pivotal role in IgAN[8]. Iptacopan (LNP023), an oral small-molecule Factor B inhibitor was evaluated in a phase IIa/IIb trial that recruited 112 IgAN patients with eGFR ≥30 ml/min/1.73 m^2 and proteinuria ≥1 g/day at screening despite maximal tolerated RAAS blockade. To be enrolled, participants were required to obtain vaccination against Neisseria meningitides A, C, Y and W-135. Participants were randomized to placebo versus 4 different BID doses of LNP023 (10 mg, 50 mg, 100 mg, and 200 mg). The study recently reported its positive results in a late-breaking abstract at the ERA-EDTA 2020 meeting. There was a statistically significant dose-response effect of iptacopan on proteinuria. At 90 days, the 200 mg BID arm reduced 24-h UPCR by 23% compared to placebo. These data supported the launch of the APPLAUSE-IgAN global trial, a phase III placebo-controlled trial designed to enroll 450 patients worldwide randomized to receive 200 mg BID of LNP023 versus placebo (ClinicalTrials.gov Identifier: NCT04578834). Importantly, this trial will include a small subset of patients (n=20) with severe renal impairment, defined as eGFR 20 to <30 ml/min/1.73 m^2.

Mannose-binding lectin associated serine protease 2 (MASP-2) is an important component of the lectin pathway that, with MASP-1, cleaves C4 and C2 into active fragments triggering formation of the C3 convertase and ensuing inflammatory effects. Targeting MASP-2 is an attractive treatment option to inhibit the lectin pathway while preserving the capacity to generate C3 convertase via the classical and alternative pathways. Narsoplimab (OMS721) is a humanized monoclonal antibody selectively targeting MASP-2. In a phase II, multicenter, clinical trial, IgAN patients with proteinuria >1 g/day despite maximal tolerated RAAS blockade and baseline eGFR >30 mL/min/1.73 m^2 were enrolled in 2 sub-studies based on whether they were corticosteroid-dependent or -independent at baseline. Interim analysis of both groups revealed the drug was safe and well-tolerated and decreased proteinuria; eGFR remained stable[18]. These encouraging data led to the launch of ARTEMIS-IGAN, a phase III clinical trial assessing the efficacy and safety of narsoplimab in IgAN patients with persistent 24-h proteinuria >1 g (ClinicalTrials.gov Identifier: NCT03608033).

Besides assessing their efficacy in IgAN, complement inhibitors must be evaluated for safety. Eculizumab considerably increases the risk of infections with encapsulated organisms, particularly meningococci. This risk is partly mitigated by administration of vaccines at least two weeks prior to the initiation of therapy. The risk of infections will ultimately depend on the level and extent of complement inhibition in addition to the duration of therapy. Other potential safety concerns are based in the knowledge that some deficiencies of the classical complement pathway increase the risk of developing systemic lupus erythematosus, so monitoring for autoimmune complications will be important[19].

Adding complement inhibitors that target the pathogenesis of IgAN to the armamentarium for treatment offers new and exciting options for patients.

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What next for the Oxford Classification?

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The Oxford Classification of IgA nephropathy (IgAN) was based on histological scoring of 265 IgAN biopsies[1,2]. An evidence-based approach was used to develop a reproducible histological classification of IgAN that improved accuracy of predicting risk of disease progression. The Oxford Classification was updated in 2016 in the light of new evidence arising from large multicentre international studies, with the addition of crescents to the scoring system[3]. Subsequently the MESTC scores have been incorporated in a risk prediction tool[4].

Further development of histological evaluation of IgAN biopsies focusses on improving the accuracy and reproducibility of the Oxford Classification in clinical practice, and supplementation of the MESTC scores using methodology not used in the development of the Oxford Classification to identify additional lesions and prognostic information in the renal biopsy.

The MEST-C scores were found to be reproducible in the original Oxford Classification study, but subsequent multicentre studies have identified high interobserver variation in the scoring of cellular lesions (M, E and C) in particular[5]. Pathologists in the Oxford Classification group achieved consistent scoring by first achieving consensus on difficult areas of interpretation, and by using a single circled PAS-stained section to score glomerular lesions in each biopsy. Clinical practice is very different with variation in sectioning and staining protocols, scoring using multiple slides and stains, and variations in approach between pathologists.

There is widespread tendency to overscore mesangial cellularity, largely a result of pathologists not following the strict guidance provided in the Oxford Classification. Overscoring M1 results in the mesangial hypercellularity losing its prognostic value5. Improvement in reproducibility of scoring endocapillary hypercellularity, which reflects glomerular inflammation, can be achieved by quantifying glomerular macrophages using immunohistochemistry (IH) for CD68[6]. The author recommends CD68 staining of all IgAN biopsies and including the maximum glomerular macrophage count in the pathology report; >6 macrophages in the most inflamed glomerulus is indicative of E1. This has been demonstrated to be a simple and highly reproducible method for identifying glomerular inflammation[6]. IH for CD68 might also be of value in differentiating crescents from pseudocrescents, and for scoring mixed cellular/sclerosing lesions.

Detection of focal glomerular lesions, in which a single lesion will determine the Oxford Classification score (as for E, C and S), has been demonstrated to be dependent on the number of slides examined, particularly when the percentage of glomeruli in the biopsy with these lesions is low[5]. Detection of very focal E and C lesions is important for accurate prognostication and further guidance on sectioning and staining protocols is required.

There is growing evidence that biopsies hold prognostic information additional to the MEST-C scores. The prognostic value of certain lesions could not be evaluated in the Oxford Classification study as the lesions were uncommon in the biopsies used to develop the classification, including necrotising glomerular lesions and thrombotic microangiopathy (TMA). The definition and reproducibility of TMA lesions has been problematic, and there is a 10-fold variation in the reported frequency of TMA in IgAN with some studies reporting TMA in over 50% of biopsies[7]. In most IgAN patients, TMA is associated with severe hypertension[8,9,10]. Several studies have demonstrated that TMA is an independent risk factor for progression to end-stage renal disease[9,10].

Digital pathology is being increasingly used to quantify inflammatory cell infiltrates and fibrosis in IgAN biopsies[6,11]. It remains to be ascertained whether image analysis and diagnostic algorithms provide added value over subjective biopsy scoring. The use of digital slide tools facilitates measurement of glomerular size, although a simple measure of the diameter of the largest glomerulus in the biopsy can be performed easily without digital pathology. Maximum glomerular diameter is associated with risk of progression to chronic kidney disease[12] and significantly improves risk prediction based on MEST-C scores[13].

The lesions evaluated in the Oxford Classification study were restricted to those that could be assessed by light microscopy (LM); the potential prognostic value of immunohistology and electron microscopy was not evaluated. Glomerular capillary wall IgA and additional presence of IgG within the deposits have been demonstrated to be associated with increased risk of disease progression. However, study of the Oxford Classification cohort indicated that these features were associated with endocapillary hypercellularity and not of independent prognostic value[14].

Immunohistology for complement components can provide important prognostic information. The intensity of glomerular C3 on immunofluorescence is associated with risk of disease progression[15] and combined analysis of C3 deposits and Oxford Classification scores has demonstrated that the hazard ratios for eGFR decline are significantly higher for C3/M1, C3/S1 and C3/C1-2 than for each lesion alone[16]. There is evidence for deregulation of the alternative complement pathway in IgAN; absence of factor H (FH) and presence of FHR5 in the glomerular deposits is associated with disease progression[17]. However, glomerular

FHR5 correlates with M1 and E1, and it remains to be ascertained whether FH deregulation is of prognostic significance independent of Oxford Classification scores. There is also evidence of lectin pathway activation in IgAN with glomerular C4d deposits being present in approximately one quarter of IgAN biopsies. The presence of C4d has been consistently associated with increased risk of disease progression[18,19]. However, there are important issues that should be addressed before routine reporting of C4d can be recommended: methodology (IF/IH) affects the detection of C4d and the distribution of C4d is highly variable (focal/diffuse; segmental/global; mesangial/capillary wall)[20].

Evaluation of these additional LM features and immunohistological findings in a risk stratification model is required to determine which will provide added value to clinical parameters and MEST-C scores. Methodological issues, and how to interpret and quantify histological changes other than the MEST-C scores, need to be addressed before firm recommendations on their reporting and use in clinical practice can be made. It was always intended that the Oxford Classification evolves according to new evidence and it is likely that the next update of the classification will incorporate at least some of the histological features discussed in this article.

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What biomarkers are on the horizon that may help risk stratify patients with IgAN?

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IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide and is characterized by formation of IgA1-containing circulating immune complexes. Extensive research had identified a number of key pathogenetic factors, including genetic, immunological and environmental factors. Due to the multifactorial pathophysiology, the clinical presentation of IgAN is variable, ranging from mild forms with minor urinary abnormalities and preserved renal function to cases that rapidly progress to end stage renal failure. Thus, early identification of patients at risk for a progressive course is urgently needed. Early risk stratification and individualized therapies would be desirable for patients with IgAN.

Elevated levels of galactose-deficient IgA1 (Gd-IgA1) seen in the circulation of many patients with IgAN are regarded as the initiating factor for the formation of circulating immune complexes. These Gd-IgA1 glycoforms are recognized by IgG autoantibodies resulting in the formation of IgA1-containing immune complexes (IgA1-ICs), some of which deposit in the kidneys. Furthermore, the concentration of immune complexes containing Gd-IgA1 is increased in the blood and urine of IgAN patients (1). Gd-IgA1 in the serum of patients with IgAN is found exclusively within immune complexes bound to IgG. Therefore, the onset and progression of IgAN is believed to require Gd-IgA1 (1st hit), as well as endogenous anti-glycan antibodies (2nd hit) and subsequent immune complexes formation (3rd hit) and glomerular depositions (4th hit) (2).

The poor prognosis of IgAN is partly due to the delayed diagnosis. The renal biopsy is the gold standard for diagnosis as well as assessment of disease activity and prognosis of IgAN. However, different timing of intervention of renal biopsy may yield variable pathological severity and chronicity on renal biopsy due to the different indication of renal biopsy in each country (3). These observations emphasize that renal biopsy is just a snap-shot, and thus has a limitation in the assessment of disease activity.

The degree of proteinuria is one of most important prognostic factors not only in IgAN but also in all renal diseases at present (4), and there have been a substantial number of clinical studies on renal disease in which both a decrease in kidney function and proteinuria have been considered as an endpoint. Therefore, many clinical guidelines recommend therapeutic indication based on the degree of proteinuria. However, it is difficult to distinguish proteinuria from acute glomerular inflammatory lesions or chronic lesions. In general, IgAN have a long chronic clinical course with combination of such acute inflammatory lesions and the common pathway based on chronic lesions. Therefore, it is hard to qualitatively discriminate proteinuria at acute phase and chronic phase, suggesting the limitation of proteinuria-based assessment of the disease activity. Taken together, disease activity assessment methods other than renal biopsy and urinalysis are needed.

Serum level of Gd-IgA1 that provided a 0.77 sensitivity and a specificity of 0.90 to distinguish IgAN patients from healthy controls by receiver operating characteristic curve analysis (5). Moreover, serum levels of a proliferation inducing ligand (APRIL), in which recently applied for clinical trials, is elevated in patients with IgAN. Although those serum biomarkers may be useful for the diagnosis of IgAN, there are substantial overlap in serum levels between patients with IgAN, other renal diseases, and healthy controls (6). A fraction of Gd-IgA1 from the glomerular deposits is excreted into the urine and thus represents a disease-specific marker of IgAN. Urinary excretion of Gd-IgA1 discriminated patients with IgAN from patients with other renal diseases. Urinary Gd-IgA1 thus may represent a disease-specific marker of IgAN (7). However, no single biomarker was sufficiently specific for IgAN at present.

The above-described clinical findings suggest that Gd-IgA1 and its related immune complex containing with anti-glycan autoantibodies are essential effector molecules in the pathogenesis of IgAN. Recent studies demonstrated that increased Gd-IgA1 levels associated with progression of proteinuria and a greater risk of deterioration of renal function in IgAN (8). In addition, the combination of high serum Gd-IgA1 levels and circulating levels of advanced oxidation protein products were correlated with progression of renal injuries in IgAN (9). Berthoux *et al*. reported that serum levels of IgG autoantibodies specific for Gd-IgA1 at the time of renal biopsy were significantly associated with clinical progression of IgAN towards end stage of renal failure (10). Among individual IgAN patients, plasma levels of IgA-ICs correlate with the phases of clinical activity, as well as the degree of hematuria and proteinuria (11). Furthermore, serum levels of Gd-IgA1 specific IgG autoantibodies correlated with disease severity, as assessed by magnitude of proteinuria (12). Moreover, improvement of urinary abnormalities was well correlated with decreased serum levels of Gd-IgA1 and Gd-IgA1 containing immune complexes (11). Those findings indicate the possibility that evaluation of not only serum levels of the autoantigen (Gd-IgA1) but also those of autoantibodies against Gd-IgA1 or immune complexes should be required as disease markers for IgAN.

Annual checking system for urine analysis is well developed from childhood in Japan. Greater part of Japanese IgAN patients are initially discovered through chance hematuria. The renal biopsy is not recommended for patients presenting with isolated hematuria. Thus, there are still many patients who show delayed intervention of therapy, resulted in deterioration of renal function. Therefore, these biomarkers may be applicable to second screening to the examinees with hematuria in a general

checkup. Hematuria generally precede to proteinuria in IgAN, such new screening system with these biomarkers may change the importance of hematuria screening.

Although accurate individual risk stratification at diagnosis and predicting treatment response remains a challenge, noninvasive and real-time examination with biomarkers on the basis of pathogenesis is critical to elucidate the efficacy of treatment and disease activity. It is suggested that a panel of serum and urine biomarkers may be helpful in addition to a recently established IgAN risk prediction tool derived from an international cohort of IgAN patients. Moreover, for the early screening of potential IgAN with isolated hematuria, those biomarkers may be applicable for early intervention of therapy resulted in reduce the risk of deterioration of renal function.

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Targeting pathogenic IgA production

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Previous studies confirmed that glomerular IgA in IgA nephropathy (IgAN) are mainly mucosal type of polymeric IgA1. Pathological role of mucosa has been thus discussed for many years in this disease. Since transient exacerbation with or without gross hematuria after upper respiratory infection are hallmark manifestation of this disease, there is no doubt of the role of nasopharyngeal mucosa. However, which mucosa is more involve remains of debate because of some discrepancy in outcomes of clinical trials for treatments targeting mucosae. Another characteristic of the glomerular IgA in IgAN is aberrant glycosylation in hinge region of IgA1. It is now widely accepted that galactose-deficient IgA1 (Gd-IgA1) is a key effector molecule of this disease (1). The complement activity of IgA is much weaker than that of IgG and IgM; therefore, immune complex (IC) formation of GdIgA1 with IgG and/or IgM has been considered to be required for full initiation of glomerular inflammation. In fact, it has been known that the amount of IgG co-deposition in the glomeruli correlates with prognosis of this disease. However, while some doubts remained because the fluorescence staining showed no co-deposition of IgG in some cases of IgAN, a recent study using a highly sensitive nanobody antibody (Ab) showed co-deposition of IgG with glomerular IgA in all IgAN cases (2). It turns out that the amount of IgG deposition may regulate the severity of the inflammation. These clinical findings indicate that one of critical therapeutic targets should be mucosal type of GdIgA1 which has an affinity to glomerulus and an avidity to IgG/IgM for the IC formation.

APRIL and BAFF, known as TNF superfamily ligands play key roles in IgA class switch recombination in mucosa under mucosal activation of Toll-like receptors (TLR) which are transmembrane proteins sensing pathogen-associated molecular patterns. In the past decade, a large genome-wide association study, GWAS, on IgAN identified candidate genes for mucosal immunity and complement regulation factor (3, 4). One of the most important genes is TNFSF13A, which encodes APRIL. On the other hand, recent GWAS on tonsillectomy identified genes for BAFF (5), suggesting its role in hyper immune response in chronic tonsillitis, but not IgAN. Recent publications have reported the elevation of the serum and tonsillar levels of APRIL in patients with IgAN, which is associated with the serum levels of GdIgA1, worse prognosis and disease activity of this disease (6-8). In accordance with these clinical findings, preclinical studies with anti-APRIL Ab, but not anti-BAFF Ab, in spontaneous murine IgAN model (gddY) revealed improvement of serum IgA levels, reduced aberrantly glycosylated IgA levels and IC formation, and improved UP accompanied by the loss of glomerular IgA and C3 depositions (9, 10). Based on such results, international clinical trials with anti-APRIL Ab and anti-APRIL receptor Ab are ongoing.

Molecular mechanisms in which nephritogenic GdIgA1 IC in IgAN selectively deposits in glomerular mesangial area remain unclear, although this process may be another target of treatment. Previous study demonstrated that an increase in endogenous IgG Ab that recognizes Gd-IgA1 in IgAN patients. At least some of these IgG were autoantibodies with amino acid substitutions in the CDR3 region of the variable region (11). Recently other mechanism of IC formation with GdIgA1 is discussed. A molecule called apoptosis inhibitor of macrophage (AIM) is a functional molecule of macrophages that is involved in wide variety of pathologies such as liver cancer and AKI. Recent study revealed this molecule was found to co-deposit with glomerular IgA in all cases of IgAN (12). Interestingly, AIM knock out gddY mice by CRISPR Cas9 revealed no nephritis even with glomerular IgA deposition. This was understood to be because there was no co-deposition of IgG and IgM, and thus no inducement of complement activity. Conversely, when recombinant AIM was administered to this disease, co-deposition with IgG/IgM and C3 occurred immediately, causing nephritis. Currently, drugs are being developed based on the concept of promoting immune cell functions with AIM. Our recent study further confirmed striking elevation of IgA autoantibody against cellular component specifically expressed by glomerular mesangial cells not only in murine IgAN but also human

IgAN (paper in revision). It also confirmed an increase of plasma cell infiltration producing IgA against the component in the target organ 'kidney'. This autoimmune process may be also critical target of this disease.

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Key unmet needs identified following the KDIGO guideline review

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The KDIGO guidelines categorically state in a practice point that to date there are no validated diagnostic serum or urine biomarkers for IgA nephropathy (IgAN). Nevertheless, over the years, researchers have been keen in searching these markers for diagnosis and for introducing a more personalized therapy for IgAN.

The vast majority of adult IgAN patients coming to a nephrologist's attention are those with a slowly progressive course, mild to moderate proteinuria, persistent microhematuria and hypertension. Since non-specific modifiers such as uncontrolled hypertension and proteinuria significantly impact the disease course, it is indisputable that supportive measures targeting either of these processes should first be initialized in all IgAN patients at risk for progressive disease. Blood pressure increases at an early stage with a significant activation of the renin-angiotensin system (RAS) in the kidneys. Even patients who have apparently normal BP have a higher BP than matched healthy individuals and exhibit subtle cardiac changes.[1] The best evidence available is for blockade of the RAS using either ACE inhibitors or angiotensin receptor blockers (ARB) that should be initiated as first-line antihypertensives in all IgAN patients exhibiting proteinuria above 0.5 g/d, irrespective of whether they are hypertensive or not. This approach is strongly supported by a level 1B recommendation in the revised KDIGO guidelines. Indeed, BP control should primarily be performed using RAS blockers since retrospective registry data showed that IgAN patients treated with an ACE inhibitor to control BP had a better preservation of renal function than IgAN patients that were not treated with ACE inhibitors or ARBs.[2] Even if BP is well-controlled (i.e. systolic BP <120 mmHg in adult patients), RAS blockers should be uptitrated to the maximum tolerated dose aiming at further reduction of proteinuria.

