An update on pathology of IgA nephropathy
Atualização em nefropatia da IgA

ABSTRACT
IgA Nephropathy (IgAN) is the commonest of the glomerular diseases in the world. Its progression rate of 30-40% of the cases em 20-30 years makes IgAN an important healthcare issue in Nephrology. Diagnosis of IgAN depends on biopsy findings, particularly at immunofluorescence microscopy. The frequency of IgAN diagnosis is variable in different populations and depends on screening and biopsy indication policies. IgAN pathogenesis is considered multifactorial; its primordial defect is the production of galactosis-deficient IgA molecules. This review paper discusses the most up-to-date aspects of the pathogenesis, pathological classification and clinical implications of IgAN.

Keywords: glomerulonephritis; glomerulonephritis, IgA; immunoglobulin A; pathology.

INTRODUCTION
IgA Nephropathy (IgAN) is a primary renal disease, or part of the syndrome of Henoch-Schoenlein Purpura, or secondary to a series of extrarrenal conditions, namely liver and gastrointestinal diseases. It was first recognised in 1968, when Berger and Hinglais reported the association of hematuria, proteinuria and upper respiratory tract infections. Renal biopsy samples of affected patients showed variable light microscopy changes, apart from proliferative and exsudative glomerulonephritis.

Immunofluorescence microscopy findings were however consistent: intense mesangial IgA deposits, with less intense deposits of IgG and C3. Such observations are seemingly coincidental with previous reports by Volhard and Fahr, in 1914, and by Bates, Jennings and Earle, in 1957, who described a focal acute glomerulonephritis, coincidental with infectious conditions, but rarely followed by overt nephritic syndrome.

Today IgAN is regarded as the commonest glomerular disease in the world. Its slow but unyielding progression causes 30 - 40% of the patients to progress to end stage renal disease (ESRD) in 20 - 30 years. Thus, alongside diabetic nephropathy, IgAN emerges as an important health-care issue in Nephrology. As opposed to diabetic nephropathy, however, IgAN tends to affect younger individuals, who are thus burdened by a lifelong chronically progressive disease. This review intends
to highlight the main and most recent features regarding the pathogenesis and pathology of IgAN.

**Epidemiology**

Diagnosis of IgAN largely depends on the screening policies and biopsy indication. In Brazil, a series of 9617 renal biopsies obtained countrywide revealed that IgAN represents 9.6% of all and 20.1% of the primary glomerular diseases. Its clinical presentation is variable: proteinuria – usually non-nephrotic – associated with macroscopic or microscopic haematuria and hypertension.

In the USA 50% of the IGAN patients older than 30 years old has Stage 3 to Stage 5 chronic kidney disease at the diagnosis. In North American and European cohorts, the male to female ratio in affected individuals is 2:1; incidence peaks in the second and third decades of life. A recent series of 600 Brazilian IgAN cases depicted a slight male predominance (1.24:1), with an average of 33 years old at diagnosis. Hematuria was the main presentation feature (72% of patients), followed by proteinuria (57% of patients), which was nephrotic in 28% of them.

**Pathogenesis**

**Inheritance pattern**

IgAN is regarded nowadays as a slowly progressive disease, with both familial and sporadic occurrence. IgAN may be inherited in an autosomal dominant pattern, with reduced penetrance. Exome analysis has so far detected several candidate genes involved in the pathogenesis of IgAN, which are implicated in: a) antigen processing, cytokine production and antigen presentation by class I major compatibility complex (MHC-I); b) mucosal immunity; c) alternative complement pathway regulation. Loci apparently involved in these pathways are 6q22-q23, 3p24-p23, 2q36, 4q26-q31 and 17q12-q22.

**Structure and production of IgA**

Human IgA is mainly produced in mucous membranes; a small fraction of it is found in circulation. Human polymeric and monomeric IgA exists as IgA1 and IgA2. IgA polymers are composed of subunits aggregated by a J-chain. Transepithelial transport of IgA to luminal surface is done by polymeric immunoglobulin receptor (pIgR), which exhibits affinity to the J-chain. After luminal secretion, part of the pIgR is retained within the polymeric IgA molecule - the so called secretory component.

