Immunohistology (IH) and Phase-Contrast Microscopy (PCM) in 310 Kidney Biopsies

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Phase-contrast microscopy (PCM) permits description of morphological structures in an unstained cryostat-section. The present report concerns our experience with this method in combination with immunofluorescence applied to kidney biopsies and its practical value for morphological diagnosis and for interpretation of fluorescent findings in various renal diseases and in transplant kidneys.

METHODS

Biopsies were obtained by percutaneous puncture, and occasionally by open biopsy or nephrectomy. The tissue was divided, or, more often, two specimens were taken.

Light microscopy. Renal tissue was fixed in Schaffer's solution for methacrylate- or buffered formol (10%) for paraffin-embedding. Semi-thin or 4μ sections were stained with haematoxylin-eosin, van Gieson, periodic acid-Schiff, Gomörri silver stain and Goldner's staining.

Immunofluorescence. Material embedded in PBS-buffered gelatine (8%) was snap frozen in liquid nitrogen and cut on a -25°C cryostat at 4μ. Preparation of slides followed standard procedures. FITC-labelled commercial antisera (Hyland Laboratories and Melyo Laboratories) against IgG, IgM, IgA, IgE, C' 3, fibrinogen, fibrin, albumin and horse-gamma-globulin were used. Sera were tested for specificity by immunoelectrophoresis. Blocking experiments for control of specificity were done.

Fluorescence microscopy combined with phase-contrast. Sections were studied with an Ortholux-Leitz microscope using phase-contrast in combination with an opak-illuminator according to PLOEM and two incidental uv-lamps (HBO 250).

The grading of immunofluorescence (Table I) is symbolised as +/-++/+++,
Table I. Synopsis of immunofluorescent findings combined with phase-contrast microscopy in different renal diseases. 
\(+/++/+++\) represents degree of immunofluorescence; (+) doubtful fluorescence; mes. = mesangial; gran. = granular

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>Immunofluorescence</th>
<th>Phase contrast</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>IgG IgM C3 IgA Fbg Proteinuria Vasculopathy</td>
</tr>
<tr>
<td>normal kidney</td>
<td>15 - - - - - - - -</td>
<td>normal structures</td>
</tr>
<tr>
<td>pyelonephritis</td>
<td>31 - - - - - - -  +/+++ 'struma like'</td>
<td>glomerular sclerosis interstitial scarring arteriosclerosis</td>
</tr>
<tr>
<td>nephrosclerosis</td>
<td>17 (+) - - - - - +</td>
<td>glomerular sclerosis arteriosclerosis</td>
</tr>
<tr>
<td>amyloidosis</td>
<td>6 ++ ++ ++ ++ ++ ++ ++</td>
<td>irregular lobulation loss of vascular structures</td>
</tr>
<tr>
<td>necrotising GN</td>
<td>3 - - (+) - ++ - diffuse mes.</td>
<td>loss of glomerular structures</td>
</tr>
<tr>
<td>Goodpasture</td>
<td>2 +++ + - - - - - linear extracapillary</td>
<td>extracapillary proliferation</td>
</tr>
<tr>
<td>diff. proliferative/</td>
<td>17 +++ ++ ++ (+) ++ +</td>
<td>smooth, swollen capillary loops</td>
</tr>
<tr>
<td>exudative GN</td>
<td>diffuse - gran. mes.</td>
<td></td>
</tr>
<tr>
<td>diff. sclerosing GN</td>
<td>26 +++ ++ +++ ++ ++ +/+ +</td>
<td>glomerular and capsular sclerosis arteriosclerosis</td>
</tr>
<tr>
<td>membrano-proliferative GN</td>
<td>4 + +++ +++ ++ ++ +/- irregular - coarse gran.</td>
<td>irregular thickening of GBM</td>
</tr>
<tr>
<td>lobular GN</td>
<td>2 +++ +++ ++ ++ +/+ irregular - gran. mes.</td>
<td>lobulation of capillary loops</td>
</tr>
<tr>
<td>focal proliferative/</td>
<td>17 /++ /++ /++ /++ ++ -</td>
<td>sometimes focal sclerosis</td>
</tr>
<tr>
<td>sclerosing GN</td>
<td>irregular - gran. sometimes focal</td>
<td></td>
</tr>
<tr>
<td>membranous GN</td>
<td>11 +++ ++ ++ ++ ++</td>
<td>general thickening of GBM</td>
</tr>
<tr>
<td>minimal lesion</td>
<td>14 - - - - - - ++ mes.</td>
<td>normal structures</td>
</tr>
<tr>
<td>hyperacute rejection</td>
<td>2 - - - - +++ - Thrombosis +++</td>
<td>glomerular/vascular</td>
</tr>
<tr>
<td>acute rejection</td>
<td>68 + + - - + - + diffuse - linear mes. diffuse - focal gran.</td>
<td>interstitial oedema cellular infiltration</td>
</tr>
<tr>
<td>chronic rejection</td>
<td>21 (+) (+) (+) + (+) diffuse - linear diffuse - focal gran.</td>
<td>intimal proliferation/sclerosis, prominent GBM</td>
</tr>
<tr>
<td>no changes</td>
<td>12 (+) (+) (+) ++ diffuse - linear</td>
<td>normal structures</td>
</tr>
</tbody>
</table>

