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Original article

The Role of Direct Immunofluorescence in Diagnosis of the Glomerular Diseases

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Abstract

Introduction. Glomerular diseases are considered the leading cause of morbidity and mortality in renal pathology patients. There are restricted approaches for the morphologic analysis of renal biopsies, such as the pathognomic morphologic changes. Deposition of immu–noglobulin (Ig) and/or complement factors in glomerulus causes injury, which leads to an inflammatory response ending in proliferative glomerulonephritis. Immunofluo–rescence (IF) is crucial in diagnosing glomerular diseases, revealing immune deposits (like IgG, IgA, IgM, com–plement) in kidney biopsies to classify conditions (e.g., IgA nephropathy, lupus nephritis) and under–stand mechanisms, often showing distinct patterns (li–near for Goodpasture's, granular for immune complex diseases) that guide treatment alongside light and electron microscopy.

The aim of this study is to present the importance of the immunofluorescent evaluation of the renal biopsies and to receive fundamental data for diagnosis of the glomerular disease

Methods. All cases of native renal biopsies performed from 2018 to 2025year in our department were re–viewed retrospectively in this study. All biopsies with an adequate biopsy specimen were estimated by light microscopy (LM) and some of them with immunofluores–cent (IF) techniques. Electron microscopy was also used to confirm the diagnosis. Clinical details were documented in a predesigned form. Hence, we could have evaluated 242 out of 493 kidney biopsies re–ceived in this period.

Results. 242 biopsies had sufficient material and were analysed with immunofluoresce. The predominant cli–nical presentation in this study was IgA nephropathy (16,1%), followed by membranous nephropathy (15,2%). The other glomerular diseases were as follows: focal–segmental glomerulosclerosis (8,2%), minimal glomerular abnormalities (7,4%), mesangial glomerulonephritis without IgA immundeposit (7,8%), extracapillar rapid progressive glomerulonephritis (6,6%), membranopro–liferative glomerulonephritis (5,3%), lupus nephritis (5,3%) and amyloidosis (4,9%). diabetic nephropathy (6,1%), glomerulosclerosis (7,1%). Other cases (10%) with several patients each, were: tubulointerstitial neph–ritis, renal lymphoma, myeloma multiplex-light chain GN, fibrillary and immunotactoid nephropaties, con–firmed with electron microscopy.

Conclusion. Direct IF is vital for classifying GN, followed by electron microscopy, which is an essential tool. This article describes a rational evaluation of kid–ney biopsies on LM in a way that guides toward the logical assessment to reach the diagnosis. Light mic–roscopy alone can misdiagnose renal disease. This is especially important in cases of early stage mem–branous nephropathy, IgA nephropathy (IgAN), Lupus nephritis and IgM nephropathy (IgMN), as these enti–ties can only be diagnosed by correlating the micro–scopic, immunofluorescence findings and clinical details.

Keywords: glomerulonephritis, renal biopsy, immunofluorescence

Introduction

Glomerular diseases are considered the leading cause of morbidity and mortality in renal pathology patients [1]. There are restricted approaches for the morpho–logic analysis of renal biopsies, such as the patho–gnomic morphologic changes. Deposition of immu–noglobulin (Ig) and/or complement factors in glome–rulus causes injury, which leads to an inflammatory response ending in proliferative glomerulonephritis [2,3,4]. Proliferative glomerulonephritis is defined by an increase in glomerular cellularity due to infiltration and proliferation of leukocytes within the glomerulus or from the proliferation of mesangial cells and pa–rietal epithelial cells [2,5,6]. In severe forms of diffuse proliferative glomerulonephritis, the crescent is formed by the obliteration of bowman space by epithelial proliferation. Recognizing this pattern on light mic–roscopy (LM) and categorizing it into different disease entities with the help of direct immunofluoresence (DIF) is essential for an accurate diagnosis.[7] Immu–nofluorescence (IF) is crucial in diagnosing glomerular

diseases, revealing immune deposits (like IgG, IgA, IgM, complement) in kidney biopsies to classify con–ditions (e.g., IgA nephropathy, lupus nephritis) and understand mechanisms, often showing distinct pa–tterns (linear for Goodpasture's, granular for immune complex diseases) that guide treatment alongside light and electron microscopy.