Despite the large body of evidence that supports the use of RAS inhibitors in the vast majority of IgAN patients, there are some unmet needs that have not been addressed in the guideline. This comprises whether RAS blockade is beneficial in normotensive IgAN patients with only moderately increased proteinuria (i.e., around 0.5 g/day). Furthermore, it is not clear whether dual RAS blockade using the combination of an ACE inhibitor and an ARB exerts similar positive effects in IgAN patients as observed in other glomerular diseases. Smaller clinical trials had demonstrated additional antiproteinuric effects through a co-administration of losartan with ACE inhibitors, or of losartan with a direct renin inhibitor (DRI) in IgAN patients.[3] An extended observation of the STOP-IgAN cohort argued against a combined ARB/ACEi regimen.[4] Proteinuria at the end of the randomized, 3-year trial phase was even higher in our patients on dual RAS blocker therapy whereas overall renal outcomes were comparable between trial participants under single and those dual RAS blocker therapy. Another RAS-blocking therapy, the direct renin inhibitor aliskiren, has been shown to reduce proteinuria at 6 months by a further 26% and suppress serum IL-6 and TGF- β levels when given to IgAN patients with proteinuria >1 g/d despite optimized ARB treatment.[5] Nevertheless, aliskiren was not pursued further due to a high incidence of hyperkalemia and hypotension among type 2 diabetics with CKD.

Another emerging supportive approach not yet addressed in the KDIGO guidelines is the addition of a sodium-glucose cotransporter-2 (SGLT2) inhibitor. In the DAPA-CKD trial, 270 IgAN patients (mostly without concomitant diabetes) with a low median eGFR around 40 ml/min received dapagliflozin on top of a RAS inhibitor.[6] Renal outcome markedly improved and the hazard ratio for the renal endpoint (50% loss of eGFR, dialysis or death from a kidney disease-related or cardiovascular cause) was 0.29 (95% CI, 0.12, 0.73) compared to placebo. Limitations of that study were its post-hoc nature, relatively few patients reaching renal endpoints, and in particular an unusually bad outcome of the placebo group. More insight into the role of SGLT2 inhibitors in the treatment of IgAN will be provided by the ongoing EMPA-Kidney trial (NCT03594110).

Besides corticosteroid, and possibly MMF and hydroxychloroquine in Chinese patients, there is a high unmet medical need for more efficacious and safer therapies in patients at high risk for progressive kidney disease. Several phase III RCTs are still ongoing in IgAN. Essentially all trials include patients with an eGFR above 30 ml/min, proteinuria usually above 1 g/d and only after RAS blockade had been optimized for several weeks or months:

1. In the NEFIGARD trial (NCT03643965), patients are randomized to placebo or enteric release budesonide (Nefecon®) based on a phase II RCT, which demonstrated that this enteric corticosteroid reduced proteinuria and eGFR loss over one year in IgAN.[7]
2. In the ARTEMIS trial (NCT03608033), patients are randomized to placebo or repeated infusions of an antibody to MASP-2, the key enzyme regulating the activity of the mannose-binding lectin pathway of complement. In a small phase II RCT, this antibody had markedly reduced proteinuria in IgAN patients.[8]
3. There are more phase II or III clinical trials to evaluate the efficacy and safety of inhibiting other arms of the complement cascade in IgAN, for example, (i) alternative pathway – using LNP023, an orally available, small-molecule inhibitor of complement factor B (APPLAUSE trial; NCT04578834); and (ii) terminal converging pathway – using Ravulizumab, a C5 inhibitor (NCT04564339).

A new-class drug of dual acting ARB and endothelin receptor antagonist (ERA) emerged as a promising novel treatment for IgAN:

4. In the phase III PROTECT trial (NCT03762850), patients are randomized to receive irbesartan or sparsentan, a dual angiotensin-II and endothelin-1 receptor blocker, based on a phase II trial in patients with focal segmental glomerulosclerosis.[9]
5. Similar to PROTECT, ALIGN (NCT04573478) also targets endothelin-1 using the specific endothelin-A receptor blocker atrasentan.

Which of these approaches is the best and safest one, and whether ultimately combinations may be useful in high risk IgAN patients should become clear in the near future.

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IgA nephropathy. A South American Perspective

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South America has an area of 17,840,000 square kilometers. Its population is estimated at more than 423 million, and ranks fifth in population (after Asia, Africa, Europe, and North America). Brazil is by far the most populous South American country, with more than half of the continent's population, followed by Colombia, Argentina, Venezuela and Peru. South America is politically divided into 13 countries. The population is formed by descendants of Europeans (mainly Spaniards, Portuguese and Italians) Africans and Amerindians. There is a high percentage of Hispanics (mixed European and Amerindian) that vary greatly in composition by place. There is also a minor population of Asians, especially in Brazil, Peru, and Argentina. Genetic admixture occurs at very high levels in South America. In Argentina, the European influence accounts for 85% of the genetic background, Hispanics for 17-31% and sub-Saharan African for 2-4%, while for instance in Colombia, the sub-Saharan African genetic background may vary from 1% to 89%, while the European one varies from 20%-79%, depending on the region. People from European descent, represents the majority of the population in Argentina (85%), Uruguay (68%), Chile (64.7%) and 48.4% in Brazil. Hispanics are the largest ethnic group in Bolivia, Paraguay, Venezuela, Colombia and Ecuador and the second group in Peru and Chile. South America is also home to one of the largest populations of Africans. This group is significantly present in Brazil, Colombia, Guyana, Suriname, French Guiana, Venezuela and Ecuador. Brazil, Peru and Argentina have the largest Japanese, Korean and Chinese communities.

In this panorama, the prevalence of IgA Nephropathy (IgAN) may depend on the particular ethnic and environmental factors that the continent presents. However, official data us scant. In general, there is no national registry on chronic kidney diseases nor official data about the prevalence of IgAN. Most of the data belong to single-center registries. According to our experience at the British Hospital in Buenos Aires, in the period 1995-2020, 1089 adult kidney biopsies were performed on native kidneys due to abnormalities in urinalysis and/or kidney function. IgAN ranked as the second most common cause of diagnosed glomerular disease after secondary focal and segmental glomerulosclerosis, representing 14.6% of all biopsies (n=159). Among all the biopsied glomerulopathies, the prevalence remained steady: In years 1996-2000 it was 15% while in years 2016-2020 it was 15.8%. The most frequent indications of kidney biopsy due to IgAN were: Asymptomatic urinary abnormalities (85%), nephrotic range proteinuria (10%), nephrotic syndrome (4.5%) and rapidly progressive glomerulonephritis (0.5%). As to gender, 77 females (48%), median age 39.41 (18-60) years and 82 males (52%), median age 33.4 (18-71) years at the time of biopsy comprised this cohort. Median estimated glomerular filtration rate was 54 (31-120) mL/min and proteinuria 1.6 (0.4-4.1) g/day. Twelve percent of patients were hypertensives (8% males vs 4% females). The most common pattern of Oxford score encountered was M1E0S1T1-C0. All patients were on RAS blockade, and 37% required immunosuppression due to proteinuria > 1g/day despite nephroprotection measures. At 25 years of follow-up, 23 subjects (14.4%, 14 males and 9 females) required renal replacement therapy. In the Uruguay glomerular disease registry, in the period 1990-2014, from a general perspective, IgAN was also the second cause of glomerular disease, following focal and segmental glomerulosclerosis. However, in the periods 2005-2009 and 2010-2014, IgA nephropathy became the glomerulopathy with the highest incidence rate, 12.11 and 12.53 cases pmp/yr, respectively. In the same two period focal and segmental glomerulosclerosis incidence rate decreased to 6.93 and 7.66 cases pmp/yr, respectively. The most common indication that led to the diagnosis of IgAN was “asymptomatic urinary abnormalities”. IgAN was most frequently diagnosed between ages 18 and 49 (mean age 33.8 years).

Since the 15th International Symposium on IgAN organized by the International IgAN Network held in Buenos Aires in 2018, the advent of industry-sponsored protocols to the region permitted Argentina and Brazil to take part in randomized controlled trials in IgAN for the first time in history. This milestone has allowed participating local centers not only to be starring cutting-edge studies, but also to develop local educational and logistic resources to qualify for global good clinical practice standards for the good of patients. In the last 3 years, over 20 patients from Argentina and 4 from Brazil have participated in several phase II and phase III randomized controlled trials on IgAN. To date, IgAN labeled drugs that have been or are being assessed include budesonide and iptacopan, while three more protocols are under local approval for due evaluation in the clinical setting in Argentina and Brazil.

Starting in 1988, South-American publications related to basic research, clinical investigations and case reports in IgAN indexed in PubMed excluding kidney transplantation includes 53 from Brazil and 23 from Argentina. However, in the last 5 years, most of the publications are related to multicentric international co-operative studies, networks and working groups.

A dual retrospective and prospective local nation data base is under development in Argentina for glomerular disease registration. The potentiality of South America in clinical research is enormous. While Argentina and Brazil have been the first nations to assess targeted drugs for IgAN, it is necessary and challenging to spread randomized controlled trials to the rest of the countries of the region.

Racial Differences in Lectin Pathway Inhibition

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IgA nephropathy (IgAN) is the most common primary glomerular disease worldwide and remains a leading cause of chronic kidney disease and end-stage kidney disease (ESKD). The diagnosis of IgAN depends on renal biopsy with a demonstration of predominant IgA1 deposition in the glomerular mesangium. However, a wide spectrum of clinical and pathologic features of IgAN have been observed, as well as the variable treatment responses, implying whether IgAN may not be the same disease across the world.

More specifically, IgAN is most prevalent in Asians, followed by Caucasians and relatively rare in Africans. More severe clinical presentation and higher risk of disease progression have been reported in Asians than Europeans. Moreover, active lesions, such as endocapillary hypercellularity and crescents, are more commonly reported in Asians than Europeans. Response to corticosteroids/immunosuppression therapy is variably reported, with greater apparent efficacy reported in the studies of Asians than Europeans.

Although a multi-hit hypothesis has been suggested for IgAN, the relative importance of each "hit" may vary in different ethnic populations and this variation may underlie the differences in presentation of IgAN. For example, increased serum levels of Gd-IgA1 have been reported in up to 90% of patients with IgAN from different cohorts across the globe. However, there is a significant overlap in Gd-IgA1 levels between patients with IgAN and healthy subjects. Many, but not all, patients with IgAN have elevated levels of Gd-IgA1 and many unaffected relatives of patients with IgAN have elevated levels of Gd-IgA1, indicating that Gd-IgA1 while potentially important in the pathogenesis of IgAN, is insufficient by itself to cause disease. Most importantly, although Gd-IgA1 levels are elevated in IgAN patients compared to population matched healthy subjects both in Chinese and Europeans, Gd-IgA1 levels in Chinese IgAN patients are lower than those found in European healthy controls, suggesting that the regulation and response to Gd-IgA1 may be different between Chinese and European patients.

Complement activation plays an important role in the development and progression of IgAN. Complement component C3 frequently co-localizes with IgA, while C1q is rarely observed, suggesting activation of complement mainly via the alternative pathway or lectin pathway in IgAN. Moreover, the presence of mannan-binding lectin (MBL) with IgA, C3, and C4d is consistent with activation of the lectin pathway in patients with IgAN. With regards to lectin pathway activation, deposition of C4d, MBL, and MASP1-3, is commonly seen in kidney biopsies of IgAN patients, associating with disease severity. MBL deposits, found in about 25–35% of IgAN patients in Caucasian cohort, have been associated with higher proteinuria, lower eGFR, and more severe histopathological lesions. In a Chinese study of 162 patients with IgAN, urinary MBL levels were significantly associated with impaired renal function and more proteinuria. A study that measured MBL2 variants and MBL levels in 749 patients with IgAN and 489 healthy controls from a Chinese cohort also found that in patients with IgAN, MBL deficiency had a higher incidence of prodromic infections and gross hematuria than those with sufficient MBL levels (100-3540 ng/ml), and that patients with high MBL levels (>3540 ng/ml) had more severe proteinuria and a higher proportion of crescents, which suggests that MBL contributes to IgAN pathogenesis through multiple mechanisms. More recently, a meta-analysis regarding glomerular C4d deposition and kidney disease progression in IgAN showed a higher risk ratio of C4d deposition in Asians than in Caucasians, and glomerular C4d deposits were associated with severe clinical and pathological characteristics. This data is consistent with the findings of the increased proportions of inflammatory lesions (endocapillary hypercellularity and/or the presence of crescents) and microangiopathic lesion in Asian patients with IgAN. It might be one of the reasons that the Asian populations with IgAN showed a higher risk of ESKD progression than Caucasian populations, because crescents and arteriolar microangiopathic lesion are independent risk factors of prognosis of IgAN.

To summarize, the wide spectrum of clinical-pathological presentation of IgAN indicates that IgAN might not be a single disease. The relative importance of each "hit", including the complement activation, may vary in different ethnic populations and this variation underlies the differences in presentation. Regarding the racial difference in complement activation, a fundamental difference in the propensity to complement mediated inflammation and glomerular injury may underlie the more severe clinical and pathological patterns in Asian IgAN population. In the future, a better understanding of pathogenic pathways operating in different ethnic populations and the discovery of better biomarkers may help more precise targeting treatment for IgAN.

FREE COMMUNICATIONS

Impact of VIS649, an APRIL-Neutralizing IgG2 Monoclonal Antibody, on Tetanus- and Diphtheria-Toxoid Vaccination-Elicited Immune Responses in Healthy Volunteers: Phase 1, Randomized, Double-Blind, Placebo-Controlled Study

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Background: BesidesVIS649, a humanized IgG_2 monoclonal antibody that binds to and blocks APRIL, is in development as a potential treatment for IgA nephropathy. The aim of this analysis was to determine if VIS649 suppression of APRIL influences antibody boost responses to tetanus and diphtheria toxoid vaccination. **Methods:** In the vaccination cohort of this Phase 1, randomized, double-blind, placebo-controlled, single ascending dose study (NCT03719443), healthy volunteers received intravenous VIS649 6.0 mg/kg or placebo (2:1 ratio), followed by tetanus/diphtheria toxoid (TENIVAC®) vaccine. Study drug was administered on Day 1; discharge was on Day 2; vaccine was administered at Week 4; participants were followed to Week 16. Blood samples were taken, and anti-toxoid IgG, IgM and IgA quantitative ELISA assays were performed. **Results:** Of 15 participants included, 14 completed the study. Following immunization, tetanus anti-toxoid IgG titers increased: mean 7.9-fold and 6.4-fold at Week 6 for placebo and VIS649 recipients, respectively. Thereafter, tetanus anti-toxoid IgG titers declined but remained above the protective threshold of 0.1 IU/mL for all participants throughout the study. Similar trends were observed for diphtheria anti-toxoid IgG titers: mean 5.5-fold and 5.1-fold at Week 6 for placebo and VIS649 recipients, respectively. There was no evidence of tetanus- or diphtheria-toxoid elicited IgM responses. In a *post-hoc* analysis, pre-existing serum tetanus/diphtheria anti-toxoid IgA titers fell between Day 1 and Week 4 in the VIS649 group. Titers were consistent with the overall suppression of total serum IgA, were boosted after vaccination in both groups, and declined faster in the VIS649 recipients thereafter. **Conclusions:** VIS649 administration did not interfere with participants' ability to mount an antigen-specific serum IgG or IgA boost response to tetanus/diphtheria toxoid vaccination. Consistent with recall vaccination exposure, there was no evidence of tetanus- or diphtheria-specific IgM responses. These data indicate that qualitative antibody responses are preserved during APRIL suppression. **Keywords:** VIS649, APRIL, Phase 1 study

Interim Results of a Phase 1/2 Trial to Investigate the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Clinical Activity of BION-1301 in Patients with IgA Nephropathy

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Background and Aims: IgA nephropathy (IgAN) is the most common primary glomerulonephritis globally, with up to 45% of IgAN patients at risk of progressing to ESKD. There are currently no approved treatments specifically for IgA nephropathy. The initiating step in IgAN pathogenesis is the excess production of galactose-deficient IgA1 (Gd-IgA1) resulting in the formation of pathogenic immune complexes that cause kidney inflammation and damage. A Proliferation-Inducing Ligand (APRIL), a TNF-superfamily cytokine, is elevated in IgAN patients and correlated with higher levels of Gd-IgA1 and proteinuria, and lower eGFR. BION-1301 is a novel monoclonal antibody which binds and blocks APRIL. Here we present interim results from Part 3 of a Phase 1/2 study that characterizes the safety, PK, PD and preliminary activity of BION-1301 delivered by IV administration in IgAN patients. Final data from Parts 1 and 2 in healthy volunteers were presented at ASN in 2020. **Methods:** Part 3 of this study (NCT03945318) is an ongoing multicenter, multicohort, open-label study in up to 40 IgAN patients. BION-1301 is being delivered IV at 450mg every 2 weeks, or SC (dose and schedule TBD), for 12 months. Key eligibility criteria include: (1) urine protein ≥0.5 g/24h, (2) stable/optimized dose of ACE-I/ARB (or intolerant), and (3) biopsy-verified diagnosis of IgAN within 10 years. **Results:** BION-1301 has been well tolerated in IgAN patients receiving IV administration to date with no SAEs observed. Durable reductions in serum free APRIL and immunoglobulins and Gd-IgA1 were observed, along with clinically meaningful reductions in proteinuria as early as 12 weeks. **Conclusion:** BION-1301 is a novel anti-APRIL monoclonal antibody being developed as a potential treatment for patients with IgAN. BION-1301 offers disease modifying potential by directly targeting the pathogenesis of IgAN. Promising early biomarker and clinical activity support the continued development of BION-1301 in IgAN. **Keywords:** BION-1301, IgA Nephropathy, Gd-IgA1, APRIL

Human Mesangial Cell Activation Induced by Endothelin-1 or IgA Nephropathy Patient-Derived Immune Complexes is Blocked by Selective ETA Antagonist Atrasentan

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Background/Objectives: Mesangial cell (MC) activation in response to IgA-immune complexes is considered the initiating intra-renal event in the pathogenesis of IgAN. However, the molecular mechanisms responsible have not been well defined. The objective of these studies was to determine the role of the endothelin A (ET_A) receptor in MC activation in response to ET-1 and IgAN patient-derived immune complexes, by using the potent and selective ET_A antagonist atrasentan. **Methods:** Primary human renal MCs in culture were treated with ET-1 at varying concentrations for up to 72 hours in the presence or absence of atrasentan. Cellular proliferation and cytokine secretion were measured, and global transcriptional responses were characterized by RNA sequencing. In addition, IgA-containing immune complexes were purified by jacalin-agarose affinity chromatography from the serum of either IgAN patients or matched healthy controls and used to stimulate cultured human MCs with or without atrasentan. **Results:** ET-1 directly stimulated human MC proliferation and IL-6 secretion, which was blocked by atrasentan in a concentration-dependent manner. RNA sequencing and gene set enrichment analysis revealed hallmarks of MC activation with ET-1 treatment including up-regulation of cell proliferation, pro-inflammatory and pro-fibrotic networks, which were blocked by atrasentan. MCs exposed to IgA-immune complexes purified from the serum of IgAN patients demonstrated hyper-proliferation relative to complexes from healthy controls (5.1 ± 0.13-fold, *P<0.01*), which was significantly attenuated by atrasentan (57 ± 6%, *P<0.01*). **Conclusion:** These studies suggest an important pathogenic role of the ET_A receptor in producing hallmark characteristics of MC activation including proliferation, inflammation and fibrosis, which can be blocked by the ETA antagonist atrasentan. These results support the therapeutic potential of atrasentan in IgAN patients, not only via its well characterized effect to reduce proteinuria, but also by potentially reducing mesangial cell activation, the initiating intra-renal event in IgAN. **Keywords:** Atrasentan, Endothelin, Mesangial Cell