representing respectively 30%, 60% and 90% positive immunofluorescence in the biopsies studied. Diffuse or focal fluorescence means all or some glomeruli respectively with positive fluorescence. Immunofluorescence (P-HK) and light microscopy (HZ) were studied independently.

RESULTS

Our findings are summarised in Table I. The following is a synopsis of our immunofluorescence findings with special emphasis on structural changes seen by PCM. Some groups of diseases are not mentioned in this paper,
eg Lupus erythematosus, multiple myeloma and a variety of other systemic diseases.

**Normal kidney (15 biopsies).** These specimens served as negative controls for the antisera and they all were found negative. By PCM, glomeruli had smooth capillary loops with open capsular space and normal Bowman's capsule. Tubules can be identified by their epithelial structures and a distinct interstitial space, as well as smaller arteries or peritubular capillaries. The internal elastic lamina of arteries is prominent and shows a blue autofluorescence.

**Pyelonephritis (31 biopsies).** Most biopsies revealed a typical combination of fluorescence negative glomeruli with focal sclerosis of capillary loops and capsule and focal distended 'struma like' tubules which showed casts with a bright (questionably specific) fluorescence mainly for IgA. Also focal interstitial sclerosis and various degrees of arteriosclerosis were found. Three biopsies had positive glomerular staining with irregular deposition of immunoglobulin and C'3. In three patients with hypertension we found fixation of C'3 in small arteries and afferent arterioles.

**Nephrosclerosis (11 biopsies).** Similar changes as seen in pyelonephritis were found in nephrosclerosis but glomerular sclerosis was more diffuse and 'struma like' tubular changes were rare. Again vascular deposits of C'3 were found in four patients with hypertension.

**Amyloidosis (6 biopsies).** All biopsies had irregular, focal or diffuse, granular or lobular depositions of IgG, IgM, IgA and/or C'3 in capillary loops and in mesangial areas. Arteriolar vessels showed segmental or diffuse staining of their walls. Blocking experiments were often negative. PCM showed loss of structure in identical regions. Thus an unusual glomerular and vascular pattern of deposits with loss of structure is highly suspicious for amyloidosis which should be confirmed by typical thioflavin-T and congo-red-fluorescence.

**Necrotising or extracapillary GN (5 biopsies).** Patients with rapidly progressive GN and anuria had two different immunofluorescence findings.

Two patients with Goodpasture's syndrome confirmed on autopsy and elution studies from the kidneys, showed linear fixation of IgG and to a lesser degree of IgM and C'3 along all basement membranes (Figure 1). PCM showed heavy extracapillary proliferation which compressed the capillary loops (Figure 1). Heavy deposits of fibrin were found in extracapillary sites. The other biopsies were negative for immunoglobulins and C'3. Only minor deposition of fibrinogen was seen in mesangial areas while PCM showed extensive loss of glomerular structures and some capsular proliferation.