The J-chain is therefore essential to IgA transepithelial transport; thus, all IgA produced in mucous membranes is polymeric. Five IgA receptors are known in humans: CFR (constant fraction receptor), ASGR (asialoglycoprotein receptor), plgR, transferrin receptor and Fc α/μ receptor. Only the latter two were found in renal tissue, exclusively in mesangial cells.

IgA1 shows peculiar molecular features that differentiate it from IgA2, IgM and IgG. A binding site with two to six O-glycans is located in the hinge region, between the first and second regions of the constant domain of IgA1 heavy chain. O-glycan synthesis has the following steps: 1- addition of N-acetylgalactosamine to serine or threonine residues; 2- binding of a galactosis molecule to the β1,3 conformation; 3- binding of sialic acid to galactosis or N-acetylgalactosamine - a step that prevents further conformational changes.

IgAN is the product of a multihit process. The primordial defect lies in the production of aberrant IgA1 molecules, which are deficient in galactosis in their hinge region O-glycan residues (Gd-IgA1). Gd-IgA1 have their hinge region N-acetylgalactosamine residues exposed. A further necessary step into the pathogenesis of IgAN is the production of antibodies - IgA or IgG - against galactose-deficient sugar residues in Gd-IgA1.

This step is assisted by toll-like receptors (TLRs), which are involved in the response to the exposure to bacterial wall and viral envelope components, and in the polyclonal activation of B lymphocytes and immunoglobulin production, independently of preceding T cell activation. The formation is IgG-Gd-IgA1 immune complexes is facilitated by Gd-IgA1 greater affinity to IgG. Gd-IgA1 bound to antiglycan antibodies are not amenable to linkage to asialoglycoprotein receptors on hepatocytes, which eases its escape from hepatic metabolism and its persistence in circulation.

In addition, the more sialylated the IgA1 molecule, the more negatively charged it is and the greater its affinity to mesangial cells and its resistance to hepatic removal. To sum up, the determinant events in pathogenesis of IgAN are the generation of Gd-IgA1 immune complexes, their affinity to mesangia and defective clearance from circulation and mesangia, and renal tissue responses.
In the mesangia, Gd-IgA1 immune complexes may bind to fibronectin, type IV collagen, CD71 or mesangial cell integrins.

Activated mesangial cells secrete extracellular matrix components; have the NF-κB signal transduction pathway activated and the nitric oxide synthase expression enhanced; secrete renal lesion mediators like angiotensin II (AT-II), cytokines (interleukin-6 [IL-6], transforming growth factor-β [TGF-β], macrophage chemotactic protein-1 [MCP-1], tumour necrosis factor-α [TNF-α]) and profibrotic factors.

TNF-α elicits a pro-apoptotic state on visceral epithelium, since podocytic TNF-α receptors 1 and 2 (TFR1 and TNFR2) are upregulated in IgAN. The sustained inflammatory stimulus causes mesangial hypercellularity, apoptosis, oxidative stress and mesangial matrix expansion.

**Pathology**

From the pathologist’s viewpoint, the diagnosis of IgAN is relatively simple: it is defined by the identification by immunofluorescence (IF) or immunoperoxidase (IP) microscopy of dominant to co-dominant granular to globular IgA deposits in mesangial areas, and less frequently (24-54% of the cases), in glomerular capillary loops. It is noteworthy that 3-16% of healthy individuals present glomerular IgA deposits of undetermined significance.

Electron microscopy (EM) is not mandatory for the diagnosis of IgAN. Nevertheless, it is used to confirm the presence of mesangial electron-dense deposits. Capillary wall and subendothelial deposits may be present, respectively, in 50% and 55% of the cases. Subendothelial deposits might be associated with mesangial interposition and basement membrane duplication. Subepithelial deposits are highly atypical and should be interpreted as a superimposed immune complex mediated disease, not related to IgAN.

At light microscopic level, however, IgAN is as highly variable as its clinical presentation and prognosis. The seminal work by Berger described focal glomerulonephritis in most of the biopsies; 10% of the cases showed no changes at light microscopy. Subsequent observations led to the demonstration of a wider range of histopathological aspects, which included segmental sclerosing lesions and endocapillary and extracapillary proliferative lesions (Figure 1).