The aim of this study is to present the importance of the immunofluorescent evaluation of the renal biopsies to receive fundamental data for diagnosis of the glo–merular disease.

Material and methods

All cases of native renal biopsies performed from 2018 to 2025 year in our department were reviewed retro–spectively in this study. The number of renal biopsies varies and was different for each year. The lower number was seen in the period of corona virus disease pandemy. All biopsies were estimated by LM and but some of them also with IF techniques. The number of immunofluorescent analysis was lower because of technical problems as the tissue sent for DIF was in–adequate. Clinical details were documented in a pre–designed form.

Renal biopsy was done and after that the tissue is embedded in the cryostat embedding medium, frozen and cut at 3 μ thickness at -22°C. Sections were taken on slides and later, the slides were rinsed in phosphate buffer saline at pH 7.2 for 10 min. The sections were treated with optimally diluted antisera (3 drops on each slide) - IgG, IgA, IgM and C3. The slides were incubated in wet chamber for 1 h in dark room, washed with PBS, and mounted with glycerine jelly. The slides were observed of the fluorescence mic–roscope. DIF reporting is done based on the nature and distribution of immune deposits, glomerular localiza–tion, the intensity of staining, and the pattern of immune complex deposits. The intensity of staining was graded subjectively from 0 to 3,0 being negative and 3 maximum intensity (mild +1, moderate +2, and marked + 3) [1,3]. After correlating clinical data with LM and IF findings, the final diagnosis was rendered.

Results

In despite of the histomorfological estimation 242 biopsies were evaluated with direct immunofluores–cence. Biopsies in transplanted patients were not in–cluded in this study. The predominant clinical pre–sentation in this study was IgA nephropathy (16,1%), followed by membranous nephropathy (15,2%) as presented in Table 1. The other glomerular diseases were as follows: focal segmental glomerulosclerosis (8,2%), minimal glomerular abnormalities (7,4%), mesangial glomerulonephritis without IgA deposition (7,8%), extracapillar rapid progressive glomerulonephri–tis (6,6%), membranoproliferative glomerulonephritis (5,3%), lupus nephritis (5,3%) and amyloidosis (4,9%). diabetic nephropathy (6,1%), glomerulosclerosis (7,1%). The rest of the cases (10%) with several patients each, were: tubulointerstitial nephritis, renal lymphoma, myeloma multiplex-light chain GN, fibrillary and immunotactoid nephropathies, confirmed with electron microscopy.

Table 1. Clinical presentation of chronic glomerulonephritis, diagnosed with DIF in this study

Mode of presentation	IgAN	MN	FSGS	MGA	MsG	MPGN	RPGN	SLE	AMD	DN	GSc
Total cases N=242	39	37	20	18	19	13	16	13	12	15	17
(%)	16,1	15,2	8,2	7,4	7,8	5,3	6,6	5,3	4,9	6,1	7,0
Nephrotic syndrom	4	30	18	16	11	10	/	9	12	12	/
Nephritic syndrom	15	/	/	/	/	/	/	/	/	/	/
Proteinuria	/	7	2	2	8	3	10	4	/	3	10
Eritruria	20	/	/	/	/	/	6	/	/	/	7

IgAN-IgA nephropathy, MN-membranous nephropathy, FSGS-focal segmental glomerulosclerosis, MGA-minimal glomerular abnormalities, MsG-mesangial glomerulonephritis without IgAdeposits, MPGN membranoproliferative glomerulonephritis, RPGN-rapidly progressive glomerulonehritis, AMD- amyloidosis, SLE-systemic lupus erithematodes, DN-diabetic nephropathy, GSc- glomerulosclerosis

The dominant deposit on IF in Lupus nephritis (LN) was C3 and IgG, but also several cases were "full house" screen. A high deposit of IgA in IgA nephropathy and IgG deposition along the GBM in membranous neph–ropathy, also deposition of IgG and C3 in membra–noproliferative GN was evaluated.

