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Utility of anti-immunoglobulin IgA, IgG, IgM, Kappa, Lambda - FITC (conjugate) cocktail in routine renal pathology practice

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Abstract

Background Immunofluorescence studies on frozen sections are an essential component in the evaluation of renal biopsies. The basic panel in most centres constitutes IgG, IgA, IgM, C3c, C1q, Kappa and Lambda light chain antibody testing. The purpose of this panel is to detect immunoglobulin or complement deposits and further subclassify the disease based on the location, intensity and pattern of immunoglobulin and complement staining. However, there are a substantial proportion of nephropathies that do not show any obvious immune-deposits on immunofluorescence. We currently, do not have any evidence-based alternative immunofluorescence panel to rule out these conditions. This study aims to evaluate the utility of anti-immunoglobulin IgA, IgG, IgM, Kappa, Lambda - FITC cocktail immunofluorescence on renal biopsy frozen sections with emphasis on its role as a primary screening panel in conjunction with C3c and C1q.

Methods Anti-immunoglobulin IgA, IgG, IgM, Kappa, Lambda light chain - FITC cocktail immunofluorescence was performed on 593 consecutive native renal biopsies along with the routine panel comprising of the individual FITC labelled IgG, IgA, IgM, C3c, C1q, Kappa and Lambda light chain immunofluorescence stains.

Results In 235 (39.6%) cases immune deposits (immune-complex mediated and monoclonal gammopathy-related) were present and the rest 354 (59.7%) cases were negative for immunoglobulin or complement deposits. Overall, the sensitivity, specificity, positive predictive value and negative predictive values of anti-immunoglobulin IgA, IgG, IgM, Kappa and Lambda - FITC cocktail in distinguishing immune-complex/immunoglobulin-mediated glomerulopathies from non-immune complex/immunoglobulin-mediated glomerulopathies were 100% each.

Conclusion Anti-immunoglobulin IgA, IgG, IgM, Kappa and Lambda - FITC cocktail when used in conjunction with C3c and C1q, can be an effective first line investigation in all native renal biopsies. Further, testing with the individual FITC labelled IgG, IgA, IgM, Kappa and Lambda light chain immunofluorescence can be performed, depending on the initial screening as described above. Overall, this algorithmic approach can save valuable resources.

Keywords Renal biopsies, Immunofluorescence, Frozen sections, Polyvalent, Cocktail antibody

Background

Native medical renal biopsies are precious tissue samples that require analysis by light microscopy (LM), immunofluorescence (IF), supplemented by immunohistochemistry (IHC), and electron microscopy (EM) for complete comprehensive diagnosis (Fogo 2003). Common indications for native renal biopsies are acute kidney injury, proteinuria, hematuria and chronic kidney disease

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(Luciano and Moeckel 2019). Kidney biopsy sometimes is quite difficult to obtain and the pathologists often have to deal with instances where only a limited tissue core is available for diagnosis. Therefore, this becomes a challenging situation to perform all the essential tests on the little tissue for a wholesome diagnosis. According to the current guidelines, if a limited tissue is available then always the clinical differential should be the guiding force for division of sample for LM, IF and EM (Walker et al. 2004). Unlike other biopsies, IF plays an indispensable role in the diagnosis of native as well as transplant medical renal diseases (Wagrowska-Danilewicz and Zeromski 2010). IF on native renal biopsy frozen sections is well known since 1960s (Steblay 1962). Fresh frozen tissue is considered as gold standard for IF (Walker et al. 2004). It requires dark field microscopy which produces high signal to noise ratio and usually displays accurate localization of immune deposits with superb resolution. This can be enhanced with experienced technicians performing excellent frozen sectioning under the supervision of well- trained renal pathologists (Walker et al. 2004). The standard IF technique for detecting immunoglobulin (Ig) and complement deposits requires a minimum of five Ig stains (IgG, IgA, IgM, Kappa and Lambda light chains) and two complement stains (C3c and C1q) with additional fibrin and C4d (Agarwal et al. 2013). Many centres also extend the basic panel by testing IgG subclasses (IgG1 to IgG4) (Huang et al. 2013) and other relevant immunostains such as C4d (in transplant biopsies) as required. The main purpose of this standard panel is to detect Ig (whole, heavy and light chains) and complement deposits in the various compartments of the kidney represented in the biopsy which has immense diagnostic utility (Fogo 2003). Both the intensity as well as pattern of distribution in different locations play a major role in the accurate diagnosis of renal pathologies (Sethi et al. 2016). A major proportion of native renal biopsies comprise of conditions with nil immune deposits. However, till date, there is no alternative except for performing the whole extensive Ig panel in all native renal biopsies. In cases with scant or no glomeruli on IF sample, paraffin embedded IF has been tried and standardised and is being performed in few setups (Singh et al. 2016). However, this requires an efficient and well trained team of technicians and pathologists as the digestion of tissue is the rate limiting step for this process, making interpretation and reporting often difficult (Solanki et al. 2019). IF on paraffin embedded tissue has been regarded as a salvage ancillary technique for cases where IF could not be performed as a routine practice (Nasr et al. 2018).