**Diffuse proliferative or exudative GN (17 biopsies).** Diffuse or focal granular
deposits of IgG and to a lesser degree C'$_3$ and IgM were found in eleven biopsies. One biopsy showed diffuse linear staining of a glomerular basement membrane with IgG and C'$_3$. Five biopsies were totally negative for immunoglobulins and had only traces of mesangial fibrinogen, while light microscopy showed mesangial proliferation. By PCM capillary loops sometimes looked 'swollen' without significant sclerosis.

**Diffuse sclerosing GN (26 biopsies).** The outstanding feature was significant glomerular sclerosis with loss of capillary structures (Figure 2). All biopsies showed irregular granular deposits of IgG and C'$_3$, often C'$_3$ alone in a garland-like pattern (Figure 2). Mesangial fibrinogen was found and signs of proteinuria with tubular casts were sometimes prominent.

**Membrano-proliferative GN (4 biopsies).** In all biopsies C'$_3$ and to a lesser degree IgM and IgG were found in a granular, irregular coarse pattern along the capillary loops and sometimes in mesangial regions. By PCM there was no heavy sclerosis but sometimes a focal prominence of basement membranes. Thus predominance of coarse C'$_3$ globulin and focal basement membrane changes with rare sclerosis seems to be rather typical.

**Lobular GN (7 biopsies).** The immunofluorescence pattern was rather
similar to that found in memhrano-proliferative GN. Focal clear cut lobulation of capillary loops could be detected by PCM.

**Focal proliferative or sclerosing GN (17 biopsies).** Morphologically defined as focal segmental with proliferation or fibrosis with some capsular adherions, these lesions are difficult to identify by PCM. Granular deposits of IgG and IgM, usually with C$_3^3$, were found in nine biopsies. Sometimes the deposits were to be shown in a focal pattern. Some cases had diffuse fixation of fibrinogen in a mesangial pattern. An IgA-IgG-glomerulopathy (see below) was found in 3 biopsies.

**Membranous glomerulonephritis (11 biopsies).** All except one biopsy showed fine granular deposits of IgG and C$_3^3$ and IgM in smaller amounts, probably on the epithelial side of the basement membrane, while mesangial regions were negative. Again one biopsy showed a diffuse smooth or linear deposition of IgG and C$_3^3$. In most cases PCM showed diffuse thickening of basement membranes. The regular deposits, like 'string of pearls', together with diffuse thickening of the basement membrane easily allow recognition of the lesion.

**Minimal change (14 cases).** By definition there were no or few structural changes to be detected. Only one biopsy had some irregular fine granular deposits of IgG but 7 biopsies (especially from young children) showed diffuse mesangial staining for fibrinogen.

**Nephropathy with mesangial IgA-IgG-deposits (13 biopsies).** This glomerulopathy, first described by Berger, is an immunofluorescent entity defined by diffuse deposits of IgA and IgG in the mesangial region.
The histological diagnosis in our biopsies was focal, sclerosing or proliferative GN (3), proliferative GN (6) and sclerosing GN (4). The structural changes detected by PCM were in agreement with these findings.

**Transplant kidneys (113 biopsies).** Biopsies are summarised on the basis of their histological changes as: hyperacute rejection with extensive thrombosis and necrosis; acute rejection with interstitial oedema, and infiltration mainly by lymphoid cells; chronic rejection with interstitial fibrosis, and mixed lymphoid-plasma cell infiltration.

**Hyperacute rejection (2 biopsies).** Immunofluorescence showed severe thrombosis with fibrinogen deposits in glomerular and peritubular capillaries and on the intima of arterial vessels. Immunofluorescence is required for rapidity of diagnosis and specific detection of fibrin.

**Acute rejection (68 biopsies).** There are two distinct immunofluorescent findings which did not correlate to the degree of cellular infiltration: (a) transplant glomerulopathy with linear (9 biopsies) or fine granular deposits (12 biopsies) of IgG, IgM and to a lesser degree of C′3. These glomerular lesions were neither accompanied by proteinuria as one might expect from the partial membranous pattern of the deposits nor were severe glomerular changes found on light microscopy. Some endothelial swelling, some widening of mesangial matrix, and discrete thickening of the basement membrane were found, but serial biopsies in two patients revealed a total loss of these deposits after several months. Only one patient with heavy garland-like deposits developed a sclerosing GN and lost his kidney.

