The effect on patient management of temporary non-availability of immunofluorescence for renal biopsy reporting

Anand Date, D S Dakshinamurthy, C K Jacob, J C M Shastry

Abstract

Delay in reporting the immunofluorescence findings on renal biopsies, owing to an interruption in supply of reagents, made possible a retrospective analysis of the effect of the lack of this information on patient management. Hospital case records of the 39 patients so affected were reviewed to determine what changes in their management took place after the immunofluorescence findings became available. The clinical, laboratory, and light microscopic findings in all except a case of pauci-immune crescentic glomerulonephritis allowed management decisions to be made that were not influenced by immunofluorescence findings. This was owing to correct prediction of the immunofluorescence findings, as in cases of IgA nephropathy presenting with recurrent haematuria; the adequacy of light microscopy in the interpretation of graft biopsies, in classifying lupus nephritis and in most cases of nephrotic syndrome; and the absence of entities identifiable only by immunofluorescence among these patients.

(*J Clin Pathol* 1998;**51**:337–339)

Keywords: immunofluorescence; therapeutic decision making

The temporary non-availability of imported reagents resulted in a delay in reporting immunofluorescence findings on renal biopsies. During this period clinicians had only the light microscopic diagnosis to assist them in decision making. Here we present the findings of a retrospective review of the effects of this deficiency on patient management.

Methods

For 38 days in 1993, immunofluorescence reagents routinely used in reporting renal biopsies in the department of pathology, Christian Medical College Hospital, Vellore, India, were not available owing to difficulties experienced by the local retailer in importing these reagents.

Renal biopsy reports during this period gave only the light microscopic findings and the pathologist's impression. Cryostat sections of tissue sent for immunofluorescence were mounted on glass slides and stored at −20°C. When the reagents became available the stored sections were stained in batches, and an additional report containing the immunofluorescence findings was dispatched.

Hospital case records were reviewed to see how the additional information provided by immunofluorescence altered management decisions made when only the light microscopic findings were available.

Indications for renal biopsy, methods and stains used—including direct immunofluorescence staining by fluorescein isothiocyanate labelled antihuman IgG, IgA, IgM, and C3—were standard, and have been described earlier.[1]

The case numbers assigned below are to simplify the presentation of findings and do not indicate a chronological sequence.

Results

During the period when immunofluorescence staining could not be done, biopsy samples adequate for light and immunofluorescence microscopy were received from 39 patients. The interval between receipt of the light microscopic and the immunofluorescence report varied from less than one week in the five most recently biopsied patients, to over six weeks in the first nine cases.

ALLOGRAFT BIOPSIES

Allograft biopsies from cases 1 to 7 were all from males, aged 16 to 56 years. Cases 1 to 4 were biopsied for an episode of renal failure occurring from 25 to 398 days after transplantation. Case 1 showed only minor mesangial alterations of doubtful significance, cases 2 to 4 showed acute cellular rejection on light microscopy, and in addition, case 4 had transplant glomerulopathy. Case 5 had an episode of renal failure 266 days after transplantation, and ultrasonographic evidence of a dilated collecting system as well as a large lymphocele. The biopsy to exclude a coexistent rejection showed only minor glomerular alterations consistent with early transplant glomerulopathy. Cases 6 and 7 were biopsied for massive proteinuria and graft dysfunction 12 and 75 months after transplantation, respectively. Both biopsies showed prominent mesangial and capillary wall thickening with some hypercellularity. There was much difference in the severity of involvement between glomeruli and lobules, suggesting a diagnosis of transplant glomerulopathy rather than recurrent or de novo glomerulonephritis.

Cases 2 to 4 were treated for acute rejection with enhanced bolus doses of steroids, case 5 improved after a nephrostomy. No specific treatment was instituted in cases 1, 6, and 7. No change in management followed immunofluorescence study, which showed only vascu-

Christian Medical College and Hospital, Vellore, India
Department of Pathology
A Date

Department of Nephrology
D S Dakshinamurthy
C K Jacob
J C M Shastry

Correspondence to:
Professor Anand Date, Department of Pathology, College of Medicine, Sultan Qaboos University, PO Box 35, Al Khod, 123 Muscat, Sultanate of Oman.

Accepted for publication
5 February 1998

lar C3 staining in cases 1 and 3, and faint diffuse glomerular IgM and C3 staining in cases 6 and 7.