We serendipitously came across a polyclonal rabbit anti-human IgA, IgG, IgM, Kappa, Lambda/ fluorescein isothiocyanate (FITC) antibody Dako (Code F0200) which was intended for the demonstration of human Ig in tissues, and may also be used for other immunofluorescence techniques. This antibody is a mixture of five purified immunoglobulin fractions of rabbit antisera individually conjugated with fluorescein isothiocyanate (FITC) isomer 1 that reacts with human IgA (α-chains), IgG (γ-chains), IgM (μ-chains), and with Kappa and Lambda light chains. It is commercially available in liquid form premixed with phosphate buffer at pH 7.2. A pan-Ig immunostain (cocktail of IgG, IgA, IgM, Kappa and Lambda light chains) akin to pan cytokeratin (a cocktail of various keratins) could prove to be an ideal screening tool when combined with C3c and C1q. This would save the valuable remnant frozen tissue, antibodies, reagents, time, effort and overall cost for further essential tests.

To the best of our knowledge there is no published literature with regards to testing of this antibody on native renal biopsies. With, this background knowledge, a hypothesis that anti-human IgA, IgG, IgM, Kappa, Lambda - FITC antibody cocktail is likely to detect Ig deposits in positive cases with Ig deposits and most importantly exclude the negative cases with absence of deposits was postulated.

Methods

To test the hypothesis, polyclonal rabbit anti-human IgA, IgG, IgM, Kappa, Lambda - FITC antibody Dako (Code F0200) immunofluorescence testing was done in conjunction with polyclonal rabbit anti-human IgG - FITC antibody Dako (Code F0202), polyclonal rabbit anti-human IgA - FITC antibody Dako (Code F0204), polyclonal rabbit anti-human IgM - FITC antibody Dako (Code F0203), polyclonal rabbit anti-human Kappa light chains - FITC antibody Dako (Code F0198), polyclonal rabbit anti-human Lambda light chains - FITC antibody Dako (Code F0199), polyclonal rabbit anti-human complement C3c - FITC antibody Dako (Code F0201), and polyclonal rabbit anti-human complement C1q - FITC antibody Dako (Code F0254) in 593 consecutive native renal biopsies from 1st April 2019 to 31st March 2020 in the division of renal pathology at NextGenPath Diagnostics, India. For all the antibodies tested, a uniform protocol was followed. The biopsies were received in Michel's transport medium, immediately washed in washing solution for 10 min, embedded in Optimal cutting temperature (OCT) medium and snap frozen. Cryosections were taken at 3 μm on hydrophilic adhesion slides. The sections were washed in phosphate buffered saline (PBS) for 10 min to remove the OCT medium. The primary antibodies were diluted in PBS. (Table 1) The labelled sections were incubated with 100–200 μl of corresponding diluted primary antibodies viz. FITC labelled IgG, IgA, IgM, C3c, C1q, Kappa light chains, Lambda light chains