Discussion

Immunofluorescence studies play an essential role in the evaluation of medical renal biopsies. Particularly, in the study of renal glomerular diseases, where it pro–

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vides fundamental data for the diagnosis, classifica–tion, and etiology of the glomerular pathologies.
We noted different incidences during different periods of time, especially for patients with membranous and IgA nephropathy. The most frequent histological forms in our study were IgA nephropathy (16,1%), as in studies from other Mediterranean countries. The inci–dence of membranous nephropathy is also high (15,2%). A multidisciplinary diagnostic approach remains essen–tial for managing glomerular diseases [8]. However, significant disparities in access to biopsy, nephronpa–thology, and serological testing hamper optimal care in many regions. Investment in diagnostic infrastructure, clinician training, and health policy reforms are critical for improving global kidney health outcomes [9,10].
Glomerular diseases are a significant contributor to chronic kidney disease (CKD) globally, accounting for up to 30% of end-stage kidney disease cases (ESKD). Diagnostic accuracy is crucial for appropriate mana–gement but remains challenging, especially in low-resource settings. Immunofluorescence (IF) is an essen–tial diagnostic tool for glomerulonephritis, because it uses fluorescent antibodies to identify and visualize immune deposits (like antibodies, complement, and antigens) in the kidney tissue. This process helps in accurately diagnosing, classifying, and determining the cause of various types of glomerulonephritis, provi–ding crucial information beyond what might be seen with a light microscope alone [2,11,12].
How immunofluorescence aids in diagnosing glome–rulonephritis? Visualizes immune deposits and detects the presence and location of specific proteins, such as IgA, IgG, IgM, and complement factor (C3), that are deposited in the glomeruli and indicate immune-mediated kidney damage [1,2,10]. Also, it classifies types of glomerulonephritis such as specific pattern and type of immune deposits found are characteristic of different forms of the disease, allowing for precise classification. For example, IgA nephropathy is identi–fied by the presence of IgA deposits, typically in the mesangium. It helps distinguish between primary forms of glomerulonephritis and those caused by other diseases, such as autoimmune disorders or infections.
Correlation with clinical findings is needed. The results from immunofluorescence staining are correlated with clinical symptoms and other laboratory findings, like blood and urine tests, to provide a complete picture of the patient's condition [7,11]. The presence or absence of certain immune deposits can also provide information about the disease's potential course and prognosis.
IF uses fluorescently labeled antibodies to light up specific proteins (antibodies, complement factors like C3, C1q) in kidney tissue. The location and appearance of deposits are important for diagnosis. Granular IgG immune subepithelial deposit along GBM was found in membranous glomerulonephritis (Figure 1).

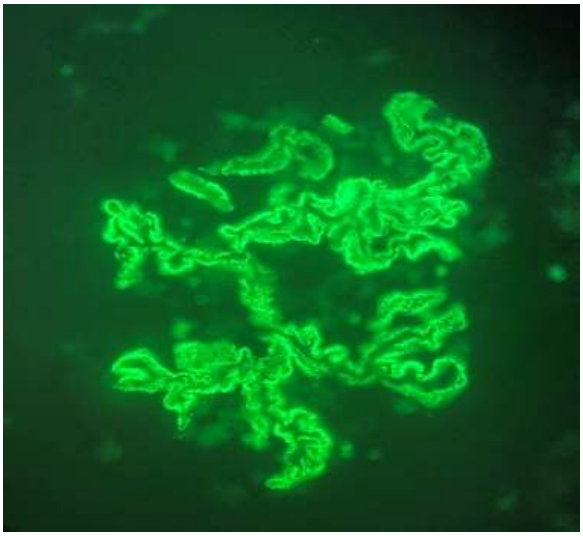


Fig.1. IgG granular subepitelial immun deposits along GBM. IgG-FITC in membranoous GN grade III