IgA-Complement immune complexes: A novel mechanism for the delivery of complement proteins to the glomerulus in IgA nephropathy

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Mesangial co-localisation of proteins from the lectin and alternative complement pathways with IgA has been reported in IgA nephropathy, and complement activation has been associated with worse clinical outcome. The origin of these complement proteins is unclear. Resident glomerular cells are capable of synthesising some complement proteins, and it has been proposed that deposited IgA containing immune complexes (IC) are capable of fixing soluble plasma complement proteins *in situ* in the glomerulus. We wished to determine whether complement proteins could be delivered to the glomerulus as part of IgA-ICs in IgAN. Novel ELISAs were established to measure serum levels of ICs containing IgA and components of the lectin and alternative complement pathways. Levels of IgA- C2, -C3, -C9, -C5b-9, -MBL, -ficolin 1, -ficolin 2, -ficolin 3, -collectin 11, -collectin10, -MASP2, -MASP 1, -Factor H (FH), -Factor H related protein 4 (FHR4) and -properdin containing IC were measured in sera from 60 patients with biopsy proven IgAN, 60 patients with other kidney diseases and 60 healthy subjects. Data were analysed using one way ANOVA or student T Test and expressed as mean +/-SEM. Circulating ICs containing IgA and each of the complement components, except FH and FHR4, were detectable in sera of from all groups. Significantly higher levels of IC containing IgA -ficolin 2, -MASP2 and -C5b-9 were seen in sera from IgAN patients. Levels of IgA-C5b-9 IC were higher in patients with progressive IgAN compared to those with a more benign clinical phenotype. Our findings support the hypothesis that in IgAN complement components are being delivered to the glomerulus integrated within IgA-IC. Importantly, the key serine protease of the lectin pathway, MASP-2, appears to be preferentially associated with circulating IgA-IC in IgAN, and may be disproportionately transported to the kidney, reinforcing the likely importance of lectin pathway dysregulation in IgAN. **Keywords:** IgA Nephropathy, Complement, Lectin pathway

Molecular Phenotyping of aberrant IgA1 O-glycosylation in serum of patients with IgA nephropathy

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Background: Patients with IgA nephropathy (IgAN) develop characteristic glomerular immunodeposits containing IgA that is enriched for IgA1 glycoforms with galactose-deficient hinge-region *O*-glycans (Gd-IgA1). Elevated serum levels of Gd-IgA1 in patients with IgAN have been well documented, suggesting a key role of Gd-IgA1 in disease pathogenesis. These observations suggest a potential prognostic role for a minimally invasive biomarker based on profiling serum/plasma IgA1 *O*-glycoforms. Here, we report a quantitative assessment of molecular IgA1 phenotype(s) in IgAN by profiling serum IgA1. **Methods:** Serum samples were collected from forty IgAN patients to form a training cohort based on high or low serum levels of Gd-IgA1. Isolation of IgA1 from sera is based on lectin-affinity chromatography followed by size-exclusion chromatography to separate IgA1 monomeric and polymeric forms and IgA1 bound in immune complexes. IgA1 *O*-glycosylation was analyzed by liquid chromatography-high-resolution mass spectrometry (LC-MS) using LTQ Orbitrap Velos MS. **Results:** Molecular phenotyping of IgA1 was performed using our established quantitative LC-MS *O*-glycoform profiling methodology. Standardization with internal calibration was implemented for LC-MS *O*-glycosylation profiles to allow comparison of results. The LC-MS analysis revealed variations in abundance of individual IgA1 *O*-glycoforms in the tested samples. Using the measured quantitative values for 12-15 IgA1 glycoform, the overall extent of galactose-deficiency is expressed as a ratio of all Gd-IgA1 glycoforms over a subset of "normalizing" galactose-complete IgA1 *O*-glycoforms. Using this ratio, samples fell into the expected ranges as patients with high versus low serum Gd-IgA1 were distinguished. **Conclusion:** Quantitative profiling of IgA1 clustered *O*-glycosylation can determine molecular IgA1 phenotype(s) and identify IgA1 glycoforms as biomarkers related to disease pathogenesis. These approaches are applicable to differential profiling of IgA1 from IgAN patients with high versus low serum Gd-IgA1 to identify pathogenic IgA1 glycoforms involved in the formation of nephritogenic immune complexes and distinguish patients at risk of disease progression. **Keywords:** Mass Spectrometry, Immune complexes, Quantitative profiling, Molecular phenotyping

Pathogenetic mechanisms involved in hematuria bouts after respiratory tract infections in IgA nephropathy

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Background: Hematuria is a common finding in patients with IgAN, mainly occurring after respiratory infections. However, the mechanisms involved in glomerular egression of erythrocytes into the urinary space are unknown. To answer this question, we induced a respiratory infection in a humanized experimental model of IgAN, the α1KICD89tg mice that express human IgA1 and its CD89 receptor. **Methods:** α1KICD89tg mice (12 weeks old) received an intranasal instillation of *Streptococcus pneumoniae* (SP, 10^7 CFU). Blood, urine and renal samples were obtained 48h after post-SP instillation. Hematuria was quantified in the urinary sediment and renal function was determined by biochemical analysis. Human-IgA1 glomerular, complement deposition and infiltranted proinflammatory cells was examined by immunohistochemistry/immunofluorescence. Circulating leukocyte populations were studied on an hemocytometer. Renal inflammatory cytokines, metalloproteases, transcription factor and markers of tubular and glomerular damage were determined in kidneys by RT-PCR and western-blot. **Results/Discussion:** SP-instillation increased hematuria, albuminuria and proteinuria induced the expression of the tubular injury markers

N-GAL and KIM-1 in α1KICD89tg mice. SP-instillation decreased expression of the slit diaphragm proteins (nephrin, synaptopodin), reduced collagen-IV content, promoted glomerular accumulation of IgA1 and proteins of complement system (C3, MBL), and increased Nrf2 activation in the glomerulus. We also observed increased number of interstitial F4/80+ macrophages, higher expression of MMP-9 and pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, CCL-2, CCL5) as well as p65/NF-κB activation in the renal tissue. Notably, increased peripheral neutrophils levels were observed in the SP-infected α1KICD89tg mice. Neutrophil depletion with anti-Ly6G mAb (200 µg/kg i.p) reduced SP-mediated hematuria, albuminuria and proteinuria, prevented loss of synaptopodin and nephrin, decreased renal inflammation and MMP-9 expression in SP-infected α1KICD89tg mice. **Conclusion:** Hematuria bouts following respiratory tract infections in the α1KICD89tg mice are caused by a neutrophil-mediated alteration of the glomerular filtration barrier leading to podocyte damage, complement deposition and loss of Collagen IV. **Keywords:** Hematuria, IgAN, MMP-9, Neutrophils, Nrf2, NF-kB, Podocyte damage, Collagen IV

BAFF-dependent IgA production do not play a pivotal role in the pathogenesis of murine IgA nephropathy

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Backgrounds: IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide, but its pathogenesis has not yet clear understood. Recent studies suggested B cell activating factor belonging to the TNF family (BAFF), which participates in the activation of B cells and class switch of IgA, as a potential disease marker of IgAN. However, the role of BAFF in the IgAN remains unclear. In this study, we investigated pathological role of BAFF in IgAN using a grouped ddY mouse which is the spontaneous murine model of IgAN. **Methods:** Mice with IgAN designated grouped ddY were treated with PBS or anti-BAFF monoclonal antibody (anti-BAFF Ab) by intraperitoneal injection every three days for four weeks. We measured the levels of urinary albumin, serum immunoglobulins, and serum IgA-IgG immune complex at the beginning and end of the treatment. The levels of serum aberrantly glycosylated IgA were also measured using biotinylated Ricinus communis agglutinin-I and Sambucus nigra bark lectin. We further assessed glomerular depositions of IgA and C3 by immunofluorescence staining and analyzed changes of B cell population in spleen and bone marrow using flow cytometric analysis. **Results:** Anti-BAFF Ab treatment significantly decreased serum levels of IgA, IgG, and IgM as compared with PBS treatment in the murine IgAN model ($p < 0.001$, $p = 0.003$, and $p = 0.002$, respectively). However, it did not affect urinary albumin excretion, serum levels of IgA-IgG immune complex, and serum levels of aberrantly glycosylated IgA. Glomerular depositions of IgA and C3 as well as B cell population in spleen and bone marrow were also not affected by anti-BAFF Ab treatment. **Conclusion:** Anti-BAFF Ab treatment was effective to inhibit the production of immunoglobulins, but not nephritogenic IgA in murine IgAN model. Our results suggest that BAFF-dependent IgA production may not play a pivotal role in the pathogenesis of IgAN. **Keywords:** IgA nephropathy, B cell activating factor belonging to the TNF family, Disease marker

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Selective ETA Antagonist Atrasentan, Rapidly Reduces Albuminuria and Downregulates Intra-renal Pro-Inflammatory and Pro-Fibrotic Transcriptional Networks in the gddY Mouse Model of Spontaneous IgA Nephropathy

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Background/Objectives: Endothelin pathway activation has been observed in kidney biopsies from IgAN patients and may be an important driver of disease progression via ETA receptor activation. The objective of this study was to evaluate the effect of short-term treatment of the potent and selective ETA antagonist atrasentan in the gddY mouse model of spontaneous IgAN, with a focus on dynamic changes in the intra-renal transcriptional profile. **Methods:** 6-week-old male gddY mice were administered atrasentan (0, 10, 20 or 30 mg/kg/day) via drinking water for 5 days. Urine albumin to creatinine ratio (UACR) was measured at baseline and on Day 4 of treatment. Kidney cortex was flash-frozen on Day 5 for RNA-seq which was analyzed by pairwise differential gene expression using edgeR's quasi-likelihood F-test (FDR adjusted p-value < 0.05). Gene set enrichment analysis (GSEA) to define hallmark pathways was performed and cross-validated to the transcriptome of kidney biopsies from IgAN patients. **Results:** Atrasentan reduced UACR from baseline by 28 ± 44 % (*p=ns*), 62 ± 8 % (p=0.0498) and 63 ± 6 %

(P=0.029) at 10, 20 and 30 mg/kg/day, respectively. High quality RNA-seq reads revealed dose-dependent effects of atrasentan on differential gene expression, including reductions in ETA target genes. GSEA demonstrated that atrasentan resulted in a down-regulation of intra-renal gene pathways associated with proliferation, inflammation and fibrosis and an up-regulation of oxidative metabolism, in the gddY mouse, reversing hallmark gene pathways observed to be dysregulated in the glomeruli of IgAN patient biopsies. **Conclusion:** In the gddY mouse model of IgAN, selectively blocking ETA with atrasentan leads to rapid reductions in albuminuria and results in intra-renal transcriptional downregulation of inflammatory and fibrotic signaling. These results support the therapeutic potential of atrasentan in IgAN patients to reduce proteinuria and kidney inflammation and fibrosis, key drivers of IgAN progression. **Keywords:** Atrasentan, Endothelin, gddY mouse

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Impact of polyclonal anti-T-lymphocyte immunoglobulins on the recurrence of IgA nephropathy after kidney transplantation: The PIRAT study

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Background and Aims: Polyclonal anti-T-lymphocyte antibodies (PATLA) immunosuppressive induction has been shown to be associated with a lower rate of IgAN recurrence compared to basiliximab after transplantation. The aim of the PIRAT study was to compare an induction by PATLA *versus* basiliximab by the mean of a randomized controlled trial. **Method:** Adults with primary IgAN, first transplantation, PRA <50% could be included in the study. Patients were randomized 1:1 to receive either PATLA (Grafalon, 18mg/kg) or basiliximab (20mg at transplantation and 4 days after). Both groups received corticoids for at least one year, tacrolimus and mycophenolic acid. Primary outcome was the clinico-histological recurrence (CHR: IgA deposition on transplant biopsy and albuminuria>300mg/d) during 5 years post-transplantation. Protocol biopsy at 5 years was highly recommended. **Results:** 117 patients were finally included in 13 French transplant centers, with 60 patients in the PATLA group and 57 in the basiliximab control group. Both groups were similar (median, PATLA vs. basiliximab, p>0.05 wilcoxon test) in term of sex ratio (4.45 vs 4.57), recipient age (47.9 vs. 47.7 years old), proportion of living donors (33% vs. 25%). Overall proportion of patients with one biopsy was 63% vs. 66%. A trend in favor to the protection by PATLA from the CHR was found (hazard ratio 0,35 [0.11–1.1], p=0.082). Biopsy proven histological recurrence was significantly lower after PATLA induction (HR 0.34 [0.16–0.76], p=0.0079). PATLA group experienced more infections (40 vs. 28 p=0.06, a lower number of biopsy-proven acute rejections (5 vs 10, p=0.17). **Conclusion:** PATLA for immunosuppressive induction was found protective from the recurrence of IgA deposition during the first 5 years after transplantation, compared to basiliximab. A similar trend, although not significant, was found about the clinico-histological recurrence which was the predefined primary outcome. **Keywords:** Kidney Transplantation, IgA nephropathy, Recurrence on transplant, Immunosuppression

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A novel ELISA method to measure levels of IgA1-specific IgG antibodies

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Background/Aims/Objectives: Increased levels of poorly *O*-galactosylated IgA1 (gdIgA1) play a central role in the pathogenesis of IgA nephropathy (IgAN). According to the multi-hit hypothesis, gdIgA1 molecules are recognised by IgA1-specific IgG and IgA autoantibodies leading to the formation of immune-complexes. The aim of this study was to develop a novel ELISA to measure serum levels of IgG molecules specific for an intact gdIgA1 with pathogenic capabilities, and to determine if these

levels could be used as a biomarker in IgAN. **Methods:** A novel ELISA was developed using as the capture antigen an intact IgA1 paraprotein which displayed high HPA lectin binding. This patient had developed a rapidly progressive glomerulonephritis which on kidney biopsy was associated with a crescentic GN with marked mesangial IgA, IgG, C3 deposition. Serum samples from 91 IgAN patients, 80 healthy subjects and 58 non-IgAN kidney disease controls were analysed. In parallel, the kidney biopsy reports were examined of the IgAN cases to evaluate IgG and or C3 co-deposition. Analysis of normally-distributed data was performed using one-way ANOVA, student's T test or Pearson's correlation and Kruksal-Wallis, Mann-Whitney tests or Spearmans's correlation were used when the data was non-normally distribute. **Results/Discussion**: No difference was seen between levels of gdIgA1-specific IgG in the cohorts. However, a significant positive correlation was observed between gdIgA1-specific IgG and IgG-IgA immune complexes which was specific for IgAN. Only 14% of the IgAN cases had IgG co-deposition in kidney biopsy, and these patients did not have elevated serum levels of gdIgA1-specific IgG. **Conclusion:** Serum levels of gdIgA1-specific IgG measured using this novel ELISA cannot be considered a biomarker in IgAN. However, this data suggests these antibodies specifically participate in the formation of immune complex formation and that they may play a pathogenic role in some IgAN patients. **Keywords:** gdIgA1, gdIgA1-specific IgG, ELISA, serum

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Analysis of the effect of TRF-budesonide (Nefecon) on urinary sCD163 in patients with IgAN from the Phase 2 NEFIGAN trial

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Background: CD163 is a hemoglobin scavenger receptor involved in limiting oxidative heme toxicity and is mostly expressed by monocytes and macrophages. The soluble form (sCD163) is recognized as a marker of M2 macrophage activation and is a potential biomarker for inflammation or disease. The exact role of CD163 and both plasma and urine sCD163 in IgA nephropathy (IgAN) is unknown. Studies have shown elevated sCD163 plasma levels in patients with IgAN and increased CD163+ macrophage infiltration in glomeruli of patients with crescentic IgAN, which suggests CD163+ infiltration may be a determinant of renal outcomes in IgAN. Herein, we assessed the effects of a novel, targeted-release investigational formulation of budesonide (TRF-budesonide [Nefecon]) on urine sCD163 and sCD163/urine creatinine levels in patients with IgAN enrolled in the Phase 2 NEFIGAN trial. **Methods:** sCD163 and sCD163/urine creatinine levels were measured using an enzyme- linked immunosorbent assay (ELISA) in 439 urine samples collected as part of the NEFIGAN trial. Urine samples for exploratory biomarker analysis were obtained at time of randomization, following 9 months of treatment with randomized therapy (placebo, TRF-budesonide 8 or 16 mg/day) and on completion of the study (12 months). Changes in biomarker levels were analyzed using log-transformed data and analysis of covariance. Statistical significance was set as $p<0.05$. **Results:** A significant reduction in urine sCD163 levels was observed with both doses of TRF-budesonide at both end of treatment and end of study compared with baseline. Only TRF-budesonide 16 mg/day resulted in a significant decrease in the ratio of sCD163/urine creatinine at both 9 and 12 months compared with baseline. **Conclusion:** These findings show that TRF-budesonide (Nefecon), which is designed to target the Peyer's patches in the gut, significantly reduces urine levels of a biomarker associated with inflammation (sCD163) in IgAN. **Keywords:** IgA nephropathy, soluble CD163, urine creatinine, inflammation, biomarker, Peyer's patches

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eGFR slope at 1 year may independently predict clinical benefit in patients with IgA nephropathy

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Background: Recent meta-analyses have separately explored the relationship of treatment effects on proteinuria reduction and rate of eGFR decline at 1 year with clinical outcomes in patients with IgAN. In this meta-analysis, we assess whether these endpoints are independently related to clinical outcomes. **Methods:** Study level data from 1299 patients in 13 IgAN studies, aggregated into 8 study groupings, were obtained from the TESTING study, STOP-IgAN (Rauen 2020) and a meta-analysis of the remaining studies published in 2019 (Inker & Heerspink). The relationship between endpoints was analysed using a 2-stage random effects model. In the first stage, separate empirical Bayes estimates (EBEs) of treatment effects were calculated for each endpoint per study and expressed as log(ratio) of geometric means for proteinuria at 1 year, difference in arithmetic means for 1-year eGFR slope and log hazard ratio for clinical outcome. In the second stage, the EBEs for clinical outcome, weighted by study size, were regressed on the EBE for proteinuria and eGFR slope at 1 year. The independent contribution of each 1-year endpoint was assessed using a model including the EBE for both endpoints. Clinical outcome was defined as doubling of serum creatinine, eGFR <15 mL/min/1.73 m^2 and ESRD, with minor modifications for the STOP-IgAN and TESTING

studies. **Results**: Separate analyses of EBE of 1-year proteinuria and 1-year eGFR slope with EBE of clinical outcome confirmed previously reported relationships: proteinuria (p=0.052) and eGFR slope (p=0.001). EBE for 1-year proteinuria and eGFR slope were highly correlated (r=-0.81). In a multivariate regression model including EBE for both endpoints, eGFR independently predicted clinical outcome (p=0.006), but proteinuria did not (p=0.174). Similar results were observed when using 6-month proteinuria. **Conclusion:** The relationship between clinical outcome and intermediate endpoints of proteinuria and eGFR slope at 1 year is only independently driven by treatment effects on eGFR. **Keywords:** IgAN, eGFR, proteinuria, clinical outcome