Table 1 Summary of antibodies' clones and titration used in the study

Antibody	Clone	Dilution
IgA/FITC	Rabbit Polyclonal	1:25
IgG/FITC	Rabbit Polyclonal	1:40
IgM/FITC	Rabbit Polyclonal	1:25
Ig Kappa light chains/FITC	Rabbit Polyclonal	1:20
Ig Lambda light chains/FITC	Rabbit Polyclonal	1:20
C3c/FITC	Rabbit Polyclonal	1:25
C1q/FITC	Rabbit Polyclonal	1:20
Polyvalent IgA, IgG, IgM, Kappa, Lambda/FITC	Rabbit Polyclonal	1:100

and Ig cocktail (IgG, IgA, IgM, Kappa and Lambda light chains) at room temperature. After an hour, the antibodies were washed in PBS, mounted on phosphate buffered glycerine and viewed under fluorescence microscope with the following specifications: excitation filter wavelengths 450 to 490 nm; dichromatic mirror cut-on wavelength—500 nm and barrier filter wavelengths 515 nm cut-on. Two histopathologists (BL and ST) independently reviewed the cases and arrived at the diagnosis on the basis of staining patterns on IF and LM findings. The positive staining was graded according to the intensity (0 to 3+). A score of ≥ 2+ was taken as positive. Any staining which was faint, non-specific and non-contributory was graded as 0 or 1. The nature of deposits was described as granular or linear and the distribution of deposits was recorded. There was complete agreement in the diagnosis of the cases along with the interpretation of IF and LM findings.

Results

Of the total 593 cases, 239 had immune deposits (Ig and complement) and 354 were devoid of Ig and complement deposits. Among the 239 that had immune-deposits, 4 cases of C3 glomerulopathy did not show any Ig deposits. Excluding the 4 cases of C3 glomerulopathy, the rest of the 235 cases had Ig deposits viz anti-glomerular basement membrane disease (5), immune-complex mediated glomerulonephritis, unclassified (7), IgA nephropathy (75), infection-related glomerulonephritis (45), lupus nephritis (37) and membranous nephropathy (57), AL amyloidosis (1), Ig light chain cast nephropathy (6), Ig light chain deposition disease (2). (Table 2) So a total of 235 out of 593 (39.6%) cases had immunoglobulin deposits. In all these cases Ig cocktail showed positive staining with an intensity of 2+ to 3+ and distribution similar to the diagnostic individual Ig. For example, glomerular capillary loop staining for anti-IgG, Kappa and Lambda light chains in membranous nephropathy was also mirrored in the Ig cocktail stain. Similarly, mesangial staining for anti-IgA, Kappa and Lambda light chains in IgA nephropathy demonstrated positive concordance with the Ig cocktail stain (Fig. 1). The 354 (59.7%) cases which did not reveal any Ig or complement deposits were acute interstitial nephritis (25), Alport's disease (1), AA-amyloidosis (3), acute pyelonephritis (17), acute tubular injury (48), acute thrombotic microangiopathy (7), collapsing glomerulopathy (5), chronic tubulo-interstitial nephritis (36), chronic thrombotic microangiopathy (9), diabetic nephropathy (76), focal segmental glomerulosclerosis (37), sarcoidosis (1), hypertensive nephropathy (28), minimal change disease (23), myoglobin cast nephropathy (4), oxalate nephropathy (6), polyarteritis nodosa (2), pauci-immune glomerulonephritis (13), Sjogren's tubulointerstitial nephritis (2) and non-diagnostic (11). These cases were also negative for Ig cocktail staining. The diagnosis for the above cases was made on the basis of findings of LM and IF with a clinico-serological correlation. EM was not available in our setup. Sensitivity, specificity, positive predictive value and negative predictive value of Ig cocktail staining compared to the panel of individual Ig stains in distinguishing cases with and without Ig deposits were calculated and found to be 100% each (Table 3).

Table 2 Distribution of immune-mediated diseases included in the study

No	Immune-mediated diseases included in the study	Number of cases (*N* = 235)	Percentage (%)
1	IgA nephropathy	75	31.9
2	Membranous nephropathy	57	24.2
3	Infection related glomerulonephritis	45	19.1
4	Lupus nephritis	37	15.7
5	Immune-complex mediated glomerulonephritis, unclassified	7	2.9
6	Ig Light chain cast nephropathy	6	2.5
7	Anti-glomerular basement membrane (Anti-GBM) disease	5	2.1
8	Ig Light chain deposition disease	2	0.8
9	AL Amyloidosis	1	0.4

