PERITUBULAR C4d DEPOSITS IN RENAL ALLOGRAFT BIOPSIES AND ANTI HLA I/II ANTIBODIES SCREENING - INCIDENCE AND CLINICAL IMPORTANCE

H. Viana, F. Carvalho, M.C. Santos, M.J. Galvão, A.R. Santos, F. Nolasco, J. Ribeiro Santos

Serviço de Nefrologia do Hospital de Curry Cabral, Lisboa, Portugal

Background: The presence of C4d peritubular capillaries deposits (PTC) is a sensitive and specific method to diagnose humoral rejection. The use of monoclonal antibody and immunofluorescence in frozen tissue section is the better method for its detection. In this study we determine the incidence and clinical importance of C4d PTC deposits in renal transplant biopsies performed in three years.

Methods: C4d staining was performed in all the biopsies with available frozen tissue obtain for allograft dysfunction between January 2004 and December 2006. We employed a monoclonal anti C4 antibody (Quidel Corporation). Routine light microscopic studies were performed simultaneously. The study was prospective since March 2005, since when a serum sample was obtained at the time of biopsy to detect the presence of circulating anti-HLA class I/ II alloantibodies by flow cytometric assay (FlowPRA screening test). In acute humoral rejection biopsies, the circulating donor specific anti donor HLA antibodies were determined by flow cytometry.

Results: We studied 109 biopsies in 86 cadaver renal transplant patients (61 M, 25 F), with an average age of 47,9 years old. Overall 16 biopsies (14,7%; n=16/109) presented with diffuse positive C4d (+C4d) in PTC. The histological diagnosis was: acute humoral rejection (n=4), chronic allograft nephropathy (n=7), acute cellular rejection (n=1), acute tubular necrosis (n=1), thrombotic microangiopathy (n=1), calcineurin inhibitor toxicity (n=1), and normal kidney (n=1). Biopsies with C4d deposits had an average time from of transplantation of 40,5 months and a median serum creatinine of 3,35 mg/dl. There was no significant difference in creatinine levels between the positive and negative groups. The incidence of +C4d was of 13,5% (n=8/59) in the group in the first 6 months and of 16% (n= 8 /50) after 6 months of transplantation (p>0,05). Half of +C4d biopsies in the first 6 month were associated with acute humoral rejection (n= 4/8). After 6 month, the majority of +C4d biopsies (n=7/8) had evidence of chronic allograft nephropathy. In this last group of 8 biopsies in 6 patients: 2 patients subsequently die and 4 patients lost there graft. The presence of C4d PTC deposits was more frequent in biopsies from HCV antibody + patients (n=8/13; Fisher's test p<0, 0001), and with a previous renal transplant (n= 5/15; p= 0.0059). The 4 acute humoral rejections (all diagnosed before March 2005) had circulating donor specific anti- HLA antibodies. Circulating anti-HLA alloantibodies were screened in serum obtained from 46 biopsies (n=20 <6 month; n=26 ≥ 6month). They were detected in 10.9% (n=5; >5% of reactivity). Anti-HLA alloantibody were detected in 7,5% (n=3/40) of the C4d negative biopsies, and 33,3% (n=2/6) of C4d + biopsies (p>0,05).There was no circulating anti-HLA alloantibody in 90,25% of the C4d negative (n=37/41).

Conclusion: The prospective evaluation of C4d PTC deposits is essential to early diagnosis of acute humoral rejection and its rapid treatment. The significance of late C4d deposits is not established, but may point to the importance of a humoral component in chronic allograft nephropathy. Our data also suggest an association between +C4d PTC deposits and 2nd transplant recipients, and the presence of anti-HCV antibodies. It is not clear if antibody to HCV is a risk factor for C4d deposits or merely indicates longer time on dialysis. Negative alloantibody screening may indicate a reduced risk for the presence of C4d PTC deposits.