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Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of VIS649, an APRIL-Neutralizing IgG2 Monoclonal Antibody, in Healthy Volunteers: Phase 1, Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose Study

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Background: APRIL may play a role in the pathogenesis of IgA nephropathy. VIS649 is a humanized IgG2 monoclonal antibody that binds to and blocks APRIL. This study evaluated safety and tolerability, and characterized the PK/PD of VIS649. **Methods:** This was a Phase 1, randomized, double-blind, placebo-controlled, single ascending dose study (NCT03719443). Sequential cohorts (0.5, 2.0, 6.0, and 12.0 mg/kg) each enrolled 9 healthy adult volunteers (4 Japanese, 5 non-Japanese) who received VIS649 or placebo (7:2 ratio). A fifth cohort enrolled 15 participants who received VIS649 6.0 mg/kg or placebo (10:5), followed by tetanus/diphtheria vaccine (TENIVAC®) challenge after 28 days. Study drug was administered intravenously on Day 1; discharge was on Day 2; participants were followed for 16–24 weeks. Safety assessments and blood sampling for PK/PD were performed. **Results:** Of 51 participants included, 47 (92.2%) completed the study. VIS649 was well tolerated, with no serious AEs. Most TEAEs were mild; their incidence and severity were not dose-dependent. No clinically relevant effects were observed on laboratory tests, vital signs, electrocardiogram parameters, or physical examinations. VIS649 had non-linear PK: t½ increased with dose; AUC increased in a greater than dose-proportional manner. Following VIS649 administration, serum IgA, galactose-deficient (Gd)-IgA1, IgG, and IgM were reversibly suppressed in a dose-dependent manner. Mean free (non-VIS649 bound) serum APRIL decreased to the LLQ at Week 1, and time-to-recovery showed a dose-response. Circulating lymphocytes were not depleted. There were no significant PK/PD differences between Japanese and non-Japanese participants. **Conclusions:** Single intravenous doses of VIS649, up to 12.0 mg/kg, were safe and well tolerated in healthy adults and suppressed free serum APRIL. Serum Gd-IgA1 decreased in parallel with total serum IgA and recovered in a dose-dependent manner following reappearance of free APRIL in serum. These data support the further development of VIS649 as a potential treatment for IgA nephropathy. **Keywords:** VIS649, APRIL, Phase 1 study

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Low serum immunoglobulin G4 levels and its potential mechanism in IgA nephropathy

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Background: Low serum IgG4 levels in IgA nephropathy (IgAN) were noticed in our preliminary experiment. We aim to verify the low IgG4 levels, and investigate the possible mechanism and significance. **Methods:** IgAN patients (n = 112) and healthy controls (HC, n = 112) were enrolled. Patients with primary membranous nephropathy, minimal change disease, or lupus nephritis were selected as disease controls (DC, n = 122). Serum and urine IgG4 levels were detected. $IgG4^+B$ and Th2 cells in the peripheral blood were measured by flow cytometry. Human primary mesangial glomerular cells (HRMC) were cultured in vitro. IgA1, IgG4, and (IgA1 + IgG4) from IgAN and HC were used to stimulate HRMC. Cell proliferation, IL-6 and TGF-β1 secretion were detected. **Results:** IgG4 and IgG4/IgG in IgAN were lower than HC and DC (all $P < 0.001$). Severe and higher-risk IgAN patients displayed lower IgG4 levels (all $P < 0.05$). The cutoff value of IgG4 in differentiating IgAN from HC and DC was 0.26 mg/mL and 0.17 mg/mL (sensitivity 98.2% and 90.2%, specificity 82.4% and 85.2%, AUC 0.941 and 0.937, $P < 0.0001$), respectively. IgG4 in IgAN were negatively correlated with Gd-IgA1 levels ($P = 0.049$). $IgG4^+B/B$ cells and Th2/Th cells of IgAN were lower than HC (all $P < 0.05$). The urine IgG4 levels in IgAN were higher than HC, but were lower than DC (all $P < 0.001$). IgAN-IgA1 could significantly promote HRMC proliferation and secretion of IL-6 and TGF-β1, the cell proliferation and IL-6 and TGF-β1 secretion in the IgAN-IgA1 + IgAN-IgG4 group were significantly lower than those in the IgA1 group (all $P < 0.05$). **Conclusions**: Low serum IgG4 levels seemed to be a potential diagnostic biomarker for IgAN. Decreased IgG4+B cells and Th2 cells may contribute to the low IgG4 levels. IgG4 may exert protective effects in IgAN. **Keywords:** IgA nephropathy, IgG4, biomarker

INTERACTIVE POSTERS

A Phase 1/2, Multicenter Trial to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of BION-1301 in Healthy Volunteers and Adults with IgA Nephropathy

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Background and Aims: IgA nephropathy (IgAN) is the most common primary glomerulonephritis globally, with up to 45% of IgAN patients at risk of progressing to ESKD. There are currently no approved treatments specifically for IgA nephropathy. The initiating step in IgAN pathogenesis is the excess production of galactose-deficient IgA1 (Gd-IgA1) resulting in formation of pathogenic immune complexes that cause kidney inflammation and damage. A Proliferation-Inducing Ligand (APRIL), a TNF-superfamily cytokine, is elevated in IgAN patients and correlated with higher levels of Gd-IgA1 and proteinuria, and lower eGFR. BION-1301 is a novel monoclonal antibody which binds and blocks APRIL. The primary objective of Study ADU-CL-19 is to assess the safety and tolerability of BION-1301 in Healthy Volunteers (HV) and IgAN patients and to secondarily assess the PK, PD, immunogenicity, and preliminary clinical activity. **Methods:** The Phase 1/2 study (NCT03945318) comprises 3 parts. Parts 1 and 2 were blinded, placebo-controlled single and multiple ascending dose designs in HV and have been completed. Part 3 is a multicenter (US, UK, South Korea), multicohort, open-label study in up to 40 patients with IgAN. Patients in Cohort 1 receive BION-1301 at 450mg IV every 2 weeks for up to 1 year. Subsequent cohorts will be given BION-1301 via SC injection. Key eligibility criteria: (1) urine protein ≥0.5 g/24h, (2) stable/optimized dose of ACE-I/ARB (or intolerant), (3) biopsy-verified diagnosis of IgAN within 10 years. **Results:** Final HV data from Parts 1 and 2 were presented at ASN in 2020. Part 3 is ongoing, and interim data from Cohort 1 are being presented at ERA-EDTA in 2021. **Conclusion**: The current design of the Phase 1/2 study, incorporating SC dosing, provides for an improved patient experience, and will enable the generation of long-term safety, PK, PD, immunogenicity, and preliminary activity data for use of BION-1301 in IgAN patients. **Keywords:** BION-1301, IgA Nephropathy, Gd-IgA1, Clinical Trial

A key role for soluble CD89 as inducer of mesangial proliferation in childhood IgA nephropathy

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Background: Childhood IgA nephropathy (cIgAN) includes a wide spectrum of clinical presentations, from isolated hematuria to acute nephritis with rapid loss of renal function. IgAN is an autoimmune disease and its pathogenesis involves galactose deficient (Gd) IgA1, IgG anti-Gd-IgA1 autoantibodies and the soluble IgA Fc receptor (sCD89). However, the implications of such factors notably sCD89 in cIgAN pathogenesis remain unclear. **Methods:** Here, we studied these biomarkers in a cohort of 67 cIgAN patients and 42 controls. **Results:** While Gd-IgA1 was only moderately enhanced in patient plasma, levels of circulating IgA complexes (sCD89-IgA and IgG-IgA) and free sCD89 were markedly increased in cIgAN. sCD89-IgA1 complexes and free sCD89 correlate with proteinuria, as well as histological markers of disease activity: mesangial, endocapillary hypercellularity and cellular crescent. sCD89 was found in patient urines. Mesangial sCD89 deposits were detected in cIgAN biopsies. Serum chromatography fractions containing sCD89-IgA1 or free sCD89 from patients induced mesangial cell proliferation *in vitro* in a sCD89-dependent manner. Recombinant (r) sCD89 binds to CD71 and induced mesangial cell proliferation *in vitro* likely through the CD71 pathway. Injection of rsCD89 induced marked glomerular proliferation and proteinuria in human IgA1 transgenic mice. **Conclusion:** Free and IgA1-complexed sCD89 are key players in mesangial proliferation. These findings reveal as essential role for sCD89 in cIgAN, defining it as a biomarker and a new therapeutic target. **Keywords:** IgA nephropathy, Glomerulonephritis, Immune complexes, Proteinuria, Renal pathology

Quantitative Analysis of IgA1 O-glycoforms in Familial IgA Nephropathy

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Background: Assessment of familial forms of IgA nephropathy (IgAN) revealed genetic control of serum galactose-deficient IgA1 (Gd-IgA1) levels. 50% of first-degree relatives of patients with familial IgAN have high serum Gd-IgA1 levels (≥95th percentile of healthy controls), but without clinical signs of IgAN. However, molecular patterns of IgA1 *O*-glycosylation in IgAN patients and their relatives, or how they might change over time is unknown. We report a longitudinal analysis of quantitative MS profiles of IgA1 *O*-glycosylation in patients with IgAN and their blood relatives in a familial cohort of IgAN. **Methods:** Serum samples were collected at several time points, (2005, 2009, and 2014) from IgAN patients and their family members, both blood relatives and married-in. Serum IgA1 was isolated using lectin-affinity chromatography. Molecular forms of IgA1 were isolated by size-exclusion chromatography. The entire range of IgA1 hinge-region *O*-glycoforms was analyzed by high-resolution mass spectrometry (LC-MS) using LTQ Orbitrap Velos MS. LC-MS data were analyzed with the Pinnacle software. **Results:** The kindred repeat collections included 2 IgAN patients, 13 blood relatives, and 7 genetically unrelated individuals (marry-ins). Using these serum samples along with a series of training samples, we performed molecular analysis of IgA1 using our established quantitative LC-MS *O*-glycoform profiling methodology. A series of internal standards to allow comparison of results were implemented for LC-MS *O*-glycosylation profiles. We expressed the degree of galactose deficiency for individual samples as a ratio of galactose-deficient to galactose-complete glycoforms of the most abundant IgA1 glycoforms in the LC-MS profiles. Using this ratio, our Familial Cohort samples fell into expected ranges. The IgAN patients and Blood relatives matched ratios seen with patients with high levels of Gd-IgA1 and the married in relatives matching ratios of healthy controls thus demonstrating the utility of our methods. **Conclusion:** LC-MS of serum IgA1 can identify Gd-IgA1 in Familial Cohorts. **Keywords:** O-glycosylation, mass spetrometry, biomarkers

Long-Term Value of the International IgAN Prediction Tool and the Role of Hematuria

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Background: Within 30 years, 20-50% of IgAN patients progress to ESRD. Identifying these patients is difficult since renal function can deteriorate after being stable for years. The International IgAN Risk Prediction Tool (IgAN-RPT) combines Oxford classified histologic lesions and clinical risk factors to predict renal outcome. This tool has however not been validated beyond 7 years of follow-up and the impact of microhematuria has not been assessed. **Aim:** Evaluating the IgAN-RPT's predictive value beyond 7 years and the addition of microhematuria to the model. **Material/Methods:** We studied long-term renal outcome of 95 IgAN patients from Karolinska University Hospital, Stockholm, who all had been included in the IgAN-RPT's derivation cohort. Median follow-up was 11.2 years. Degree of microhematuria was assessed by automated microscopy or urinary dipstick. Primary outcome was defined as 50% decrease in eGFR from baseline or ESRD. Patients were divided in quartiles based on predicted risk. **Results:** The mean predicted 7-year risk for increasing quartiles were 1.47%, 3.95%, 8.95% and 33.17% and the recorded 7-year-outcome 0%, 0%, 0%, 45.8%. During continued follow-up 0%, 4.2%, 21.7% and 75.0% of patients reached the primary outcome. High-degree microhematuria was a predictor of the primary outcome in univariate analysis ($p = 0.14$, thus below the threshold of 0.2 for variables included in the IgAN-RPT) but not independent of the model. **Conclusion:** The IgAN-RPT identifies long-term high- and low-risk patients which can guide decisions on the frequency of clinical control visits and the selection of high-risk patients for clinical trials. The predictive value of hematuria at diagnosis might be attenuated by treatment effects which is difficult to control for in retrospective studies. However, recent evidence supports the value of hematuria as an indicator of active and smoldering inflammatory activity which should be a target for future therapies to improve renal outcome. **Keywords:** IgA nephropathy, Risk prediction, Progression of renal disease, Microhematuria

Co-culture with plasmacytoid DC and B cell enhance IgA production through TLR9/APRIL signaling in murine model of IgA nephropathy

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Background: Our recent study revealed that chronic Toll-like receptor 9 (TLR9) stimulation induced a proliferation-inducing ligand (APRIL) expression on naïve B cells and such APRIL+B cell may contribute to nephritogenic IgA production in IgA nephropathy (IgAN). On the other hand, APRIL from TLR9 activated dendric cell (DC) is generally known to be involved in B cell maturation and IgA class switching. Present study aimed to evaluate the role of TLR9/APRIL signaling in DC in IgA production of murine IgAN. **Methods:** Splenic DCs and B cells from grouped ddY (gddY) mice, which are known as the spontaneous IgAN model, and Balb/c mice were isolated using magnetic cell sorting system. In addition, DCs were further divided into three DC subsets; i.e., plasmacytoid DCs (pDCs), CD8+ conventional DCs (cDCs), and CD11b+cDCs by cell sorter. We co-cultured these isolated DCs and B cells with or without CpG-ODN, a synthetic oligonucleotide TLR9 ligand, and IgA in the culture supernatants was measured by ELISA. We also measured the expressions of TLR9 and APRIL in each DC. **Results**: The gddY-derived, but not Balb/c-derived, DCs could dramatically enhance IgA production by co-culture with B cells derived from both gddY and Balb/c mice, and further enhance under CpG-ODN stimulation. Moreover, pDCs from gddY mice strongly induced the IgA production in B cells, compared with cDCs. The expressions of APRIL and TLR9 in the pDCs were higher than those in cDCs. **Conclusion**: Present findings suggest that the gddY DCs, especially pDCs, strongly enhance IgA synthesis from B cells through TLR9 and APRIL signaling. **Keywords:** Dendric cell, Plasmacytoid dendric cell, APRIL, TLR9, IgA nephropathy

Transforming MEST-score to prognostic staging in IgA-nephropathy

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Background: The Oxford classification/MEST-score is an established histopathologic prognostic tool for patients with IgA-nephropathy (IgAN). The objective was to derive a simple model, based on MEST score, enabling pathologist to provide clinicians with more precise and applicable prognostic information in the pathology report. **Methods:** A total of 306 patients with biopsy proven primary IgAN and up to 28 years of follow-up was included. The biopsies were retrieved from *The Norwegian Kidney Biopsy Register* (NKBR) and reclassified according to the Oxford classification. Study end point was end-stage renal disease (ESRD). Cox regression statistics were applied to calculate an adjusted hazard ratio (HR). Three models were derived to subgroup patients into different risk classes: Model A based on histology, model B based on a composite score calculated from the adjusted HR, and model C based on quartiles. **Results:** Patients were recruited from an all-Norwegian cohort, 234 (76.5%) were male and the mean age at time of biopsy were 37.4 years. Mean patient follow-up was 16.5 years (range 0.2 - 28.1). In total 61 (20%) patients reached ESRD during the study period. In univariate analysis all M, E, S and T lesions are associated with increased risk of ESRD, but in multivariate analysis only S and T lesions were associated with poor outcome. ROC analysis at 15 years showed that model A and model B performed just as well as MEST score, with an AUC at 0.85. *Harrel c index* were 0.82 for MEST score and 0.80 model A and B. We found model B to be suitable in clinical practice since it is most user-friendly. **Conclusion:** Patients can be divided into risk classes based on their MEST score from the diagnostic kidney biopsy, enabling pathologist to provide clinicians with valuable prognostic information at time of diagnosis without loss of prognostic prediction. **Keywords:** IgA Nephropathy, Prognosis, Prediction model, MEST-score

Characterization of different molecular forms of aberrantly glycosylated IgA1 in the circulation of patients with IgA nephropathy

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Background: IgA nephropathy (IgAN) is an autoimmune disease wherein IgA1 molecules with some hinge-region *O*-glycans deficient in galactose (Gd-IgA1) are bound by IgG autoantibodies in immune complexes (IC) that deposit in the kidneys and induce injury. Glomerular immunodeposits are enriched for Gd-IgA1. However, not much is known about the distribution of different Gd-IgA1 molecular forms in the circulation. **Methods:** Total IgA1 was isolated from sera of 17 patients with IgAN (7 Caucasians, 10 African Americans) by lectin-affinity chromatography and different molecular forms of IgA1 were separated by size-exclusion chromatography (SEC). Gd-IgA1 was detected by lectin ELISA. Serum IgA1-IC were isolated by SEC and their biological activity assessed by cellular proliferation assay with primary human mesangial cells (MC). IgA, IgG, and complement C3 were analyzed by SDS-PAGE/immunoblotting. **Results:** Total serum IgA1 was represented by three molecular forms: monomeric, polymeric, and bound in IC. The predominant form, monomeric, represented ~88-92% of total IgA1, whereas polymeric IgA1 represented ~8-12%. IgA1 in IC was the least abundant form, representing <0.4% of total IgA1. Relative representation of Gd-IgA1 was highest in IC, followed by polymeric forms, and lowest in monomeric forms. Gd-IgA1 in IC had minimally sialylated *O*-glycans, whereas polymeric and monomeric forms were substantially sialylated. Compared to African Americans, Caucasian patients had higher content of Gd-IgA1 in polymeric and monomeric forms of IgA1 (P=0.027 and 0.048, respectively). IgA1-IC isolated by SEC from sera of IgAN patients had molecular mass >700 kDa and stimulated proliferation of MC. These IC consisted of polymeric IgA1, IgG, and complement C3. **Conclusions:** Analyses of different molecular forms of IgA1 revealed that biologically active IC in the circulation of IgAN patients contain Gd-IgA1 associated with IgG and C3. Gd-IgA1 in these IC was polymeric and had minimally sialylated *O*-glycans. These findings support the pathogenic role of Gd-IgA1-IgG IC in IgAN.
Keywords: molecular forms, Gd-IgA1, immune complexes, autoantibody (IgG), C3

Integrin α1β1 is involved in mesangial-cell activation by IgA1-containing immune complexes from patients with IgA nephropathy