NEPHROTIC SYNDROME

Nephrotic syndrome, isolated or relapsing, was the indication for renal biopsy in cases 8 to 18, whose ages ranged from 58 months to 57 years, and included six females. Biopsies from cases 8 to 11 showed no significant abnormality on light microscopy and were diagnosed as minimal change disease. Case 12 had membranous glomerulonephritis. Cases 13 to 16 had focal segmental glomerulosclerosis of varied severity, and cases 17 and 18 had mesangial hypercellularity. Case 18 had been on non-steroidal anti-inflammatory drugs and indiginous medication for arthritis. In addition to nephrotic syndrome, case 19 had chronic renal failure with normal sized kidneys. The biopsy showed extensive sclerosis of glomeruli. Cases 8 to 18 were treated for an adequate duration with an optimum dose of steroids. Case 19 required haemodialysis. Immunofluorescence study confirmed the light microscopic diagnoses with cases 8 to 11 showing no staining. Case 12 had typical diffuse, uniform, finely granular, contiguous capillary wall deposits of IgG and C3. Cases 13 to 19 showed either no staining or trapping of IgM and C3 in areas of segmental or global sclerosis. There was no change in treatment following receipt of this information.

NEPHRITIC AND HAEMATURIC PRESENTATIONS

Nephritic and haematuric presentations were the indications for biopsying cases 20 to 27, whose ages ranged from 8 to 49 years. Cases 23 and 27 were males. Biopsy from case 20 showed acute proliferative glomerulonephritis with an occasional crescent. No specific treatment was given, even after immunofluorescence studies which showed no positive staining, since the patient was showing clinical recovery.

Cases 21 to 23 with a rapidly progressive illness had crescentic glomerulonephritis and were treated by pulse methyl prednisolone. Case 22 had many hyalinised glomeruli in the biopsy and required haemodialysis. Immunofluorescence revealed pauci-immune disease in case 21; granular capillary and mesangial deposits of C3 and IgG in case 22; and IgA, C3, and weak IgM staining suggesting IgA nephropathy in case 23. There was no change in treatment following the immunofluorescence findings in cases 22 and 23. Case 21 died of pneumonia before cyclophosphamide could be added to the immunosuppressive treatment already being given.

Cases 24 to 26 had recurrent gross or microscopic haematuria suggesting IgA nephropathy. The clinical impression was supported by the biopsy findings of a mesangial proliferative disease, and no specific treatment was given. Confirmation of the diagnosis by immunofluorescence, with typical arborising mesangial IgA and C3 deposits, did not alter the management.

Case 27 had membranoproliferative glomerulonephritis and was given frusemide and enalapril. Immunofluorescence showed granular C3, IgG, and IgM deposits. No change was made in treatment.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus was diagnosed in cases 28 to 33, all women aged 15 to 32 years. Light microscopy of their renal biopsies showed WHO class IIB changes in two cases, class IV abnormalities in two, and class V lesions in two. All had been diagnosed as having systemic lupus erythematosus clinically and serologically. The biopsies were performed to obtain a histological classification of the disease.

Patients with class II and class V lesions were treated with steroids; class IV lesions with steroids and azathioprine. Immunofluorescence study did not influence the treatment and showed coarse granular capillary and or mesangial deposits of C3, IgG, IgM, and IgA in all biopsies except in case 32 with membranous lupus glomerulonephritis, which showed only IgG and C3 deposits.

DIABETES MELLITUS

Diabetes mellitus was present in cases 34, 35, and 36.

Case 34, a 30 year old man, presented with hypertension and diabetes of recent onset followed by nephrotic syndrome without retinopathy. Biopsy showed diffuse intercapillary glomerulosclerosis. Treatment consisted in controlling the hypertension and diabetes. Immunofluorescence was negative and treatment was continued unaltered.

Case 35, a male aged 53 years, had non-insulin-dependent diabetes mellitus and coronary artery disease. Renal function deteriorated rapidly following coronary artery bypass surgery. A renal biopsy showed advanced diabetic glomerular disease and the patient was put on haemodialysis, which continued after the negative immmunofluorescence report was received.

Case 36, a 61 year old man with hypertension and non-insulin-dependent diabetes mellitus of 20 years' duration, was biopsied because he developed gross haematuria and nephrotic syndrome. Biopsy showed mild intercapillary glomerulosclerosis with focal proliferative changes and polymorphonuclear leucocytic infiltrates suggesting superadded acute proliferative glomerulonephritis. Immunofluorescence confirmed this impression, showing granular capillary deposits of C3 with less intense staining for IgG, but did not change the management of the patient, which consisted only of control of diabetes and hypertension.

SYSTEMIC VASCULITIS

Systemic vasculitis was diagnosed clinically in case 37, a 22 year old woman who presented with nephrotic syndrome, fever, peripheral cyanosis, and hypertension of one month's duration. Renal biopsy showed a focal proliferative glomerulonephritis consistent with the

diagnosis; negative immunofluorescence provided further support for this, but did not predicate a change in the immunosuppressive treatment already instituted.

