

C4d PRESENCE IN KIDNEY ALLOGRAFT BIOPSY: SENSITIVITY AND SPECIFICITY OF IMMUNOPEROXIDASE VS IMMUNOFLUORESCENCE

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Objectives

- Evaluate the sensitivity/specificity of immunoperoxidase method in comparison with the standard immunofluorescence.

Methods

- Retrospective review of 87 select biopsies made for allograft dysfunction.
- Immunofluorescence (IF) was performed in frozen allograft biopsies using monoclonal antibody anti-C4d. (Figure 1)
- The indirect immunoperoxidase (IP) technique was performed in paraffin-embedded tissue with polyclonal antiserum. (Figure 2)
- Biopsies were independently evaluated by two nephropathologist according Banff 2007 classification.

Immunofluorescence in frozen tissue

- Aceton fixation
- Ab. monoclonal Ms/Hm anti-C4d– 1/30 (Quidel®)
- Ab. IgG Hs/Ms – 1/50 (Vector®)

Immunoperoxidase in paraffin-embedded tissue

- Hydrogen Peroxide Block (Thermo Fisher Scientific®)
- Ultra V Block (Thermo Fisher Scientific®)
- Ab. polyclonal Rb/Hm anti-C4d – 1/30 (Serotec®)
- UltraVision ONE HRP Polymer (Thermo Fisher Scientific®)
- DAB+ (Dako®)

Methods

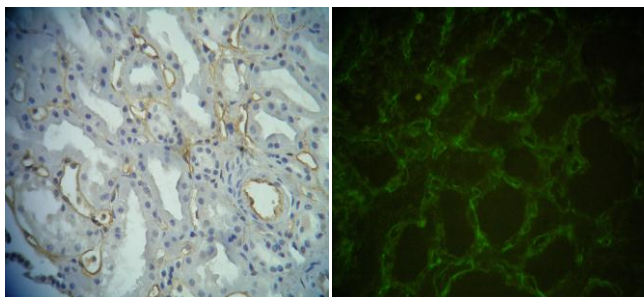
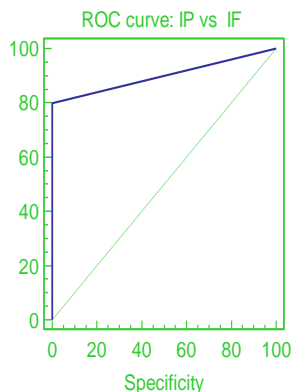


Figure 1

Figure 2

Results

- By IF, peritubular C4d deposition were detected in 60 biopsies and absent in 27 biopsies.
- The evaluation of biopsy by IP was less precise due to the presence of background and unspecific staining.



Results

- We find 13.8% (12/87) of false negative and Banff classification concordance in 79.3% (69/87) of cases.
- The ROC curve study reveal a specificity of 100% and sensitivity of 80.0 % of IP method in relation to the gold standard (area under curve:0.900; 95%. Confidence interval :0.817-0.954; $p=0.0001$).

Banff Classification C4d		Cases
Immunofluorescence	Immunoperoxidase	n =87
Diffuse	Diffuse	33 (37.9%)
Diffuse	Focal	6 (6.9%)
Diffuse	Negative	3 (3.4%)
Focal	Focal	9 (10.3%)
Focal	Negative	9 (10.3%)
Negative	Negative	27 (31.0%)

- The IP method presents a good specificity, but lesser sensitivity to C4d detection in allograft dysfunction.
- The evaluation is more difficult, requiring more experience of the observer than IF method.
- If frozen tissue is unavailable, the use of IP for C4d detection is acceptable.