Single grade classification schemes were proposed by Lee and Haas. Lee’s and Hass’ classification schemes admit five classes, to which pertain histopathological findings of the glomerular and tubulo-interstitial compartments, with an emphasis on the formers (Table 1).

To these schemes, Alamartine added, in 1990, a semi-quantitative classification scheme, which yielded a global optical score. Whilst this system relatively succeeded in establishing reasonable clinical-pathological correlation, semi-quantitative scoring systems are quite demanding and have limited reproducibility. The simplicity and similarity with the WHO classification of lupus nephritis made the single grade schemes widespread and accepted amongst pathologists.

In attempts to predict clinical course, guide treatment selection, stratify patients for clinical trials and predict the outcome, members of the Renal Pathology Society (RPS) and of the International IgA Nephropathy Network (IIGANN) assembled to propose a consensual and evidence-based histopathological classification of IgAN.

Efforts concentrated on 1- identifying and defining which lesions are characteristic of IgAN; 2- determining which were the lesions least prone to sampling and reproducibility limitations; 3- correlating lesions and clinical outcome. After the analysis of 265 renal biopsy samples of IgAN, from patients followed for 5 years, mesangial hypercellularity (M), segmental sclerosis/adhesions/synechiae (S), endocapillary hypercellularity (E) and tubular atrophy/interstitial fibrosis (T) emerged as the most reproducible parameters, with strong and independent correlation with clinical outcome.

According to the Oxford Classification of IgAN, M, E and S are binary variables (0 - absent; 1 - present). Tubular atrophy and interstitial fibrosis are graded as T0, T1 and T2 (respectively, 0-25%, 26-50% or > 50% of affected cortical tissue). Ideally, the anatomic pathology report of an IgAN biopsy should include a detailed microscopic description, including quantitative inflammatory and scarring changes: total number of glomeruli and the number of glomeruli affected by necrosis, endocapillary proliferation, cellular and/or fibrous crescents, segmental and/or global sclerosis and the extension of tubular atrophy and interstitial fibrosis (Table 2). The diagnosis line of the report should mention the M, E, S and T scores.
**Figure 1.** Protean histologic appearances of IgAN. A- Mild mesangial proliferation (M1 lesion; circle): 4-5 mesangial cell nuclei clustered within a mesangial space, PAS (Periodic Acid Schiff), 100x. B- Segmental sclerosis (S1 lesion), with segmental closure of capillary walls, mesangial matrix expansion and capsular adhesion, PAS, 400x. C- Endocapillary hypercellularity (E1 lesion), displaying closure of capillary walls by inflammatory cell infiltration and endothelial swelling. Haematoxilin and Eosin, 100x. D - Circumferential extracapillary cell proliferation characteristic of a cellular crescent. PAS, 100x.

<table>
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<tr>
<th>Table 1</th>
<th>Haas and Lee’s Single-Graded Classification Systems</th>
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<tr>
<td><strong>Haas Subclasses</strong></td>
<td><strong>Lee Grades</strong></td>
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<tr>
<td>I</td>
<td>Absent to minimal mesangial hypercellularity.</td>
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<tr>
<td>II</td>
<td>Focal segmental glomerulosclerosis-like pattern.</td>
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<tr>
<td>III</td>
<td>Focal proliferative glomerulonephritis (&lt; 50%) with or without crescents.</td>
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<tr>
<td>IV</td>
<td>Diffuse proliferative glomerulonephritis (&gt; 50%) with or without crescents.</td>
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<tr>
<td>V</td>
<td>≥ 40% of globally sclerotic glomeruli or tubular atrophy/interstitial fibrosis, with or without active glomerular lesions.</td>
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<td>Table 2</td>
<td>Histological Parameters of Oxford’s Classification of IgAN</td>
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<td><strong>Histological Parameter</strong></td>
<td><strong>Score</strong></td>
</tr>
<tr>
<td>M</td>
<td>Mesangial proliferation</td>
</tr>
<tr>
<td>E</td>
<td>Endocapillary hypercellularity</td>
</tr>
<tr>
<td>S</td>
<td>Segmental sclerosis/synechiae</td>
</tr>
<tr>
<td>T</td>
<td>Tubular atrophy/interstitial fibrosis</td>
</tr>
<tr>
<td>0</td>
<td>M0 (absent)/M1 (present in &gt; 50% of glomeruli)</td>
</tr>
<tr>
<td>1</td>
<td>E0 (absent)/E1 (present)</td>
</tr>
<tr>
<td>2</td>
<td>S0 (absent)/S1 (present)</td>
</tr>
<tr>
<td>3</td>
<td>T0 (0-25%<em>/T1 (26-50%</em>)/T2 (&gt; 50%*)</td>
</tr>
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*relative to cortical extension Pathology report should ideally include: % of globally sclerotic glomeruli % of crescents % of glomeruli with necrosis % of glomeruli with endocapillary hypercellularity.