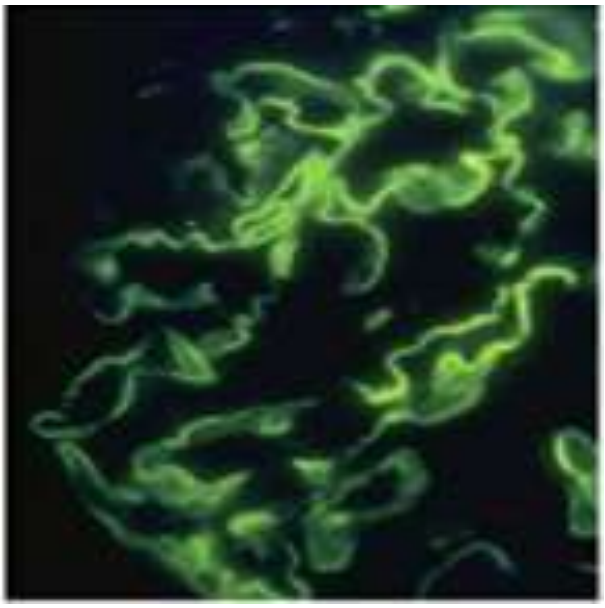


Fig. 2. Linear immun deposit along GBM-IgG-FITC in Goodpasteure syndrome

Linear pattern suggests antibodies directly attacking the glomerular basement membrane, which is common in Goodpasture's Syndrome (anti-GBM disease) (Figure 2).
Granular pattern indicates immune complexes deposited in the glomerulus, typical in lupus nephritis or IgA nephropathy.
Deposits in the mesangium (IgA nephropathy) versus capillary loops (membranous nephropathy) help diffe–rrentiate diseases. Different IgA, IgG, IgM, C3 patterns help diagnose specific diseases like IgA Nephropathy (most common), Membranous Nephro–pathy, or C3 Glomerulopathies (e.g., Dense Deposit Disease).
The diagnostic approach to glomerular diseases by integrating clinical evaluation, laboratory findings, imaging techniques, and histopathological assessments.

Primary glomerulonephritis comprises several renal-limited diseases that can cause haematuria and pro–teinuria, CKD, nephrosis and ESKD. The most common of these are IgA nephropathy (IgAN), primary mem–branous nephropathy (PMN), Focal Segmental Glo–merulosclerosis (FSGS) and Minimal Change Disease (MCD). Although rare, these diseases cause a signi–ficant burden to health care systems, given the high cost of treating ESKD with dialysis or transplantation. Until recently, the pathogenesis of primary glomeru–lonephritis has remained obscure. However, recent advances in understanding of how these diseases evolve has led to the introduction of novel therapeutic agents. Trials are underway or have recently completed that have huge implications for the standard of care for the primary glomerulonephritidies, and should dramatically reduce the number of patients who progress to ESKD. This article reviews according to the international Kidney Disease Improving Global Outcomes (KDIGO) guide–lines for the treatment of glomerular diseases, as well as recent research on pathogenesis and treatment [12].

Conclusion

Direct IF is vital for classifying the type of glomeru–lonephritis, followed by electron microscopy, which is an essential tool. Using the IF technique and compa–ring it with LM and clinical details, we evaluated the patients in our center. Immunofluorescence proved an important diagnostic tool in reaching the exact diag–nosis in various types of GN.

Light microscopy alone can misdiagnose renal disease. This is especially important in cases of early stage membranous nephropathy, IgA nephropathy, Lupus nephritis and IgM nephropathy, as these entities can only be diagnosed by correlating the microscopic, immunofluorescence findings and clinical details.

Conflict of interest statement. None declared.

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