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Background: Integrin β1 is involved in multiple glomerular functions, including mesangial-cell (MC)-driven remodeling in glomerulonephritis. We investigated the roles of two major subtypes of integrin β1, α1β1 and α5β1, in MC activation by galactose-deficient IgA1 (Gd-IgA1)-containing circulating immune complexes (CIC) from patients with IgA nephropathy (IgAN). **Methods:** CIC were isolated from sera of patients with IgAN by size-exclusion chromatography. Primary human MC were incubated with CIC for 15 min at 37°C, with or without inhibitors. Cell lysates were analyzed directly, or after immunoprecipitation (IP) with integrin β1-specific antibody, by SDS-PAGE/ immunoblotting for IgA and IgG and phosphorylated ERK1/2. **Results:** The lysates from MC incubated with CIC and the corresponding IP products contained IgA and IgG. IgA and IgG content was significantly reduced by an inhibitor of integrin α1β1 (obtustatin), but not by an inhibitor of integrin α5β1 (RGD), and partially inhibited by a tyrosine-kinase inhibitor (dasatinib). CIC induced phosphorylation of ERK1/2; this activation was inhibited by obtustatin, RGD, dasatinib, and a Chinese herbal medicine, ShenPing decoction (SP) that has been used to treat IgAN patients in Shanghai. In IP products from CIC-activated MC, cytoskeleton-associated protein talin, known to activate ERK1/2, was associated with integrin β1. Binding of talin to integrin β1 was blocked by SP. **Conclusions:** Integrin α1β1 is involved in CIC-mediated MC activation and uptake of IgA-IgG. CIC activation induced integrin α1β1 to bind talin and activate ERK1/2. SP inhibited these processes. CIC also activated ERK1/2 through other pathways that were inhibited by integrin α5β1 inhibitor RGD and dasatinib. **Keywords:** Galactose-deficient IgA1, Circulating immune complex, Integrin, MAP kinase activation, Pathogenesis of IgA nephropathy, Signaling of mesangial cells

Long-term outcomes of IgA nephropathy patients with less than 25% crescents and mild proteinuria

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Background: Whether immunosuppressive therapy in IgA nephropathy (IgAN) with less than 25% crescents (C1) and mild proteinuria can improve the renal outcome is still unclear. **Methods:** We recruited 140 IgAN patients with C1 and proteinuria < 1 g/24h who received supportive care (n = 52) or steroid-based immunosuppressive therapy (n = 88) in Xijing Hospital from July 2008 to December 2016. Proteinuria progression was defined as proteinuria ≥ 1 g/24h at the last follow-up or time-average proteinuria (TA-P) increased ≥ 50% compared the baseline proteinuria during the follow-up period. **Results:** The median of proteinuria in all patients was 575.5 mg/24h, the fraction of glomeruli with crescents was 7% (5%, 12%) and follow-up times was 69.1 months. The proteinuria progression (12.5% vs 28.8%; P = 0.02) and the rate of renal function decline (0.5 (- 1.5, 3.2) vs – 0.7 (- 3.5, 0.5) ml/min per 1.73 m^2; P = 0.01) during the follow-up period in steroid-based immunosuppressive therapy group were lower than supportive care group. Multivariate logistic regression analyses showed steroid-based immunosuppressive therapy (OR = 0.310, 95% CI 0.106-0.905, P = 0.03) significantly reduced the risk of the proteinuria progression after adjusting age, sex, mean arterial blood pressure, proteinuria, estimated glomerular filtration rate (eGFR), mesangial hypercellularity score > 0.5 (M1), endocapillary hypercellularity present (E1), segmental glomerulosclerosis present (S1), tubular atrophy/interstitial fibrosis > 25% (T1-2), the fraction of glomeruli with crescents and renin-angiotensin system blockers (RASB). The matched cohort showed similar results. The incidence of adverse events were similar between the two groups. **Conclusions:** Steroid-based immunosuppressive therapy maybe reduced the risk of the proteinuria progression and the rate of renal function decline of IgAN patients with C1 and proteinuria ≤ 1 g/24h. **Keywords:** IgA nephropathy, Crescent, Proteinuria, Prognosis, Immunosuppressive

The benefit of combining corticosteroid therapy with tonsillectomy in pathologically advanced IgA nephropathy

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Background and Aim: The meta-analysis from China in Oncotarget 2017 showed that T1 or T2 of Oxford classification (T1/2) was independently associated with resistance to corticosteroid therapy in IgA nephropathy (IgAN). Our previous pathological sub-analysis of a RCT of tonsillectomy combined with corticosteroid therapy showed that, in IgAN patients with T1/2, combining corticosteroid therapy with tonsillectomy had 20.0-fold greater benefit for disappearance of proteinuria than corticosteroid monotherapy (Katafuchi, CEN 2016), whereas the benefit of that for long-term renal survival in those with advanced histological changes was not known. Therefore, we evaluated the association between combining corticosteroid therapy with tonsillectomy and renal survival in patients with T1/2, using a dataset from Japanese nationwide prospective cohort study. **Patients and methods:** Of 1,130 patients registered for the multicenter prospective cohort study which had been conducted by the Progressive Kidney Disease Study Group, Japan Ministry of Health, Labor, and Welfare since 200, those with T1/2 were enrolled in this study. Primary outcome was defined as 1.5 times increase in serum creatinine concentration from baseline. Control group for survival analysis was defined as the patients not receiving corticosteroid therapy within one year after renal biopsy. **Results:** In 216 patients enrolled, mean eGFR was 47.2 ml/min and median level of proteinuria was 1.33 g/day. During the median follow up of 4 years, 57 patients (26.4%) reached primary outcome. Multivariate Cox regression analysis revealed that corticosteroid monotherapy (n=66) did not significantly improve renal prognosis compared to control (n=79) (hazard ratio [HR] 0.53; 95% confidence interval [CI] 0.25 to 1.08). In contrast, corticosteroid therapy combined with tonsillectomy (N=71) demonstrated significantly improved renal survival compared to control (HR 0.35; 95%CI 0.13 to 0.87). **Conclusion:** The benefit of combining corticosteroid therapy with tonsillectomy was confirmed in IgAN patients with severe pathological changes in a Japanese nationwide prospective cohort. **Keywords:** tonsillectomy, corticosteroid therapy, T1/T2 of Oxford classification, Japanese IgA nephropathy prospective cohort study

Clinicopathological prognostic stratification for renal survival in the Japanese IgA nephropathy prospective cohort study (J-IGACS)

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Background/Aim/Objectives: Baseline characteristics of IgA nephropathy (IgAN) are different between East Asia and Europe. Since the recent study from Korea reported that the international risk-prediction tool showed not as good performance (C-statistics 0.69) as previous validation (Nephrology 2021), a prognostic model fitting IgAN patients in East Asia may be needed. We aimed to evaluate the association between risk group for end-stage renal disease (RG) proposed from Japan in 2011 and renal outcome, in a dataset from nationwide prospective cohort study (J-IGACS). **Methods:** The patients were registered between April 1, 2005 and August 31, 2015 at 44 facilities throughout Japan. The primary outcome was a 50% increase in serum creatinine from baseline or dialysis induction. RG was defined by a combination of the clinical grade (CG) and the histological grade (HG), and categorized into four groups, RG I-IV based on the odds ratio for dialysis induction in our previous retrospective study (Clin Exp Nephrol 2018). CG was classified into three levels by proteinuria and eGFR, and four levels of HG corresponded to HG I; <25%, HG II; 25 – <50%, HG III; 50 – <75%, and HG IV; ≥75% of glomeruli exhibiting crescents and segmental/global sclerosis. **Result/Discussion:** The enrolled 991 patients showed 75.4 ml/min as mean eGFR, 0.58 g/day as median of proteinuria and 358/377/165/91 patients in RG I/II/III/IV. The number (%) of patients with corticosteroid therapy and tonsillectomy was 634 (64.0%) and 425 (42.9%), respectively. During the median follow up of 5.5 years, 87 (8.8%) patients reached primary outcome. RG was significantly associated with the primary outcome (hazard ratio [95% confidence interval]; II 2.78 [1.12–6.93], III 7.14 [2.90–17.6], IV 33.4 [14.1–79.0], I as reference). The discrimination performance was good (C-statistic 0.81, 95% confidence interval 0.76–0.86). **Conclusion:** RG predicted renal survival with good discrimination in a Japanese nationwide prospective cohort. **Keywords:** clinicopathological prognostication, corticosteroid, tonsillectomy

Evaluation of pharmacological mechanism of hydroxychloroquine in murine IgA nephropathy

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Background: Toll-like receptors (TLR) 9 and 7 are supposed to be involved in the synthesis of aberrantly glycosylated IgA which is the key effector molecule in IgA nephropathy (IgAN). Recently, several clinical trials suggested hydroxychloroquine (HCQ) which is known to suppress TLR9/7 signal might be effective to reduce proteinuria in patients with IgAN. However, its pharmacological mechanism is not fully clarified. In present study, we evaluate whether HCQ can suppress renal injury *via* blocking TLR9/7 signal in murine IgAN model. **Methods**: In order to elucidate the effect of HCQ in IgAN, we administered HCQ (60mg/kg/day for 4wks) to gddY mice which are known as a murine model of IgAN (Control: N=6, HCQ: N=6). We analyzed serum levels of IgA and aberrantly glycosylated IgA, the amount of proteinuria and renal injuries after administration of HCQ. In addition, to assess the effect of HCQ in the production of IgA and aberrantly glycosylated IgA *in vitro*, we cultured splenocytes of gddY mice ($3x10^6$ cells) for 72 hours under the stimulation of TLR9 ligand (CPG-ODN 5μM) and TLR7 ligand (imiquimod 5μg/ml), respectively, with/without HCQ (50μM). Level of total IgA, aberrantly glycosylated IgA, IL-6 in the culture supernatant were measured. **Results:** Treatment with HCQ decreased serum levels of IgA and aberrantly glycosylated IgA. Renal immunofluorescence analysis showed attenuation of mesangial IgA/IgG/C3 deposition. Stimulation with CPG-ODN or imiquimod could enhance production of total IgA, aberrantly glycosylated IgA and IL-6 in splenocytes. Contrary, treatment with HCQ could suppress all of these productions. **Conclusion**: Present data suggested that HCQ might be effective to inhibit the production of aberrantly glycosylated IgA which stimulated by TLR9/7 signal. However, the exact pharmacological mechanism needs to be further verified. **Keywords:** Hydroxychloroquine, Toll-like receptor 9, Toll-like receptor 7

FcαRI-mediated neutrophil activation contributed to the pathogenesis of IgA vasculitis with nephritis

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Background: IgA vasculitis (IgAV) is a systemic small-vessel leukocytoclastic vasculitis characterized by IgA-immune deposits in the vessel wall and neutrophil infiltration, which usually associated with kidney and gastrointestinal manifestations. Previous studies suggested the neutrophil-to-lymphocyte ratio increased in the patients with IgAV, which indicated that neutrophils have been activated in IgAV. The IgA Fc receptor (FcαRI) mainly express on the neutrophils and act as a regulator of inflammation responses of IgA. Here, we investigate the potential role of FcαRI-mediated neutrophils activation in the pathogenesis of IgAV. **Methods:** In this study, we enrolled 16 adult patients with IgAV and 13 healthy controls between September 2020 and March 2021 and all of the patients had renal involvement (IgAV-N). The expression of FcαRI on the surface of neutrophils in peripheral blood was detected by flow cytometry. *In vitro*, neutrophils isolated from healthy controls peripheral blood were challenged with IgA1 containing immune complexes (IgA1-ICs), derived from IgAV-N patients and healthy controls, respectively, with or without MIP8a, an anti FcαRI antibody. After that, the indicators for neutrophils activation, reactive oxygen species (ROS), release of neutrophil extracellular traps (NETs) and lactoferrin, were detected. **Results:** IgAV-N patients have significantly higher FcαRI expression on the surface of neutrophils than healthy controls (P<0.05). Incubation of neutrophils with IgA1-ICs derived from IgAV-N patients resulted in increased production of reactive oxygen species (ROS), increased release of neutrophil extracellular traps (NETs) and lactoferrin than IgA1-ICs from healthy controls. Moreover, blocking FcαRI on the neutrophils significantly reduced the production of ROS, NETs and lactoferrin from neutrophils induced by IgA1-ICs. **Conclusion:** IgAV-N patients had elevated expression of FcαRI on peripheral neutrophils. The IgA1-ICs derived from IgAV-N patients could effectively activate neutrophils, which process at least partly induced by FcαRI.Our findings suggested FcαRI-mediated neutrophils activation contributed to the pathogenesis of IgAV-N. **Keywords:** IgA vasculitis with nephritis, FcαRI, neutrophil

The Comparison of Efficacy and Safety in High-Risk IgA Nephropathy with Low-dose and Full-dose Prednisone Treatment – A Prospective Randomized Controlled Trial

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Rationale & Objective: The outcomes of glucocorticoid treatment for immunoglobulin A nephropathy (IgAN) patients, especially for patients whose proteinuria is between 1 to 3.5g/24h, are still controversial. This study aims to explore the efficacy and safety of low-dose prednisone in the treatment of high-risk IgAN patients. **Study Design:** Single-center, prospective, randomized, controlled clinical trial. **Setting & Participants:** The 84 biopsy-proven IgAN patients with proteinuria between 1–3.5 g/24h were enrolled. **Interventions:** All the patients were randomly assigned into 2 groups: 1) Low-dose prednisone treatment group: 0.5 g of methylprednisolone intravenously for three consecutive days at the beginning of the course and repeated in the 4th month, oral prednisone at a dose of 15mg every other day for 6 months. 2) Full-dose prednisone treatment group: 0.8–1.0mg/kg/d of prednisone (maximum 70 mg/d) for 2 months then tapered by 5mg every 10 days for the next 4 months. **Outcomes:** Primary outcome was change in 24h proteinuria and complete remission rate at each follow-up. **Results:** At the end of the trial, the rates of patients with a 50% reduction in proteinuria levels between the two groups were no significant difference (93% vs. 90%, P = 0.645). The total dosage of glucocorticoid was approximately 4305 mg vs 6900 mg between the two groups (60 kg/person, 30 days/month) respectively. More patients in the full-dose prednisone group presented infections and Cushing syndrome than that in the low-dose prednisone group. **Conclusions:** The full-dose prednisone therapy in high-risk IgAN patients did not significantly improve the outcome than that in the low-dose prednisone treatment, and more adverse effects were observed among the patients who received the full-dose prednisone therapy during the 6-month study phase. **Trial Registration:** Registered in the Chinese Clinical Trial Registry (approval number ChiCTR1800014442). **Keywords:** IgA nephropathy, prednisone, proteinuria, complete remission, adverse event, Clinical trial

Complement Factor H and THBD rare variants are overrepresented in a French IgA Nephropathy cohort

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Background and Aims: Complement activation through alternative and lectin pathways have been described to impact the pathogeny of IgA nephropathy. We hypothesized in this study that rare variants of alternative pathways regulatory genes could be overrepresented and could play a role at initiating the disease and could harm the prognosis of IgA Nephropathy. **Method:** Patients with biopsy proven IgA nephropathy with markers of severity with available DNA sample were included. All coding sequences of *CFH*, *CFI, MCP, C3, FactorB*, *THBD* and *CFHR5* genes were analyzed by next generation sequencing. We defined a variant as rare when its minor allele frequency was below 0.1 % in the general population. Frequencies were compared to a French volunteer's cohort (n=80) and a European large control cohort (n=503). **Results:** We screened 128 patients with IgA N, with: median age 42.4 yo, proteinuria (median) 1.4 g/day, hypertension 66%, median eGFR 48.7 mL/min/1.73 m^2. The median follow-up was 99 months and 58% of patients progressed to ESRD. We identified rare variants in 10.2 % (n=13) including 1 patient with two rare variants. Patients with IgA N have high rates of rare variants in CFH (n=9/128; 7 %) versus normal controls (n=9/503; 1.8 %) (p=0.004). Six patients carried the pathogenic variant in THDM gene p.Ala43Thr (6/128) versus 5 in 508 controls population (p=0.01). No difference in term of hypertension, proteinuria, eGFR, Oxford classification, vascular score at diagnosis was noticed between patients without any rare variant compared to patients with at least one rare variant. The progression through ESRD was not different between groups. **Conclusion:** In this cohort of Caucasian IgA nephropathy patients, rare variants of *CFH* and *THBD* were found significantly overrepresented compared to a French and European control cohort. Rare variants of alternative pathway regulatory genes were not associated with particular severity or prognosis. **Keywords:** IgA nephropathy, complement, genetics

An increasing incidence of IgA nephropathy and Henoch-Schönlein purpura during the 23 years of biopsy survey in the Czech Republic

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Background: We describe data on 14,510 renal biopsies gathered by the Czech Registry of Renal Biopsies over a period of 23 years. **Methods:** We assessed the main demographic, clinical and histological data of individuals who underwent renal biopsies of native kidneys in 31 centers in the Czech Republic (population 10.3 million) during the period 1994–2017. **Results:** We evaluated 14,510 renal specimens of the Czech registry of renal biopsies. The most frequent biopsy-proven diseases were primary (55.0%) and secondary (29.7%) glomerulonephritides (GN). Tubulointerstitial nephritis (TIN) was observed in 7.1% and vascular diseases in 5.0%. The samples were non-diagnostic in 6.2%. Among primary GN the most frequent diagnoses were IgA nephropathy (IgAN) (41.7%), focal segmental glomerulosclerosis (FSGS) (13.4%) and membranous GN (MGN) (10.0%). Among secondary GN, systemic lupus erythematosus (SLE) represented 23%, hereditary diseases 16.7% and necrotizing vasculitis (NV) 19%. The incidence for the period 1994–2017 (per million population) was: primary GN 52.3, secondary GN 25.5, IgAN 20.3, MGN 4.7, SLE 4.0, FSGS 7.4, MCD 7.4, NV 3.2 and diabetic nephropathy 2.3. We noticed increasing incidence of IgAN (from 9.55 to 20.3) as well as HSP (from 0.76 to 1.99) (Henoch-Schönlein purpura) during the period of 23 years. The frequency of serious complications (symptomatic hematoma, gross hematuria, blood transfusion) was approximately 2.3%. **Conclusions:** This report provides representative population-based data on native biopsy-proven renal diseases in the Czech Republic. Over the 23 years of nationwide biopsy survey, we noted an increasing incidence of IgAN as well as HSP. *Study was supported by grants PROGRES Q25/LF1 and DRO VFN 64165 from the Ministry of Health of the Czech Republic and by the Research Infrastructure NanoEnviCz from the Ministry of Education, Youth and Sports of the Czech Republic under Project No. LM2015073.* **Keywords:** Glomerulonephritis, Renal biopsy, IgA nephropathy, Henoch-Schönlein purpura

TRF-budesonide (Nefecon) positively impacts serum and urinary biomarkers involved in interstitial fibrosis in patients with IgAN: analysis from the Phase 2 NEFIGAN trial