Although crescents was a highly reproducible variable, it was not originally included in the original classification. Even though 45% of the cases of the original series depicted crescents, the average percentage of glomeruli with extracapillary proliferation in affected cases was 9%; in none of the cases was this percentage higher than 55%.

In the Oxford Classification cohort the presence of crescents was not independently associated with progression to ESRD or with a decline of the estimated glomerular filtration rate greater than 50% during follow-up (combined event). Further studies on crescentic IgAN point out that serum creatinine is a strong independent predictor of risk for ESRD: a value of 6.8 mg/dL is considered the upper threshold for immunosuppression indication. However newer observations in larger cohorts emphasise the role of crescents as predictors of a higher risk of a combined event. The presence of crescents in ≥ 25% of glomeruli, seems to be independently associated with a combined event, in the presence or absence of immunosuppressive treatment. These recent data support the incorporation of a crescent score to the original classification (Haas et al., personal communication).

Despite its shortcomings, the Oxford classification of IgAN was validated by several different studies pertaining to diverse ethnic groups, the most extensive of which is the VALIGA Study. The VALIGA study highlighted the importance of chronic tubulointerstitial lesions, segmental sclerosis and...
mesangial hypercellularity as predictors of the rate of loss of renal function.  

Both the VALIGA Study and the Oxford Classification original cohort failed to demonstrate that endocapillary proliferation was an independent predictor of rate of renal function deterioration.  

Endocapillary proliferation - capillary loop narrowing and occlusion by endothelial cell swelling, macrophage and neutrophilic infiltration and mesangial proliferation - is observed in approximately one third of the cases of IgAN and is mostly focally distributed.  

More recent evidences point out that the only predictors of time to ESRD are baseline estimated creatinine clearance (eGFR), baseline proteinuria, endocapillary hypercellularity (odds ratio = 3.41 for E1 compared to E0) and tubular atrophy/interstitial fibrosis.  

The low predictive value of E1 in previous studies might stem from interaction between the lesions and immunosuppressive therapy: in the VALIGA study cohort, 39% of the patients had received angiotensin receptor blockage and 10% had received steroids prior to the biopsy. These data suggest that patients with endocapillary hypercellularity in IgAN might benefit from immunosuppression.  

At the First Oxford Conference on IgA Nephropathy, in June 2014, close scrutiny of the VALIGA Study data revealed low interobserver reproducibility for the M and E variables (interobserver correlation coefficient [ICC] = 0.49 and 0.34, respectively; Roberts, personal communication). Given the low reproducibility of prognostically valuable lesions, cell labeling studies are already underway and are expected to provide a less subjective measure of endocapillary hypercellularity.  

In addition, the development of e-learning tools for continuous training and regular assessment of pathologists involved in IgAN diagnosis was proposed in 2014 and is to be launched in 2016 (Soares et al., personal communication).

**Conclusions**

IgAN emerges as an important worldwide healthcare issue. From the pathogenetic point of view, it shares similarities both with allergic and autoimmune disorders. The lack of reliable animal models hinders experimental studies about the disease. Nevertheless, deeper understanding of the interactions in the mucosa-kidney axis, of the role of complement and growth factors might pave the way into acknowledging which mechanisms are responsible for its clinical and pathological characteristics. The development of the Oxford Classification of IgAN and its constant efforts for improvement are bound to ameliorate the communication of relevant findings between pathologists and clinicians and to strengthen clinico-pathologic correlations.

**Conflicts of interest**

None to declare.

**References**

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Pathology of IgA nephropathy