ACUTE RENAL FAILURE

Acute renal failure with delayed recovery was the reason for biopsy in cases 38 and 39, a three year old boy and a 36 year old man. Renal failure in the child was preceded by fever and loose stools. The peripheral blood showed schistocytes and thrombocytopenia. Renal biopsy showed focal tuft solidification, isolated glomerular infarction, acute tubular necrosis, and a small focus of cortical necrosis typical of the haemolytic-uraemic syndrome of bacillary dysentery. The child was treated by peritoneal dialysis. The adult developed acute renal failure four weeks after the onset of acute hepatitis. Biopsy showed acute tubular necrosis. The patient was treated by haemodialysis. Immunofluorescence was negative in both cases and did not contribute to management.

Discussion

This study was made possible by the prevarication of the local retailer of the imported reagents, and our gullibility, which resulted in complete depletion of these reagents before supplies from an alternate source could be obtained. In retrospect, the immunofluorescence findings did not alter the management of patients significantly, since the findings were those expected from the clinical, biochemical, and light microscopic findings, or were not significantly different.

Were these findings merely a result of a fortuitious presentation of patients least likely to benefit from immunofluorescence, or is immunofluorescence really not needed for patient management? The answer lies between these extremes. The diagnoses represented here were those seen most commonly in our patient population.[1 2] However, cases of stage I membranous glomerulopathy, type 2 membranoproliferative glomerulonephritis, antiglomerular basement membrane antibody disease, and WHO class IIA lupus glomerulonephritis, in all of which the diagnoses are dependent on immmunofluorescence, were not included. These rare conditions together account for less than 1.8% of all biopsied cases in this hospital.[3] IgA nephropathy, also an immunofluorescence dependent diagnosis, is more common, accounting for 4.2% of the cases.[3] However, three of the four cases (Nos 24 to 26) of IgA nephropathy included in the present series had such typical clinical presentations and compatible light microscopic findings that the pretest probability of the condition being anything else was so low that the diagnosis was safely presumed. The fourth case (No 23) had crescentic glomerulonephritis and was treated with immunosuppressive drugs. The unexpected diagnosis of IgA nephropathy did not require a change in management. The biopsy from case 21 with crescentic glomerulonephritis was pauci-immune, suggesting a vasculitic aetiology. This finding would have resulted in the addition of cyclophosphamide to the immunosuppressive treatment already started if the patient had not died of pneumonia.

Most allograft biopsies can be dealt with quite adequately without immunofluorescence, as can cases of lupus glomerulonephritis, except those with normal histology on light microscopy. An earlier study of 841 consecutive biopsies, excluding transplant patients, showed that the overall diagnostic inadequacy that would occur without immunofluorescence was 8.9%.[3] The possible effect on management, as presented here, was not determined.

It is important to assess the contribution that tests such as immunofluorescence and electron microscopy make to patient management in the developing world, or at times of financial stringency in medical care in the industrialised countries. Assessment need not await a serendipitous occurrence as described above. Clinical and biochemical, light microscopic, immunofluorescence, and electron microscopic findings can be abstracted from patient's records and presented to clinicians sequentially for their therapeutic recommendations, and the effect of additional information on patient management assessed.

Such an analysis could identify clinical and histological groups most likely to benefit from the information provided by immunofluorescence, performed on stored tissue. The pattern of disease encountered in an area would determine how many cases required this test and the number and location of laboratories needed to perform it.

Since all the biopsies were reported by a single experienced renal pathologist, the value of immunofluorescence in correcting light microscopic impressions was not assessed. It can be difficult to assign cutoff points between normal and minor degrees of capillary wall thickening or mild mesangial hypercellularity. At times the cellularity of a segmental lesion may be so equivocal that differentiation between segmental sclerosing and necrotising proliferative lesions can be difficult, especially for less experienced pathologists. In such situations immunofluorescence findings may correct or strengthen light microscopic impressions.

The experience described above has not led to abandoning the use of immunofluorescence for renal biopsy diagnosis in this centre. The resulting diagnostic inadequacy, although involving only a small percentage of patients, is unacceptable in a tertiary referral centre offering postgraduate training in pathology and nephrology. However, it has provided us with a more realistic view of the contribution of this expensive test to patient management.

1 Date A, Raghavan R, John TJ, *et al.* Renal disease in adult Indians: a clinicopathologic study of 2827 patients. *Q J Med* 1987;**64**:729–37.
2 Date A, Unni JC, Raghupathy P, *et al.* The pattern of medical renal disease in children in a south Indian hospital. *Ann Trop Paediatr* 1984;**4**:207–11.
3 Date A, Pant M. How useful is immunofluorescence in tropical renal pathology? *Trans R Soc Trop Med Hyg* 1990;**84**:599–601.