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Background: IgA nephropathy (IgAN), the most common form of primary glomerulonephritis worldwide, has serious outcomes with ESRD developing in 30–50% of patients. The safety and efficacy of a novel, targeted-release investigational formulation of budesonide (TRF-budesonide [Nefecon 16 mg or 8 mg/day]), designed to deliver the drug to the Peyer's patches in the distal ileum, was assessed in the Phase 2 NEFIGAN trial. We have previously shown that serum PRO-C6, a marker of collagen type VI formation, correlated positively, and urinary C3M/creatinine, a marker of collagen type III degradation, correlated negatively with the extent of interstitial fibrosis in renal biopsies of patients with IgAN. Here, we assessed serum and urine biomarkers related to renal fibrosis and histological findings in renal-biopsy specimens from patients with IgAN from the NEFIGAN trial. **Methods:** We evaluated 150 patients from the NEFIGAN trial treated with TRF-budesonide (Nefecon 16 mg or 8 mg/day) or placebo during the follow-up of 12 months. Serum and urine samples were analysed for biomarkers related to renal fibrosis using novel enzyme-linked immunosorbent assays. Data were log-transformed prior to analysis and compared between arms using ANCOVA with log(baseline) values as a covariate. **Results:** Serum PRO-C6 was significantly decreased by both doses of TRF-budesonide after 9 months of treatment, but there was a rebound 3 months after TRF-budesonide withdrawal suggesting the potential benefit of prolonged treatment in at least some patients with IgAN. Only TRF-budesonide 16 mg/day resulted in a significant increase in urinary C3M/creatinine after 9 months of treatment compared with values at randomization, an effect that persisted even 3 months after TRF-budesonide withdrawal. **Conclusion:** TRF-budesonide may decrease the degree of interstitial fibrosis by diminishing collagen formation and stimulating collagen degradation. These effects may contribute to the long-term preservation of renal function in patients with IgAN. **Keywords:** Immunoglobulin A nephropathy, Peyer's patches, Interstitial fibrosis, Renal biopsy, Biomarker, Serum PRO-C6, Urinary C3M/creatinine

Galactose-deficient IgA1-containing immune complexes deposit in mesangium mediated by endothelial cell injuries

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Background: Galactose-deficient IgA1 (Gd-IgA1) plays a crucial role in the pathogenesis of IgA nephropathy (IgAN). However, the pathogenic role of mesangial Gd-IgA1-containing immune complexes (ICs) remains unclear. The endothelial surface glycocalyx modulates microvascular function. Loss of the glomerular endothelial glycocalyx is known to be involved in albuminuria. Here, we examined whether the deposition of Gd-IgA1-containing ICs in the mesangium may lead glomerular endothelial cell dysfunction in this disease. **Method:** Gd-IgA1 and recombinant anti-glycan IgG were used to form ICs to inject into nude mice. The renal microvascular endothelial glycocalyx removal of the injected nude mice was evaluated by real-time glycocalyx imaging. Human renal glomerular endothelial cells (HRGECs) were used to assess the potential capacity of Gd-IgA1-containing ICs to activate endothelial cells. **Results:** After co-culture of Gd-IgA1-containing ICs with HRGECs, mRNA expression levels of endothelial adhesion molecules (ICAM-1, VCAM-1 and E-selectin) were significantly upregulated ($P<0.01$). Expression levels of proinflammatory mediators (TNFα and IL-6) that are able to induce the expression of the adhesion molecules on endothelial cells were also increased ($P<0.01$). Nude mice injected with Gd-IgA1-containing ICs showed podocyte and endothelial injuries with IgA, IgG, and C3 deposition in the glomerular capillaries and the mesangium. Moreover, albuminuria and hematuria were also induced. Real-time glycocalyx imaging showed that renal microvascular glycocalyx was decreased immediately after the injection of Gd-IgA1-containing ICs and then mesangial IgA deposition was increased. **Conclusion:** Present data suggest that Gd-IgA1-containing ICs may induce glomerular endothelial injuries resulting in mesangial deposits. **Keywords:** Galactose deficient IgA1, endothelial cell, glycocalyx

Association of Microhematuria with Outcomes in Adult Patients with IgA Nephropathy

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Aims: We aimed to analyze the association between microhematuria and outcome of adult patients with IgA nephropathy (IgAN). **Methods:** 129 adults with IgAN followed up for a median duration of 54.5 (IQR: 24.25-92.75) months were included in this study. Urinary sediment analyses during the bouts of macrohematuria were not taken into consideration. Microhematuria was described as ≥5 RBCs/hpf and classified as mild (5-9 RBCs/hpf), moderate (10-19 RBCs/hpf), or severe (≥20 RBCs/hpf). Study outcome (event) was defined as at least a 50% reduction in baseline eGFR or development of stage 5 chronic kidney disease (eGFR <15 ml/min/1.73 m^2). **Results:** Usage of ACEi/ARBs [75/81 (92.5%) vs 45/48 (93.75%), p=0.803], fish oil [30/81 (37%) vs 19/48 (39.5%), p=0.773], azathioprine [16/81 (19.7%) vs 10/48 (20.8%), p=0.882] and mycophenolic acid derivatives [14/81 (17.2%) vs 11/48 (22.9%), p=0.434] were comparable among the patients with and without microhematuria. Corticosteroids were more frequently used in patients with microhematuria [41/81 (50.6%) vs 17/48 (35.4%)], although this difference was not statistically significant (p=0.093). Overall 30 patients (23.2%) reached the study outcome, and there were no differences between patients with (19, 23.4%) and without (11, 22.9%) microhematuria (p=0.944). Kaplan-Meier analysis revealed that event free survival rates were similar across study groups: 77.1% for patients without microhematuria; while 80% for mild, 77.3% for moderate, and 72.7% for severe microhematuria (p=0.436). Microhematuria did not predict the study outcome in multivariable Cox regression analyses [HR: 1.847 (95% CI: 0.696-4.904), p=0.218]. Throughout the follow-up, microhematuria disappeared (dropped below 5 RBCs/hpf) in 43 patients (53%), 8 of whom (18.6%) reached the study outcome as compared to 11 patients (28.9%) with persistent microhematuria (p=0.273). Disappearance of microhematuria was not a predictor of study outcome, as well [HR: 0.386 (95% CI: 0.068-2.180), p=0.281]. **Conclusion:** Microhematuria is not associated with renal outcomes of adult patients with IgAN. **Keywords:** microhematuria, prognosis, IgA nephropathy

Clinical significance of intensity of glomerular galactose-deficient IgA1 deposition in IgA nephropathy

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Background: Galactose-deficient IgA1 (Gd-IgA1) have a crucial role in the pathogenesis of IgA nephropathy (IgAN). Recently, it was reported that Gd-IgA1 specifically deposit on glomeruli of primary IgAN using Gd-IgA1-specific monoclonal antibody (KM55 mAb). However, the association between the intensity of Gd-IgA1 deposition and histological severity and clinical parameters are not clarified. **Methods:** We performed immunostaining with and KM55 mAbs in 141 patients diagnosed with IgAN at Juntendo University Hospital. We quantified the intensity of glomerular Gd-IgA1 by Image-J software, and analyzed its association with histological findings. We also analyzed the association of intensity of glomerular Gd-IgA1 with serum levels of Gd-IgA1 and creatinine, urinary Gd-IgA1, and proteinuria. **Results:** Glomerular Gd-IgA1 was positive in all 141 primary IgAN cases. We divided patients into tertiles according to the amount of Gd-IgA1 deposition by the Image-J software (low, middle, and high groups). The level of proteinuria in the high-intensity group was significantly higher than that in the other two groups (low vs high; *P<0.05*, middle vs high; *P<0.05*). Moreover, the level of urinary Gd-IgA1 was also higher in the high-intensity group (middle vs high; *P<0.05*). The percentage of acute lesions such as cellular crescents was significantly higher in the high-intensity group (low vs high; *P<0.05*). **Conclusion:** Present study suggested that high intensity of glomerular Gd-IgA1 deposition is associated with histological severity, especially acute lesions. Thus, glomerular Gd-IgA1 staining may be considerable index for therapeutic intervention. **Keywords:** KM55, Galactose-deficient IgA1, histological severity

Clinical Pharmacology and Population Pharmacokinetic/Pharmacodynamic Modeling of Lectin Pathway Inhibition by Narsoplimab (OMS721)

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Background: IgA nephropathy (IgAN) is a glomerular disease in which immune complex deposition on mesangial cells activates the lectin pathway of complement. Hematopoietic stem cell transplantation-associated thrombotic microangiopathy (HSCT-TMA) results from endothelial injury associated with HSCT, which activates the lectin pathway. Narsoplimab, an inhibitor of mannan-binding lectin-associated serine protease-2 (MASP-2), the effector enzyme of the lectin pathway and an activator of the coagulation cascade, is being studied in clinical trials for treatment of IgAN and HSCT-TMA. **Methods:** Lectin pathway activation pharmacodynamic (PD) effect was measured by inhibition of *ex vivo* C4d deposition in four clinical studies: Phase 2 trial in IgAN (NCT02682407), open-label pivotal trial in TMAs (NCT02222545), and two Phase 1 healthy volunteer studies. Sparse pharmacokinetic (PK) sampling was performed in the IgAN and TMA studies, while intense PK sampling was performed in healthy populations. A population PK/PD (2-compartment) model was built on the subset of single and multiple narsoplimab IV dose regimens used in these clinical studies. **Results:** The final population PK model with covariates comprised linear 2-compartment distribution and elimination, together with a non-linear elimination component described by a Michaelis–Menten term. Distribution volumes were consistent with monoclonal antibodies, indicating that narsoplimab is distributed in the blood and hydrophilic extravascular space. Total clearance (CL) of narsoplimab was concentration-dependent, with K_m values estimated to be ~5.7 µg/mL. The estimated CL for patients ranged from 0.1146–0.1286 L/h. The estimated terminal half-life ($T_{1/2}$) in healthy volunteers was 198 hours after 6 weekly IV doses of 4 mg/kg. The population covariates affecting the disposition of narsoplimab were albumin level, patient status, body weight, dose, and the presence of anti-drug antibodies (ADA). **Conclusion:** PK/PD analysis indicated a difference in exposure between patients and healthy volunteers. Fixed dosing of narsoplimab inhibited lectin pathway activation for ~1 week in the IgAN population. **Keywords:** IgA nephropathy, Lectin pathway inhibition, Narsoplimab, Pharmacokinetics, Pharmacodynamics

Differential pathway activation in Ig-producing cells from IgAN patients and healthy controls mediated by cytokine stimulation

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Objectives: Some cytokines enhance synthesis of galactose-deficient IgA1 (Gd-IgA1) in immortalized IgA1-producing cells derived from peripheral blood of patients with IgAN. Several studies have pointed to a dysregulated cytokine signaling, but little is known about how these signaling pathways influence critical transcriptional response in Ig-producing cells. Using single-cell transcriptomics, we analyzed pathway responses in immortalized Ig-producing cells derived from IgAN patients and healthy controls (HC) in response to a mixture of cytokines. **Materials and Methods:** A mixture of cytokines mimicking those produced by T-follicular helper (Tfh) cells (IL-4, IL-6, IL-21, CD40L; 50 ng/mL) was used to stimulate immortalized Ig-producing cells from IgAN patients and HC for 30 min before single-cell transcriptomic analysis. Gd-IgA1 was determined by lectin ELISA. Standard data processing using Seurat was performed. Putative markers for conserved and differentiating genes in unstimulated vs. stimulated were analyzed for pathway differences using the GSEA MSig database. **Discussion:** Tfh cytokines mediated overproduction of Gd-IgA1 in IgAN cells. We found significant overlap in the pathways (6 in the top-10) identified from the conserved gene group analysis in Ig-producing cells from IgAN patients and HC. In the differential gene sets for healthy control, we found the top pathways were immune function related, such as "response to cytokine (FDR<10^-49). In the differential gene sets for IgAN, we found no cytokine or immune related stimulation pathways between that should have been different after cytokine stimulation. **Conclusion:** Cytokine stimulation in Ig-producing cells from HC elicited an upregulation in immune-response pathways, but no significant response in cytokine or immune pathways was found in Ig-producing cells from IgAN patients. This difference suggests a significant difference in how IgAN Ig-producing cells respond to cytokines. Further investigation is needed to determine the role of these pathways in driving Gd-IgA1 overproduction medited by cytokine stimulation. **Keywords:** IgAN, Gd-IgA1, Transcriptomics, Pathway, Single-Cell, Cytokine

A New Epigenetically-Driven Mechanism Regulating the IL-6 Levels in IgA Nephropathy

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Background/Aims/Objectives: Recently, by a genome-wide screening for DNA methylation, several regions with altered methylation were identified in IgAN patients. In particular, a hypermethylated region was identified encompassing Vault RNA 2-1 (VTRNA2-1), a non-coding RNA, that resulted downregulated in IgAN patients. VTRNA2-1 is known to be a specific inhibitor of PKR, an interferon-inducible double-stranded RNA-dependent kinase. Suppression of VTRNA2-1 activates PKR and its downstream pathways. For these reasons we aim to study the PKR/CREB pathway regulating the IL-6 secretion, a cytokine with a key role in mediating the production of deglycosylated IgA in IgAN patients. **Methods:** PBMCs were isolated from IgaN patients, IgAN subjects with kidney transplant (T-IgAN), patients transplanted for causes other than IgAN (TP), and Healthy subject (HS). The expression of VTRNA2-1 was studied in RT-PCR. Total levels of PKR, phospho-PKR (pPKR), CREB, phospho-CREB and IL-6 proteins were evaluated by ELISA. **Results/Discussion:** We further validated the gene expression levels of VTRNA-2-1 showing that the expression of VTRNA2-1 in IgAN subjects is strongly reduced compared to healthy subjects. We found also that VTRNA2-1 expression levels in T-IgAN were highly downregulated compared to TP. We found in IgAN patients, in correspondence of very low levels of VTRNA-2-1, a significant increase level of pPKR, leading, in turn, to increased levels of pCREB and of IL-6 levels, significantly higher in IgAN patients compared to HS. Interestingly, PKR is activated by direct binding of double or single-stranded RNA. Therefore, commensal bacteria or viral infections may play an important role in IgAN also exacerbating this pathway. **Conclusions:** For the first time we identified a mechanism of regulation explaining the high IL-6 levels in IgAN patients, based on the epigenetic downregulation of the non-coding RNA VTRNA-2-1 and on the activation of PKR/CREB pathway. This mechanism may be amplified by commensal bacteria and virus or by infections. **Keywords:** DNA methylation, Vault RNA, non-coding RNA, IL-6, PKR

An artificial neural network (ANN) tool to predict ESKD in immunoglobulin a nephropathy (IgAN). performance analysis

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Background: We have developed and validated an ANN tool to predict end stage kidney disease (ESKD) in patients with idiopathic IgAN (KI May 2021). Our tool is based on two different ANNs. The first network predicts ESKD development while the second predicts the time frame to reach this outcome. Over the past 15 years many tools have been developed to predict ESKD in patients with IgAN but their performance is unknown. **Aim:** We have evaluated the performance of our tool. **Methods:** First, we unified the IgAN patients of the study cohort (948) and the test cohort (168) included in our previous study because the AUC values were 0.82 and 0.84, respectively, and the performance errors were 19.41% and 19.05%, respectively. Thus, we had a retrospective cohort of 1,116 patients. Next, we analyzed the performance of our tool and the causes of discordance between the predicted and observed ESKD. **Results:** Discordance was observed in 216 patients (19.35%). In 77 patients who were predicted not to reach ESKD, but had this outcome, 25 patients with proteinuria >0.5 g/day did not receive therapy after kidney biopsy or had a late treatment with RASBs alone; 52 patients did not respond to therapy (RASBs alone or combined with corticosteroids). In 139 patients, who did not develop ESKD and our tool predicted this outcome, there was an improvement of the disease after therapy. Thirty-six of the 139 patients had nephrotic proteinuria with GFR<50 mL/min/1.73 m^2 and had a favorable outcome after corticosteroid therapy. **Conclusions:** Our tool could help physicians to determine the prognosis of the disease as well as could help patients to plan for their future. The incorrect prediction of ESKD in a low percentage of patients may be due the positive effect of therapy even in severe cases with baseline poor prognosis. **Keywords:** Clinical Decision Support System, Immunoglobulin A Nephropathy, End-Stage Kidney Disease, Chronic Kidney Disease., Kidney Biopsy

Randomized clinical study to evaluate the effect of personalized therapy on patients with immunoglobulin a nephropathy (IgAN)

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Introduction: IgAN is the most common biopsy-proven glomerulonephritis in the world. Approximately 40% of IgAN patients reach end-stage kidney disease (ESKD) 20 years after their kidney biopsy. The high prevalence of ESKD shows that IgAN has a high economic impact in the countries because renal replacement therapy is costly. This challenge is one more reason to move from generalized therapy for all patients to personalized therapy. Many randomized controlled trials (RCTs) have been conducted, stratifying IgAN patients based on the laboratory findings. In contrast, data from the kidney biopsy has been used only for clinical diagnosis. **Aim:** We have designed a RCT to study personalized therapy in biopsy-proven IgAN patients with active and chronic renal lesions. **Methods:** Our clinical study of IgAN (CLIgAN) is a multicentre, prospective, controlled and open-label randomized clinical trial based on patient's stratification at the time of their kidney biopsy (ClinicalTrials.gov NCT 04662723). We will consider, first, the type of renal lesions followed by serum creatinine values, eGFR and proteinuria. Primary and secondary end points have been established. Second, we will determine whether personalized therapy can slow the decline of the renal function and delay the ESKD. **Results:** We will enroll 132 IgAN patients with active renal lesions (66 patients per arm) in the first RCT (ACIgAN). They will receive corticosteroids combined with renin-angiotensin system blocker (RASB) or RASB alone. Two hundred ninety-four IgAN patients with chronic renal lesions at high or very high risk of CKD (147 patients per arm) will be enrolled in the second RCT (CHRONIgAN) in which they will receive sodium-glucose cotransporter -2 inhibitor (SGLT2-i) combined with RASB or RASB alone. **Conclusion:** Using this approach we hypothesize that patients could receive personalized therapy based on renal lesions to ensure that the right drug gets to the right patient at the right time. **Keywords:** Randomized controlled trial, Immunoglobulin A nephropathy, Kidney biopsy, Corticosteroids, Renin-angiotensin system blockers, Sodium-glucose cotransporter 2 inhibitors

Probing IgA1 using selective digestion with the IgA1 protease from Streptococcus oralis to identify the pathogenic form of IgA1 in IgAN

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Background/Aims/Objectives: Poorly *O*-galactosylated IgA1 (gdIgA1) molecules play a pivotal role in the pathogenesis of IgAN. Selective removal of gdIgA1 using IgA1 proteases could in principle reduce both the circulating pool of IgA immune complexes and mesangial IgA deposits. The hinge region of the IgA1 molecule renders it susceptible to digestion by bacterial IgA1 proteases (IgA1P). *O*-glycosylation of the IgA1 hinge region protects against this digestion. *Streptococcus oralis* synthesises both a β-galactosidase and a neuraminidase to remove galactose and sialic acid before digesting the IgA1 hinge with IgA1P. The aim of this study was to investigate whether we could use the IgA1P of *Streptococcus oralis* to identify specific pathogenic fractions of IgA1 within the circulating IgA pool. **Methods:** The β-galactosidase, neuraminidase and IgA1P were isolated from a 24-hour culture of *Streptococcus oralis* by size exclusion chromatography following ammonium sulphate precipitation. IgA1 isolated from serum and saliva and subfractions of serum IgA1, eluted from a bespoke jacalin agarose column using variable concentrations of galactose solution and with known HMC stimulating capability, were incubated with the glycosidases and IgA1P. Levels of digested and intact IgA1 and gdIgA1 were detected by western blot using anti human IgA or the lectin from *Helix pomatia* (HPA) respectively. **Results/Discussion**: Complete digestion of serum and saliva IgA1 observed by the incubation with the pool of enzymes, but not with IgA1P alone. Undigested IgA1 was also showing to contain gdIgA1. In the IgA1 subfractions displaying the greatest HMC activation capability this gdIg1 was more evident than the digested gdIgA1. This was not seen in the other IgA1 subfractions from the same individual, suggesting HMC activation is associated with a specific configuration of IgA1 hinge *O*-glycans. **Conclusion:** Further characterisation of the *Streptococcus oralis* IgA1P-resistant gdIgA1 may identify important biochemical features responsible for the pathogenicity of IgA1 in IgAN. **Keywords:** IgA1P, Streptococcus oralis, gdIgA1