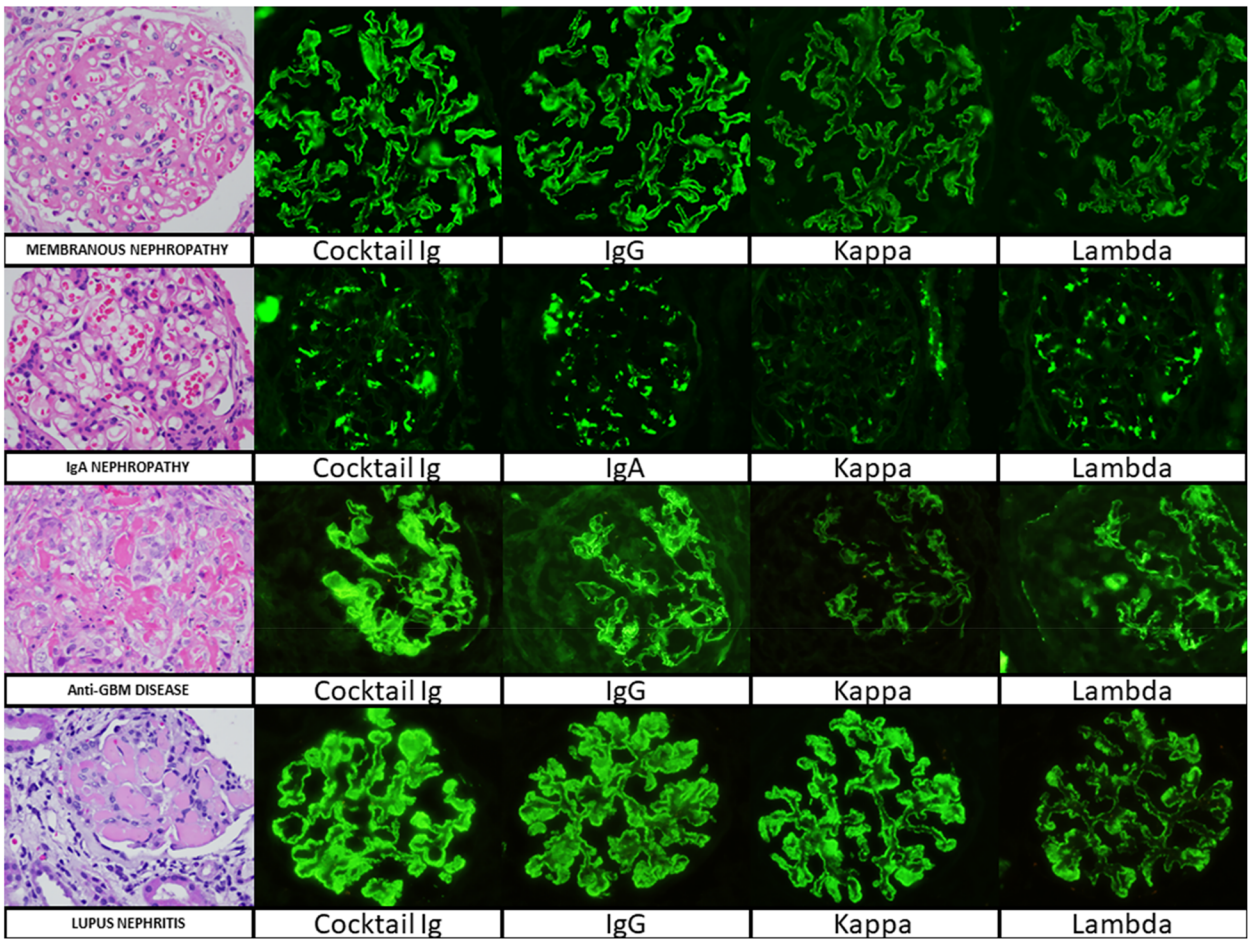

Fig. 1 A comprehensive image panel comprising of light microscopy images (hematoxylin and eosin stain, 400x) and corresponding IF images (400x) with polyvalent cocktail immunoglobulin (Ig), IgG, IgA, Kappa, Lambda positive (≥ 2 +) staining patterns in representative cases of Membranous nephropathy, IgA nephropathy, Anti-GBM disease and Lupus nephritis, respectively

No significant background staining was seen in the cases hampering the diagnosis.

