IMMUNOHISTOCHEMISTRY FOR COMPLEMENT PRODUCT C4D IN RENAL ALLOGRAFTS: EFFECT OF FORMALIN FIXATION ON FREQUENCY OF DETECTION OF CIRCULATING DONOR SPECIFIC ANTIBODIES

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Objectives: Immunohistochemistry for C4d has become a routine method for diagnosis of antibody-mediated acute rejection in allograft kidneys. Initial studies utilized fresh frozen tissue, which is not always available. In recent years, several medical centers have started using paraffin fixed tissue, but a comparative evaluation of the ability of both methods to predict donor specific antibodies (DSA) has only been performed in few studies.

Methods: 44 allograft kidney biopsies from 43 patients transplanted in our institution had paired frozen section (FS) and paraffin embedded (PE) tissues available for evaluation. Data on anti-HLA including circulating donor specific antibodies was obtained. Peritubular capillary staining (PTC) was graded as negative, focal, and diffuse. Additionally, staining of glomerular (GBM) and tubular basement membrane (TBM) was evaluated.

Results: Diffuse and focal PTC staining were found in 17/44 (39%) and 7/44 (16%) of FS versus 8/44 (18%) and 10/44 (23%) of PE respectively. 35% of biopsies with diffuse C4d on FS showed focal staining on PE. Positive DSA were detected at variable times post-transplant in 14/44 (32%) of patients and were associated with diffuse, focal, and negative staining in 11/14 (79%), 1/14 (7%), and 2/14 (14%) of FS versus 5/14 (36%), 6/14 (43%), and 3/14 (21%) of PE respectively.

Diffuse C4d PTC in FS was seen in 79% (11/14) of patients with a history of DSA. In PE diffuse C4d was present in 35% (5/14) of patients with a history of DSA, however, this rate of detection was increased to 79% (11/14) by combining diffuse and focal PE stain. Regarding staining outside of the PTC, diffuse global GBM deposits were observed in 18/44(41%) of FS versus 8/44(18%) of PE, while staining of TBM was seen in 21/44(48%) of FS compared with 2/44(4.5%) of PE. GBM and TBM deposits did not correlate with any specific morphologic diagnosis such as chronic transplant nephropathy or BK virus nephropathy respectively.

Conclusion: Diffuse PTC C4d deposits are better detected in FS than in PE. However, if focal C4d staining patterns in PE are also considered, the sensitivity with respect to detecting donor specific antibodies becomes comparable to FS.