Unravelling the complexity of pathogenic immune complexes in IgA nephropathy

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Background/Aims/Objectives: Mesangial deposition of IgA containing immune complexes is a key trigger in the pathogenesis of IgA nephropathy (IgAN). Due to the complex nature of these immune complexes, it has been difficult to clarify the relative contribution of IgA1 and the other proteins in these complexes to mesangial cell activation. Most studies have focused on delineating the contribution of IgG, complement proteins or sCD89 in these complexes in combination with poorly *O*-galactosylated IgA1 (gd-IgA1). A comprehensive proteomic analysis of mesangial cell activating immune complexes has not been performed. Therefore, the aim of this study was to to identify proteins enriched in fractions of serum IgA1 that show clear pathogenic capability in IgAN. **Methods:** A novel jacalin purification method was developed to separate serum IgA1 into fractions based on their relative affinity for jacalin. Human mesangial cells (HMC) were exposed to each of the generated IgA1 fractions for 24 hours and the level of IL-6 measured in the supernatants. Each fraction was also analysed for the content of proteins reported to be associated with outcome in IgAN: secretory IgA, gd-IgA1, IgG-IgA immune-complexes by ELISA and western blotting. LC-MS/MS was performed to determine the protein content in those IgA1 fractions associated with the highest and lowest HMC IL-6 response. Paired Student's T test with Benjamini-Hochberg correction was applied for the statistical analysis. **Results/Discussion**: Intriguingly, none of the classical proteins associated with IgAN were enriched in IgA1 fractions exhibiting the greatest HMC IL-6 response. We did, however, identify 23 enriched proteins whose presence was associated with an exaggerated HMC IL-6 response. **Conclusion:** Proteomic analysis of IgA1-containing complexes has identified a number of novel serum proteins that are likely to contribute to the pathogenic potential of circulating immune complexes in IgAN. **Keywords:** immune-complexes, proteomic, Human Mesangial Cells, pathogenic

Clinicopathological features, risk factors, and outcomes of immunoglobulin A nephropathy associated with hepatitis B virus infection

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Background: Hepatitis B virus (HBV) infections are associated with an increased risk of kidney diseases. However, the effects of HBV infection on the prognosis of immunoglobulin A nephropathy (IgAN) are unclear. **Methods:** A total of 838 patients with biopsy-confirmed IgAN were enrolled in this retrospective cohort study. The patients were categorized into either affected by IgAN and HBV infection (HBsAg-IgAN) or by primary IgAN with no sign of HBV infection (P-IgAN). A 1:1 propensity-score matching was performed between the two groups, followed by a Kaplan–Meier survival analysis, to compare the prognoses, and a Cox regression analysis, to identify factors influencing the HBsAg-IgAN outcomes. **Results:** A total of 176 pairs of patients were successfully matched. A significant difference in the systolic blood pressure and urea, serum creatinine, uric acid, and 24-h urine protein levels was observed between the groups. A renal pathological analysis also revealed a significant difference in the mesangial hypercellularity between the groups. During a median follow-up period of 2.4 years, Kaplan–Meier analysis also revealed a significant difference in renal survival between the groups. Furthermore, multivariate Cox analysis confirmed that HBV infection is an independent risk factor for IgAN progression (hazard ratio [HR] 2.096; 95% confidence interval [CI] 1.091–4.026). Finally, the HBsAg-IgAN patients who received treatment with renin-angiotensin-aldosterone system inhibitors had a better overall prognosis than those who received immunosuppressive therapy and antiviral treatment. **Conclusion:** Our results indicate that the clinicopathological features and outcomes of patients with IgAN differ significantly between those with and without HBV infection, and that HBV is an independent risk factor for IgAN progression. **Keywords:** Immunoglobulin A nephropathy, Hepatitis B surface antigen, Risk factor

POSTERS

Relevance of serum Gd-IgA1 levels in South Asian IgAN- prospective longitudinal cohort (GRACE-IgANI)

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The role of serum Gd-IgA1 in a prospective longitudinal South Asian IgAN cohort (GRACE-IgANI) and the impact of immunosuppression is not known. Serum Gd-IgA1 levels were measured in baseline and longitudinal sera (1year, 2year) in IgAN patients, and at baseline in disease controls and healthy controls using KM55 ELISA (IBL international GmBH, Germany). Serum immunoglobulins were measured in a fully automated immunoturbidometric assay (Cobas 8000) (Roche Diagnostics GmbH Mannheim, Germany). Serum immune complex and serum secretory IgA were measured by in-house sandwich ELISAs. Serum Gd-IgA1 levels were not diagnostic of IgAN in our cohort. Baseline serum Gd-IgA1 levels had strong positive correlation serum IgA. Baseline serum Gd-IgA1 levels were significantly elevated in MEST-C S1 and T1/T2 scores. Baseline serum Gd-IgA1 levels were significantly elevated in high-risk baseline group categorized by Tanaka *et al*scoring and in high-risk prediction group categorized by Barbour *et al.* But it did not have significant association with actual composite outcome at 3 years both in the treatment group I (low-risk group without IS) and the treatment group II (high-risk group with IS). There was a significant decrease in Gd-IgA1 levels in treatment group II at 1year post IS and the 1year Gd-IgA1 levels was significantly associated with CO at 3 years, but the AUC showed only moderate discrimination. The longitudinal change in Gd-IgA1 levels paralleled the change in serum IgA levels for the same period. Serum immune complex and serum IgG levels did not show any significant change from baseline to 2 years and along with serum secretory IgA levels did not have any significant association with renal outcomes at 3 years. Baseline serum Gd-IgA1 levels were not diagnostic of IgAN in our cohort and the longitudinal change paralleled the serum IgA levels with 1year Gd-IgA1 levels having only moderate discrimination for CO. **Keywords:** Serum Gd-IgA1, IgA Nephropathy, Serum immune complex, Serum secretory IgA, Serum immunoglobulins, Composite Outcomes, MEST-C score, KM55 ELISA

To be or Not to be- Placebo controlled Immunosuppression trials? Insights from prospective longitudinal GRACE-IgANI cohort

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The role of placebo controlled immunosuppression (IS) trial in high-risk South Asian IgAN is still controversial. The effect of IS on longitudinal biomarkers can be surrogate end-points for assessing its impact on CO. Serum APRIL levels were measured in baseline and longitudinal sera (1year, 2year) by ELISA. Serum Gd-IgA1 levels were measured in baseline and longitudinal sera (1year, 2year) using KM55 ELISA. Serum immunoglobulins were measured by immunoturbidometric assay. Highly sensitive CRP (hsCRP) was measured in baseline and longitudinal sera (1year, 2year) by nephelometry. Serum levels of TNF-RI, CD27 & BCMA were quantified with a Luminex multiplex assay. CO was defined as ≥50% fall in eGFR (CKD EPI) from baseline and/or eGFR (CKD EPI) <15ml/min/1.73m^2 or RRT/death. Partial remission (PR) in proteinuria was defined as decrease of 24-hour urine protein by 50% from baseline and <3g/day if nephrotic at baseline OR proteinuria <1.5g/day if non-nephrotic at baseline for at-least three months. Out of 201 IgAN patients, 37% of patients reached composite outcome (CO) in 3 years in the GRACE-IgANI cohort. Treatment group II (high-risk group with IS) had 146 patients. The mean time to PR in proteinuria was significantly longer in the CO group (10 vs. 6months, p= 0.005). Achieving less than <1g/day at 6month significantly increased renal survival (36 vs. 22months, p<0.001). There was significant longitudinal increase in serum APRIL levels from baseline to 2year in patients with favourable renal outcome. The longitudinal decrease in serum Gd-IgA1 levels at 6month paralleled the serum

IgA levels. There was significant association between baseline inflammatory markers (TNF-R1, CD27 & BCMA) and CO at 3 years whereas baseline elevated hsCRP levels showed a global decrease over time. Placebo-controlled trial in this high-risk population entails ethical considerations hence assessing the known impact of IS on surrogate end-points can potentially guide rational therapy. **Keywords:** Serum Gd-IgA1, IgA Nephropathy, Luminex, ELISA, Composite Outcome, Serum APRIL, CD27, Serum TNF-R1

Long term validation of new prediction score of IgA nephropathy in a French Caucasian cohort

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Background and Aims: The severity of IgA nephropathy is extremely heterogenous. The early stratification of risk to progress to ESRD is crucial. In this aim, International IgA Nephropathy Network recently developped a risk prediction tool to allow an estimation of the probability to progress. The objective of our study was to validate this prediction tool (i) in our French Caucasian retrospective cohort (ii) on long term prognosis (>10 years). **Method:** All biopsy-proven IgA Nephropathy patients from Saint Etienne retrospective cohort for whom proteinuria, mean arterial pressure, CKD-EPI based GFR and adequate Oxford classification at diagnosis were available have been included for analysis. Both models with (model 1) or without ethnical variable (model 2) were evaluated separately through discrimination (Nagelkerke's R^2, c-statistics) and calibration statistical processes (calibration plots at 15 and 20 years). **Results:** A total of 473 patients have been included, with a median age of 38.9 years old, proteinuria of 0.5g/day, a mean CKD-EPI of 81,6 mL/min/1,73m^2. The median follow-up was 12.4 years (IQR 6.4; 19.0). Discrimination performances were R^2 and AUC 0.280 and 0.829 for model 1, 0.289 and 0.834 for model 2. Analysis of deviance found significant greater performances of model 2 over model 1 ($p<0.001$). Calibration performances were obtained from the correlation coefficient between predicted and observed risk along decile groups of predicted risk. The model 1 had a R^2 of 0.944[0.776 ;0.987], and model 2 displayed a R^2 of 0.957 [0.825 ; 0.990] at 15 years. After 15 years the prediction from model 2 was aberrant. R^2 from model 1 at 20 years was 0.942 [0.769,0.986]. **Conclusion:** In our study we validated the IINN tool to predict the risk to progress to ESRD and/or 50% eGFR decline (i) in a French Caucasian cohort and (ii) even at 15 and 20 years. **Keywords:** IgA nephropathy, International prediction tool, Prediction model, External validation, Long term, Caucasian Cohort

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Identification of Proteins Associated with IgA1-containing Circulating Immune Complexes in Patients with IgA Nephropathy

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Background: IgA1-containing immune complexes (IgA1-ICs), consisting of galactose-deficient IgA1 (Gd-IgA1) bound by IgG specific for Gd-IgA1, are central to the pathogenesis of IgA nephropathy (IgAN). We have shown that Gd-IgA1 alone is not sufficient to induce mesangial-cell proliferation and that additional serum proteins are required for IgA1-ICs to become nephritogenic. To elucidate the composition of IgA1-ICs, we have developed a novel proteomic-bioinformatic workflow to identify proteins in IgA1-ICs in patients with IgAN. **Methods:** IgA1-ICs from sera of 20 patients and 14 healthy controls were isolated by lectin affinity chromatography followed by size-exclusion chromatography (SEC). Quality-control test confirmed that most IgA1-ICs and free IgA1 were captured by affinity chromatography. IgA1-ICs were separated by SEC from monomeric and polymeric IgA1. After IgA-specific protease and LC-MS sequence-grade trypsin digestion, each IgA1-IC sample was analyzed by liquid chromatography coupled online with mass spectrometry (LC-MS). After standard proteomic database searches, LC-MS results were extensively curated by use of *Scaffold perSPECtives* to identify proteins enriched in IgA1-ICs of IgAN patients vs. healthy controls. Additional comparisons included polymeric and monomeric IgA1. **Results:** Eighty-nine proteins were identified in IgA1-ICs samples from IgAN patients, with a false discovery rate of 1%. After proteomic-bioinformatic curation, we generated a list of 41 proteins with high-confidence identification that were uniquely enriched in the IgA1-ICs from patients with IgAN. Using Principle Component Analysis, we confirmed that protein content differentiated the three molecular forms of IgA1, monomeric, polymeric, and IgA1-IC. Pathway analysis indicated that proteins in IgA1-ICs were part of the complement

cascade, with seemingly more enrichment in the regulation of complement, and the plasma lipoprotein pathway. **Conclusion:** Our new workflow enabled targeted identification and evaluation of proteins associated with IgA1-ICs in IgAN. These proteins represent new targets to be evaluated for their roles in formation and activity of nephritogenic IgA1-ICs in IgAN. **Keywords:** Complement, Immune complexes, Targeted proteomics, Mass spectrometry

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Atrasentan in Patients with Proteinuric Glomerular Diseases (The AFFINITY Study)

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Background: Glomerular diseases are a leading cause of morbidity and mortality worldwide and are characterized by proteinuria, a strong predictor of disease progression and end-stage kidney disease. Currently, there are limited therapies and despite the recent approval of sodium glucose co-transporter 2 inhibitors (SGLT2i), residual risk remains. It is known that endothelin A (ETA) receptor activation drives glomerular injury, inflammation, and fibrosis through a common pathogenic pathway. Atrasentan, a potent and selective ETA antagonist that has shown clinically significant reduction in proteinuria in a study of over 5,300 patients with diabetic kidney disease (DKD), represents a promising therapy to reduce proteinuria and preserve kidney function in proteinuric glomerular diseases. **Objective:** Global, phase 2, open-label basket study to study efficacy and safety of atrasentan in patients with proteinuric glomerular diseases. **Methods:** Patients in the United States, Australia, South Korea, Spain, Italy, and the United Kingdom with proteinuric glomerular diseases will be enrolled in a basket study to receive 0.75 mg atrasentan orally for 52 weeks. Four cohorts are planned: IgAN, Alport syndrome, FSGS, and DKD. Patients must be receiving a maximally tolerated and stable dose of RASi and patients with DKD must also be on a stable dose of a SGLT2i. Proteinuria must be present in all patients: IgAN (urine protein creatinine ratio [UPCR] between 0.5 and < 1.0 g/g), FSGS (UPCR > 1.5 g/g), Alport syndrome (UPCR > 0.5 g/g), and DKD (urine albumin creatinine ratio [UACR] ≥ 0.5 g/g). Participants will have study assessments over 1 year with options for remote study visits using telemedicine and home health visits. The primary objective is to evaluate the change in proteinuria (IgAN, FSGS, AS) or albuminuria (DKD) from baseline to Week 12. Key exploratory objectives include changes in eGFR from Baseline to Week 52 and changes in audiology assessments in patients with Alport syndrome. **Keywords:** Atrasentan, Endothelin, Phase 2 Clinical Trial, IgA Nephropathy, Alport Syndrome, FSGS, Diabetic Kidney Disease

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The Association between Hematuria and Renal Outcomes in Immunoglobulin A Nephropathy: a Systematic Review and Meta-analysis

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Background: For patients with immunoglobulin A nephropathy (IgAN), damaged renal function, hypertension and proteinuria are established as high-risk long-term prognostic factors. But the long-time outcomes of these IgAN patients having normal renal function present with only isolated microscopic hematuria, with minimal or without proteinuria are controversial. **The aim** of this meta-analysis was to clarify the effect of hematuria on renal outcomes in IgAN. **Methods:** Observational cohort studies reporting associations between various forms of hematuria and renal outcomes among IgAN patients were identified from the PubMed and Embase databases. The pooled adjusted risk ratios (RRs) were computed with random effects models. **Results:** Sixteen studies encompassing 8260 patients with IgAN were included. Persistent hematuria was an independent risk factor for end-stage renal disease (ESRD) or a 50% decline in eGFR (RR, 1.83; 95% confidence interval [CI], 1.32-2.53; P = 0.004). Initial microscopic or mild hematuria was associated with an 87% increase in the risk of ESRD (RR, 1.87; 95% CI, 1.40-2.50; P < 0.001), while macroscopic hematuria was associated with a 29% decrease in the risk of ESRD (RR, 0.71; 95% CI, 0.63-0.80; P < 0.001). **Conclusions:** Among IgAN patients, hematuria, including persistent hematuria and even initial mild or microscopic hematuria, was associated with a higher risk for renal diseases progress and ESRD. However, independent of other classical predictors, initial macroscopic hematuria was a protective factor for IgAN. **Keywords:** Hematuria, IgAN, Systematic Review, Meta-analysis, Renal Outcomes

A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study of Atrasentan in Patients with IgA Nephropathy (The ALIGN Study)

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Background: IgA nephropathy (IgAN) is the most common primary glomerulonephritis. Up to 40% of patients with IgAN are at risk of progressing to end-stage kidney disease (ESKD), proteinuria is the strongest predictor of progression. Endothelin A (ETA) receptor activation drives proteinuria, along with kidney inflammation and fibrosis. Atrasentan, a potent and selective ETA antagonist, has been studied extensively in >5,000 patients in a global phase 3 outcome clinical trial in patients with diabetic kidney disease who were on a maximum tolerated dose of RAS inhibitor (RASi). Results showed a 35% reduced risk of the primary composite outcome of doubling of serum creatinine or ESKD (95% CI: 0.49, 0.88; P = 0.005). The most common adverse event was fluid retention. Selective ETA blockade represents a promising approach to reduce proteinuria and preserve kidney function in patients with IgAN at high risk of progression. **Objective:** A global, phase 3, double-blind, placebo-controlled study is in progress to determine the effect of atrasentan in IgAN patients at high risk of kidney function loss. **Methods:** Approximately 320 patients across North America, South America, Europe, and Asia-Pacific with biopsy-proven IgAN will be randomized to receive 0.75 mg atrasentan or placebo daily for 132 weeks. Patients will continue receiving a maximally tolerated and stable dose of a RASi as standard of care. The study will also include patients that are unable to tolerate RASi therapy. Additional eligibility criteria include urine protein creatinine ratio (UPCR) ≥1 g/g and eGFR ≥30 mL/min/1.73 m^2. Participants will have study assessments over two and a half years with options for remote study visits using telemedicine and home health visits. The primary objective is to evaluate change in proteinuria at Week 24. Secondary objectives include change from baseline in eGFR, safety, and tolerability, and quality of life. **Keywords:** Atrasentan, Endothelin, Phase 3 Clinical Trial, IgA Nephropathy

Children with IgA nephropathy and Henoch-Schoenlein purpura in Czech registry of renal biopsy (CRRB) 1994–2018

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Aims and Objectives: This study aims to analyze children with IgA nephropathy (IgAN) and with Henoch-Schönlein purpura nephritis (HSP) in the CRRB. It includes practically all renal biopsies (RB) of native kidney in children in the Czech Republic (covering about 10.5 mil. population) performed in 11 pediatric centers. During 1994-2018 time period, 15,382 RBs were carried out in the Czech Republic, of which 2,680 RBs were in children and adolescents under 18 years of age (17.4%). **Results:** IgAN, the most common diagnosis, was found in 587 (21.9%) RBs, (66.8% of boys), mean age was 13.7±3.9 years. HSP was found in 110 (4.1%) children (boys 53.6%), mean age 11.4±4.6%, p<0.0001. The following data on arterial hypertension (HT) at the time of RB were found: IgAGN 15.7% of children and HSP 28.2%, p<0.01. Macroscopic hematuria: IgAN 34.9% and HSP 28.2%. Since 2001 we have body height and weight at our disposal. Therefore we can use the eGFR: IgAN 1.6±0.5, HSP 1.7±0.6 n.s. (ml/s/1.73m^2). Mean proteinuria: IgAN 0.05±0.07, HSP 0.06±0.06 n.s. (g/m^2/h). BMI: IgAN 20.5±4, HSP 19.2±4.5 p<0.05. **Conclusions:** IgAGN is the most common diagnosis in the CRRB. HT is associated with known risk factors for progression of biopsy-proven GN such as GFR or proteinuria. Children with HSP have more severe findings. HSP is an acute disease, while IgAN is a chronic, slowly progressive glomerulopathy. The Registry of RB provides important information about the epidemiology of glomerulonephritis in our region, indications for performing RB, and represents a basis for cooperation in this field. **Keywords:** IgA nephropathy, Henoch-Schönlein purpura nephritis, children, renal biopsy