Discussion

This study is a novel attempt to assess the value of Ig cocktail immunofluorescence in renal biopsies of varied etiologies. IF plays a major diagnostic role in the field of renal pathology. Direct IF on fresh frozen tissue is a validated ancillary tool for demonstrating Ig and complement deposition in renal biopsies (Solanki et al. 2019). IF tissue is first surrounded with OCT and snap frozen and further cryosectioned at 3-4 μm. The sections are incubated with commercially available individual fluorescein tagged antibodies. Approximately, 1–2 h are required for the whole process (Fogo 2003). Use of polyclonal anti-IgA, IgG, IgM, Kappa, Lambda - FITC cocktail antibody can efficiently bring down the precious tissue wastage and also the turnaround time can also

Table 3 Truth table for calculating sensitivity, specificity, positive and negative predictive values:

		Standard IF		
		Ig deposits	**No Ig deposits**	**Total number**
Test Ig CocktailTest Ig Cocktail	**Ig deposits**	235	0	235
	No Ig deposits	0	354	354
	Total	235	354	593

reduce significantly. Similar use of pancytokeratin antibody in immunohistochemistry is advocated in small valuable lung biopsies with epithelioid undifferentiated lung neoplasms (Yatabe et al. 2019). Pancytokeratin being a robust and specific antibody is widely used in oncopathology for the early determination of lineage of tumor cells (Selves et al. 2018).

With the overall sensitivity, specificity, positive predictive value and negative predictive value, we recommend that Ig cocktail could be incorporated in the evaluation of renal biopsies as primary screening tool along with C3c and C1q. Based on this preliminary outcome, other immunostains can be performed in order to save valuable resources such as remnant biopsy tissue (that can be used for further studies), cost and manpower. This Ig cocktail may be advantageous in rapidly progressive glomerulonephritis cases where the early diagnosis of immune-mediated kidney injury plays an important role. This can guide the pathologists as well as the clinicians for the judicious use of steroids and other immunosuppressive therapies.

Polyclonal anti-IgA, IgG, IgM, Kappa, Lambda - FITC is also particularly useful for the fluorescent treponemal antibody (FTA) test and for the demonstration of antinuclear antibodies (ANA) as well as other human autoantibodies. Moreover, the same principle can be applied for the skin biopsies that use similar Ig and complement immunostain panel akin to renal biopsies. However, this needs to be validated by a separate study. Our study has a few limitations as well. All the biopsies were first time diagnostic native renal biopsies and hence, the results cannot be extrapolated to post-therapy native renal biopsies and transplant biopsies. EM was not available in our set up which plays an essential role in potentiating the diagnosis in many renal diseases. The phenomenon of antibody entrapment (IgM and C3) in the segmental sclerosed glomeruli in cases of FSGS was also seen with this cocktail antibody which might lead to false positive reporting if not seen in conjunction with LM findings. Applicability of this cocktail antibody for IF on paraffin embedded tissue need validation through separate studies. Hence, we emphasize that the utility of this cocktail antibody should be viewed only as a screening tool to rule in or out the immune-mediated injury.

Conclusion

In the era of genomics and proteomics, IF still holds a strong position for the routine kidney biopsy interpretation and this cocktail antibody can be regarded as an efficient screening tool for the same. Through our study on a significantly large sample, we propose the use of cocktail antibody for the early and cost effective screening of renal biopsies. Anti-immunoglobulin IgA, IgG, IgM, Kappa and Lambda - FITC cocktail when used in conjunction with C3c and C1q, can be an effective first line investigation of all native renal biopsies. Further, testing with the individual IgG, IgA, IgM, Kappa and Lambda light chain immunofluorescence can be performed depending on the screening resultf indicated. Overall, this algorithmic approach can save valuable resources.

Abbreviations

EM	Electron microscopy
FITC	Fluorescein isothiocyanate
IF	Immunofluorescence
LM	Light microscopy
OCT	Optimum cutting temperature

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Authors' contributions

ST analysed and interpreted the patient data and contributed in writing and editing the manuscript, BLG conceptualised, analysed and edited the manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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