(b) A more severe clinical finding was acute transplant vasculopathy of arterial vessels. In ten biopsies we found heavy deposits of IgM, C′3 and

![Figure 3. Chronic transplant vasculopathy (PCM) without any deposits (IH)](image-url)
some IgG in all sections of arterial walls, often accompanied by thrombosis of peritubular capillaries. Three kidneys had to be removed for loss of function. Interstitial infiltration, oedema, and some swelling of arterial walls, can be evaluated by PCM.

**Chronic rejection (21 biopsies).** Again the biopsies showed some irregular granular deposits mainly of IgM, and in 6 biopsies there was linear staining for IgG, IgM and C'. Some of these cases showed a diffuse thickening of basement membrane on PCM and light microscopy. Most of the larger vessels revealed various degrees of intimal proliferation, sometimes with extreme narrowing of the vascular lumen (Figure 3). These vessels were negative for immunoglobulins and for fibrinogen.

**DISCUSSION**

Our findings in 310 renal biopsies studied by fluorescence microscopy revealed the advantages as well as the drawbacks of PCM and IF. Irreversible lesions such as glomerular sclerosis, thickening or focal splitting of the basement membrane, vascular sclerosis and interstitial infiltration or scarring can be identified by PCM with some experience. On the other hand it is difficult to detect proliferation of mesangial or epithelial cells with this method. Obviously PCM is not meant to replace light microscopy but it was very helpful when insufficient amounts of tissue were secured for regular histology. This is of special importance in transplant kidneys where most relevant morphological changes can be detected by this technique.

Also we found PCM examination stimulating for the immunopathologist for interpretation and discussion of immunofluorescent findings. In individual cases there often is no clear correlation between the extent of immunohistological findings, morphological changes, clinical signs of functional disturbance, and progression of the disease. For example, intense linear fixation of immunoglobulins in some transplant kidneys may occur without morphological changes or functional lesions and in others there may be progressive GN with anuria and only a few fibrinogen deposits. It would be quite misleading to make a diagnosis of 'severe anti-basement-membrane glomerulonephritis' in such a transplant patient and of 'negative immunofluorescence' in a patient with anuria without adding the morphological findings. Description of renal biopsy specimens therefore should combine immunohistological and structural characteristics, for example, 'linear deposits of immunoglobulins without structural changes' for a transplant kidney, and in a case of necrotising GN, 'some glomerular fibrinogen deposits in mostly destroyed glomeruli'.

In general a description of immunofluorescence, with its 'morphological background' has to be discussed with light microscopy and the clinical course of the disease.
ACKNOWLEDGMENTS

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OPEN DISCUSSION

K. LANGE (New York): I wonder whether you have tried, as we have, to use just the dark field illuminator and to try dark field fluorescence microscopy. You get exactly the same picture and it is simpler than moving over to phase contrast. If one uses stronger sera than you have, one can then quite easily distinguish two points.

KRAUSE: Thank you, we will try this out.

C. L. HALL (Birmingham): Is it possible using these techniques to distinguish in transplanted kidneys when the kidney is failing or has a recurrence of nephrotic syndrome, between a recurrence of the native kidney disease and a new disease affecting the transplant?

KRAUSE: I cannot distinguish this. In our group we had 32 patients — one patient had a non functioning kidney and it had to be removed. It was shown by immunofluorescence that there were very heavy globular deposits similar to those found in chronic sclerosing glomerulonephritis. The lesion was also apparent on light microscopy. Initially I think it is difficult for one to distinguish the so-called transplant 'glomerular fatigue' by either immunofluorescence or phase contrast. This is a reversible condition, and we have seen cases where the globular deposits disappeared and we were left with only a little thickening of the basement membrane. Therefore I would say that initially it is impossible to distinguish between so called transplant glomerular fatigue and recurrent nephritis.

A. LEATHERM (London): Have you had the opportunity to study any of these kidneys which have shown a linear deposition of immunoglobulin, to see whether the antibody has antibasement membrane properties?
KRAUSE: I have shown a case of Goodpasture's syndrome on which we did elution studies. The eluted protein we obtained stained a normal human kidney — thus in my opinion, proving the antibody activity.