Risk factors of elevated serum homocysteine in patients with IgA nephropathy

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Background: Clinicopathological risk factors of elevated serum homocysteine (Hcy) among IgA nephropathy (IgAN) patients were not yet well understood. The aim of this study was to investigate risk factors of elevated serum Hcy in IgAN patients. **Methods:** 310 IgAN patients were enrolled in this single-center retrospective study. According the median level of Hcy of 12.1μmol/L, 154 patients were assigned to high Hcy group and 156 patients in low Hcy group. Hcy > 12.1μmol/L was defined as hyperhomocysteinemia (HHcy). Demographic and clinicopathological data were collected and analyzed. Spearman's rank correlation and logistic regression analysis was performed to detect the association between Hcy and clinicopathological features. **Results:** The median age was 34.00 (27.00, 46.00) years. The level of Hcy was gradually increased with the progression of CKD stage (P<0.001). Compared to low Hcy group, the level of age, mean arterial pressure (MAP), serum creatinine (Scr), blood urine nitrogen (BUN), triglyceride (TG), complement 4 (C4), erythrocyte sedimentation rate (ESR), T score was significantly higher in high Hcy group, while the female rate, estimated glomerular filtration rate (eGFR), superoxide dismutase (SOD), immunoglobulin M (IgM) was significantly lower in high Hcy group. Hcy levels were positively correlated with age, MAP, 24-hour urine protein, Scr, BUN, TG, C4, ESR, T score, but negatively related with eGFR, SOD, IgM. Multivariate logistic regression model showed that male (OR=2.300, 95%CI=1.195-4.428, P=0.013), age (OR=1.028, 95%CI=1.002-1.054, P=0.035), high Scr (OR=1.016, 95%CI=1.005-1.028, P=0.006) and pathologic T (OR=2.183, 95%CI=1.002-4.754, P=0.049) were independent associated with HHcy in IgAN patients. **Conclusions:** IgAN patients with elevated serum Hcy displayed more severe clinicalpathologic characteristics. Male, age, high Scr and Pathologic T were risk factors of elevated serum Hcy in IgAN patients. **Keywords:** IgA nephropathy, homocysteine, hyperhomocysteinemia, clinicopathological characteristics

The clinicopathological characteristics and outcomes of IgA nephropathy with predominant lambda or kappa light-chain deposition

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Background: IgA nephropathy (IgAN) patients with monoclonal light-chain deposition may be at potential risk of hematological progression. However, whether the clinical characteristics of the patients with the predominant lambda or kappa light-chain deposition were consistent with monoclonal light-chain deposition are limited to anecdotes. **Methods:** The predominant lambda or kappa light-chain deposition was defined as the deposition intensity of kappa or lambda being ± and the other deposition intensity being ≥ 2+. We reviewed 19 consecutive IgAN patients with predominant lambda or kappa light-chain deposition between January 2016 and January 2020. **Results:** The patients had a median age was 32 years. The median proteinuria was 0.9 g/day. The median eGFR was 79.8 ml/min per 1.73 m^2. One patient had mild abnormal FLC ratio of 1.67, but serum immunofixation electrophoresis showed polyclonal immunoglobulin. All 19 patients had mesangial proliferative glomerulonephritis. Eighteen patients showed lambda light chain-dominated deposition. In electron microscopy, organized structures in dense deposits were not observed in all patients. Nine patients with proteinuria ≥1.0 g/day received corticosteroids and immunosuppressant. The median follow-up time was 14 months. The rate of proteinuria remission was 50%. After 1:1 matching, the terms of pathological characteristics and outcomes were not significantly different between the study groups. **Conclusion:** The result for IgAN patients with predominant kappa/lambda light-chain deposition seemed to be the same as the IgAN patients with light-chain codeposition. However, due to this being a single-center study with a small size, further multicenter studies and long-term follow-up are needed to confirm our findings. **Keywords:** IgA nephropathy, chain light, immunofluorescence

Serum and urine biomarkers related to renal fibrosis predict renal outcome in Czech patients with IgA nephropathy

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Background: IgA nephropathy (IgAN), the most common primary glomerulonephritis worldwide, has serious outcomes with end-stage renal disease developing in 30–50% of patients. Clinical predictors such as proteinuria, hematuria, hypertension as well as renal fibrosis may play a role in IgAN onset and/or progression. We evaluated serum and urine biomarkers related to renal fibrosis and histological findings in renal-biopsy specimens from patients with IgAN. **Methods:** We assessed 134 patients with biopsy-proven IgAN who were assessed at time of diagnosis for estimated glomerular filtration rate (eGFR), proteinuria, microscopic hematuria, hypertension, then followed prospectively (mean follow-up 6 years). Serum and urine samples collected at diagnosis were analyzed for biomarkers related to renal fibrosis using a novel enzyme-linked immunosorbent assay as well as histological evaluation of renal tissues at time of kidney biopsies were assessed. Linear discriminant analysis, logistic regression model were used for statistic processing. **Results:** We found serum and urine biomarkers such as PRO-C6, PRO-C3, serum LG1M and urinary C3M which completely differentiated patients with IgAN according to renal parameters at the onset into three groups [S-Creatinine (µmol/L): 1. group <= 120, 2. group <= 180, 3. group > 180] which correlated with the level of histological fibrosis in kidney biopsies ($P<0.05$, accuracy of classification 100 %) and exactly predicted renal outcome of patients with IgAN ($P<0.05$). **Conclusion:** In conclusion, serum and urine biomarkers related to renal fibrosis such as PRO-C6, PRO-C3, serum LG1M and urinary C3M/creatinine predicted renal outcome of patients with IgAN. Future studies are needed to validate these preliminary findings and to determine the power of these urinary and serum markers for assessment of responses to treatment. **Keywords:** IgA nephropathy, biomarkers, PRO-C6, PRO-C3, LG1M, C3M/creatinine

The role of complement C3 activation in clinical presentation and prognosis of IgA nephropathy – national multicenter study in children

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Aim: The aim of this study was to asses the role of complement C3 in renal biopsy and serum for the prognosis of IgAN in children. **Methods:** This retrospective study consisted of 148 children from Polish IgAN, IgAVN Registry in Children. Exclusion criteria: incomplete clinical data, <8 glomeruli in kidney biopsy, secondary IgAN, IgAVN. Proteinuria, serum albumin, creatinine, GFR, IgA, C3, C4 were analyzed twice: at the beginning and at the end of the observation. Renal biopsy deposits of IgA, G, M, C3, and C1 were analyzed, as well as the Oxford MEST-C classification. Depending on mesangial C3 deposits, the parameters were analysed in groups: A (C3≤+1, n=88) and B (C3> +1, n=50), and serum C3 level – in groups: N (normal, n=135), D (decreased, n=13). The study endpoint was abnormal glomerular filtration rate (eGFR < 90ml/min). **Results:** The mean age of

diagnosis of IgA nephropathy was 11±4.29 years. Renal biopsies were performed on average 1.2±1.77 years after initial symptoms, follow-up was 45±34.8 mths. There were no significant differences between the sum of MEST-C in the A and B. Significantly lower serum C4 levels were observed in group D than in group N. Cox proportional hazards survival curves analysis showed shorter survival time of kidneys with normal GFR in children in group B than A. In the analysis of survival curves, survival time was also affected by female sex (K>M), later time of disease diagnosis, and normal GFR at disease onset but not reduced C3 levels at disease onset. **Conclusions:** The severity of mesangial C3 deposits in renal biopsy>+1 has an impact on the length of renal survival from normal GFR in children with IgAN. Decreased serum C3 is not a prognostic factor in this group of children, but perhaps this finding needs to be confirmed in a larger group. **Keywords:** complement C3, IgA nephropathy, children

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Longitudinal changes of IgA1 O-glycoforms in IgA nephropathy

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Background and Aims: Aberrantly glycosylated IgA is associated in the pathogenesis of IgA nephropathy (IgAN). We previously established a high-throughput method for analysis of IgA1 HR *O*-glycoforms using liquid chromatography-high-resolution mass spectrometry (LC-HRMS). To identify the molecular-level characteristics of IgA1 hinge-region (HR) glycoform(s) in IgAN and to assess their changes after therapy (with or without corticosteroids (CS)), we profiled IgA1 HR glycopeptides from sera collected at two time points (before and after therapy) from Japanese IgAN patients. **Method:** Of the 10 Japanese IgAN patients recruited, 4 received CS treatment (CS group) and 6 have not (non-CS group). Japanese healthy volunteers (HC, n=10) were recruited as controls. Serum IgA1 was purified by affinity chromatography from HC and IgAN patients before and after therapy. IgA1 HR glycosylation heterogeneity was analyzed by LC-HRMS. The relative abundance (RA, %) for each glycopeptide was calculated as percentage to the total IgA1 HR glycopeptide. The amount of each glycopeptide was then calculated by multiplying serum IgA concentration (mg/dL) by RA. **Results:** Approximately 60% of IgA1 HR *O*-glycoforms in IgAN patients and HC were galactose deficient (Gd) *O*-glycoforms; these glycoforms contained one to three Gd-glycan(s), designated as 1 Gd-glycoform, 2 Gd-glycoform and 3 Gd-glycoform, respectively. In IgAN patients, the RA of non Gd-IgA1 glycoforms was elevated (P=0.002). The amounts of non Gd- and 1Gd-glycoforms were higher in IgAN patients compared to HC (each P<0.001). After several years of follow up (2.77 years (1.44-3.85)), the RA of non Gd-glycoforms decreased in CS group of IgAN patients (P=0.039) whereas it remained unchanged in the non-CS group (P=0.488). The amount of non Gd-glycoforms exhibited similar trends, *i.e.,* decreased in CS group (P=0.068) whereas it remained unchanged in the non-CS group (P=0.943). **Conclusion:** IgA1 HR *O*-glycoforms altered by treatment may serve as a biomarker(s) for monitoring patients' responses to therapy. **Keywords:** O-glycosylation, Longitudinal changes, mass spectrometry

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Total number of functional glomeruli at the biopsy diagnosis and disease outcomes in patients with IgA nephropathy: A retrospective observational study

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Background/Aims/Objectives: The total functional glomeruli at diagnosis may vary among IgA nephropathy (IgAN) patients due to several factors, including the innate number at birth, disease progression from the onset to the diagnosis, comorbidities and aging. Using a newly established method, we estimated the total glomeruli per kidney in IgAN patients and cross-sectionally studied the relationships with clinicopathological factors (CKD stages, hypertension, heavy proteinuria and MESTC histopathological scores) known to predict disease outcomes (Kidney 360, 2021). We examined the impact of the total glomeruli on disease outcomes during follow-up in IgAN patients. **Methods:** The total glomeruli per kidney was estimated by combined cortical volume assessments of unenhanced computed tomography images and stereology-based measurements of the glomerular density at biopsies. The impact of the total number of non-globally sclerotic glomeruli (NSG) and globally sclerotic glomeruli (GSG) on primary (≥30% reduction in estimated glomerular filtration rate [eGFR] and/or ESRD) and secondary (proteinuria <0.3 g/day 1-year post-diagnosis) outcome measures was retrospectively examined. **Results/Discussion:** Among 222 patients (43 years old, 62% male, eGFR 61 mL/min/1.73 m^2, average 5.8 years of follow-up), total NSG and GSG ranged from 78,000-

2,028,000 and 0-1,528,000 per kidney, respectively. Comparisons of tertile groups categorized based on NSG showed significant associations of lower NSG with the primary outcome (Log-rank test, p for trend <0.001) and higher NSG with the secondary outcome. The hazard ratio for the primary outcome adjusted for clinical confounding factors (age, gender, eGFR <60 mL/min/1.73 m^2, proteinuria ≥1.0 g/day, hypertension, RAS inhibitor use, corticosteroid use) was 1.11 (95% confidence interval, 1.01-1.19) per 100,000 decrease in NSG (Cox proportional hazard model, p=0.03). Total GSG per kidney was not associated with any disease outcomes. **Conclusion:** Total NSG per kidney on diagnostic biopsies is associated with kidney outcomes independently of known clinical prognostic factors in patients with IgAN. **Keywords:** Glomerular number, kidney biopsy, kidney outcome

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Histomorphometric analysis of tonsillar components in patients with IgA nephropathy: A cross-sectional study for the correlations with clinical and renal histopathological finding

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Background/Aims/Objectives: Disruption of the mucosal immune system, especially in palatine tonsils (PT), has been postulated to be involved in the pathogenesis of IgA nephropathy (IgAN) due to their beneficial effect on renal function outcomes after tonsillectomy (Hirano et al. JAMA Netw Open, 2019). Morphologically and functionally, PT comprises three major compartments; the reticulated crypt epithelium, the extrafollicular area (EFA), and the lymphoid follicles (LF) consisting of the mantle zone (MZ) and the germinal center (GC). The association between the histological changes of PT in IgAN and its clinical and renal histopathological findings has not been fully investigated. We have conducted the study to address this question. **Methods:** Eighty-seven patients with IgAN who underwent tonsillectomy within one year after renal biopsy were included in the study. As a comparison, tonsils from age-matched patients (N=27) with recurrent tonsillitis (RT) were employed. Serial sections were stained with anti-HLA-DR, CD3, and cytokeratin antibodies, to identify each compartment, and the area for positive immunohistochemistry was measured using computed image analysis. **Results/Discussion**: The mean ages at tonsillectomy were 35 years in both groups. In the comparison with RT, the mean size of LF and GC in IgAN patients was significantly smaller than in RT (p<0.0001). Similarly, the relative area of LF (%LFA) was smaller in patients with IgAN than RT (22.8% vs. 31.4%, *p*<0.0001). Contrarily, mean EFA was larger in patients with IgAN compared to RT patients. Comparisons of tertile groups categorized based on %LFA showed a significant association of lower %LFA with amounts of urinary protein excretion (*p*=0.006). Frequencies of patients with S1 and C1+2 according to the Oxford classification increased in fewer tertile groups of %LFA. Further, the frequencies of global glomerulosclerosis or crescent were inversely correlated with %LFA (ρ=-0.46, *p*<0.0001). **Keywords:** Tonsils, lymphoid follicles, extrafollicular area, Histomorphometric analysis

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Three-year clinical outcomes of the first South-Asian prospective longitudinal observational IgA nephropathy cohort (GRACE-IgANI)

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Glomerular Research and Clinical Experiments-IgA Nephropathy in Indians (GRACE-IgANI) is the first prospective South-Asian IgAN cohort with prespecified objectives, and protocolized longitudinal follow-up. Out of 201 incident adults with kidney biopsy-proven primary IgAN recruited into GRACE-IgANI, 195 patients (97%) had completed 3year longitudinal follow-up in September 2020. Rapid progressors (RP) were defined as average annual fall in eGFR ≥5ml/min/1.73m^2. Composite outcome (CO) was defined as ≥50% fall in eGFR from baseline and/or eGFR (CKD EPI) <15ml/min/1.73m^2 or RRT/death. The use of RASB was consistent (66-75%) throughout and a short course IS was given to patients with proteinuria ≥1g/day and/or renal impairment (73%). 76 patients (39%) were RP and 72 patients (37%) had CO at 3 years. At each scheduled follow-up proteinuria <1g/day significantly increased time to CO. The ROC curve of average annual decline in eGFR ≥5ml/min/1.73m^2 had

82% sensitivity and 89% specificity for CO and a good discrimination from 1 year (AUC 0.81) onwards. The significant predictors for CO were MEST-C T2 score (HR 4, 95% C.I. 2-9, p<0.001) at baseline, haemoglobin ≤12 g/dL (HR 2, 95% C.I. 1-5, p=0.02) at 6months, 24-hour urine protein ≥1g/day at 6months combined with serum albumin ≤4 g/dL (HR 2, 95% C.I. 1-5, p=0.02) at 1year, and fall in eGFR ≥5ml/min/1.73m^2 at 1year (HR 4, 95% C.I. 2-8, p<0.001). Mortality was 6% in the cohort. In the high-risk baseline group given IS, there was sustained reduction of proteinuria to <1.5g/day in 6month and < 1g/day in 1year in patients with favorable renal outcome and the time taken for proteinuria remission significantly impacted the eGFR decline and the CO. The South-Asian IgAN had CO in 37% of patients at 3year and longitudinal clinical variables along with baseline MEST-C T2 score predicted poor renal outcome. **Keywords:** IgA nephropathy, Rapid progressors, MEST-C score, Composite outcome, Immunosuppression, Glomerulonephritis

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The prognostic value of immunosuppressive therapy in IgA nephropathy with stage 3 or 4 chronic kidney disease

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Background: It is debated whether IgAN patients with heavy proteinuria and renal insufficiency benefit from more aggressive treatment consisting of corticosteroids or combined with immunosuppressive agents. **Methods:** A retrospective study was performed between January 2008 and December 2016 on patients with IgAN who had urinary protein excretion > 1.0 g/d and an eGFR between 15–59 ml/min/1.73 m^2 were included. These patients were assigned to receive supportive care alone or supportive care plus immunosuppressive therapy. The primary outcome was defined as the first occurrence of a 50% decrease in eGFR or the development of ESRD. **Results:** All included 208 patients were followed for a medial of 43 months, 92 (44%) patients experienced the primary outcome. Cumulative renal survival was better in the immunosuppression group than in the support-care group (P < 0.001). The median annual rate of renal function decline in the immunosuppression group was -2.0 (-7.3 to 4.2) ml/min/1.73 m^2, compared with -8.4 (-18.9 to -4.1) ml/min/1.73 m^2 in the supportive-care group (P < 0.001). In multivariate Cox regression analyses, immunosuppressive therapy was associated with lower risk of progression to ESRD, independent of age, sex, eGFR, proteinuria, mean arterial pressure (MAP), renal histologic findings and the use of renin-angiotensin system inhibitors (RASi) (HR = 0.335, 95% CI 0.209-0.601). Among the adverse events, infection requiring hospitalization occurred at similar rates in both groups (P = 0.471). **Conclusion:** Immunosuppressive therapy attenuated the rate of renal function decline and was associated with a favorable renal outcome in IgAN patients with heavy proteinuria and renal insufficiency, and the side effects were tolerable. **Keywords:** IgA nephropathy, survival analysis, immunosuppressive, stage 3 or 4 chronic kidney disease, prognostic

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Plateletcrit in IgA nephropathy

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Aims: Our aim is to explore new biomarker of IgA nephropathy progression. **Methods:** We test plateletcrit of patients of IgA nephropathy and the relationship between it and disease severity was analyzed. **Results:** We found that patients with severe proteinuria have lower plateletcrit. **Conclusion:** Plateletcrit can be a new biomarker of IgA nephropathy progression. **Keywords:** plateletcrit, IgA nephropathy, biomarker

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