



Borderline Changes in Renal Allograft Protocol Biopsies: Intensive Therapy And Graft Outcome

KEYWORDS

Kidney transplantation; Acute rejection; Borderline change; Prognosis

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ABSTRACT *Objective* To evaluate the relationship between borderline changes and graft outcome in recipients of cadaveric kidney transplantation. | *Methods* All biopsies were systematically evaluated according to Banff schema. PAP four-layer immunohistochemical staining was applied in the detection of CD4+, CD8+, CD68+, CD25+ cell, PCNA infiltration and HLA-DR protein synthesis. Patients with borderline change were analyzed by "intent to treat" and were followed for a minimum of 3-year. | *Results* Forty-six recipients with "borderline changes" were diagnosed within 3 months after transplantation. Patients by "intent to treat"(Treated group) had a significant decrease in early acute rejection episodes and a lower serum creatinine at one month than did patients in the untreated group. In borderline changes biopsies, there were more infiltrating CD4+,CD8+,CD68+,CD25+ cells than those in normal allografts, but still significantly less than those in allografts with acute rejection. | *Conclusion* The results of this study suggest the early protocol biopsy and the treatment of borderline with corticosteroids may lead to better histologic and functional outcomes in renal transplant recipients.

INTRODUCTION

Clinical or subclinical acute rejection in three months after renal transplantation is the strong precipitating factors of early chronic pathologic changes and later rejection^[1,2,3]. Therefore, sufficient and powerful immunosuppressant should be used at postoperation and control for non-T cell-mediated inflammatory response may relieve or suspend these changes^[4,5]. The research report of subclinical rejection is few, and the final results are not the same either^[6,7]. So far, the pathology, immunopathogenesis character, renal transplantation tissue and the influences on the renal function have not caused enough attention yet. There is no final conclusion on whether these kinds of recipients need intensive immunosuppressive treatment as well as its influence on prognosis of renal allograft^[8,9]. The long-term follow-up visit on 46 renal allograft recipients who were diagnosed as "borderline changes" is intended to investigate clinical evolution process of borderline change and its impact on long-term effects of renal transplantation.

MATERIAL AND METHODS

Subjects

From January 2002 to February 2012, our hospital operated total 205 renal transplantation surgeries. All the renal transplant recipients received protocol biopsies after surgery in one month and three months. 46 cases in 458 transplanted renal biopsy specimens were diagnosed as borderline changes, and those who accompanied with or did not graft dysfunction are research objects in this paper (borderline changes group). Meanwhile, 120 recipients who had normal renal function without acute rejection and 29 acute rejection recipients in the same period were listed as normal control group and acute rejection group. ATN(acute tubular necrosis), CsA(cyclosporine A) nephrotoxicity, infection and surgical complications were all ex-

cluded in selected objects.

Immunosuppressive regimen

All the patients use mycophenolate mofetil (MMF)+CsA+Pred (Prednisone) as "trigeminy" inductive and maintenance immunosuppressive program. MMF1.5 g/d; CsA: when urinary volume > 100ml/h in six hours after surgery, and serum creatinine reduced to 1/2 of pre-transplantation, patients should begin to take CsA 4mg/kg/d, and add to 8mg/kg/d when renal function returns to normal. Afterwards, adjust dose according to whole blood CsA trough concentration (200-400ug/L); corticosteroids: before the recovery of transplanted kidney blood circulation in surgery, and in the first and second day after surgery, use MP 1.0g, 0.5g and 0.5g by intravenous drip, oral Pred 80mg/d on the third day after surgery, and reduce 10mg day by day till to 20mg/d as maintenance. Anti-rejection and intensive treatment regimen: methylprednisolone(MP) 1.0g/day for 3 consecutive days.

Pathological studies

Protocol biopsies were performed months 1 and 3 after transplantation in all cases, and a renal core biopsy was performed if clinically indicated in cases of acute rejection and subclinical rejection after intensive treatment. Biopsies taken for immunofluorescent proceeding, frozen, embedded, and sectioned using standard techniques. Routine HE, PSP and PAM-Masson staining of microscope specimens, frozen section of renal tissues (4um) are used for immunohistochemical staining. Apply monoclonal antibodies of specificity with marker protein (all purchased from DAKO company), adopt PAP four-layer staining method to inspect renal cortex interstitium CD4+, CD8+, CD68+, CD25+, cells and PCNA as well as positive value of HLA-

DR. Calculate quantity of stained positive cells in stained positive renal tubules or renal interstitium of cortex separately, and the experimental result is respectively presented by positive renal tubules number or positive cells quantity/mm² of renal cortex interstitium.

Clinical diagnostic criteria of acute rejection

Fever of undetermined origin after renal transplantation, urinary volume reduces to 25-50% of the original, serum creatinine level presents advanced rising, transplanted kidney has distending pain with hypertension and weight gain. Acute rejection should be confirmed by renal biopsy.

Pathological diagnosis of acute rejection and borderline change

The pathological diagnosis of allograft acute rejection and borderline change on renal biopsy specimens in this research should be made according to pathological changes of glomerulus, tubules, vessel of renal interstitium and infiltration of interstitium mononuclear cell, according to the Banff classification scheme [10]. Diagnosis of borderline change [11]: mild renal tubular inflammation (infiltrating cells <4/tubule section t1 level), and/or interstitium inflammatory cell infiltration is less than 50% (i1 or i2 level), and no morphological changes on vasculitis.

Determination of serum creatinine

Test BUN (blood urea nitrogen), serum creatinine and other biochemical indexes with Hitach-7150 automatic biochemical analyzer. The renal function index is based on the level of serum creatinine. Give dynamic viewing on the change of serum creatinine in postoperation of renal transplantation, and pre-and post renal biopsy. Transplanted kidney dysfunction means serum creatinine can not drop to normal level (SCr \geq 130 μ mol/L) after renal transplantation.

Statistical analysis

The measurement data is represented by mean \pm standard deviation ($x \pm s$), T-test is used in comparison among groups. Enumeration data is represented by case number, chi-square test is used in comparison among groups; $P < 0.05$ has significant difference, $P < 0.01$ has obviously significant difference.

RESULTS

Demographics

There was no significant difference in ages, warm ischemia time, cold ischemia time, the standard of complement-dependent cytotoxicity (CDC) and panel reactive antibodies (PRA) among three groups. The causes of end-stage renal disease (ESRD), duration and methods of renal replacement therapy (RRT) between the groups have not obvious difference as well (Table 1.).

The occurrence time of borderline change and its clinic

The borderline changes of 46 patients mostly occurred within 3 months after surgery (42,91.3%), and among them, 32 cases in 1 month after surgery (69.6%), 10 cases in 1 month to 3 months after surgery (21.7%), and 4 cases in 6 months after surgery (8.7%). 31 cases with borderline changes were found through protocol biopsies, and the renal function was normal and stable in renal biopsy. 15 cases of them were found through emergency renal biopsy because of short-time abnormal renal function and progressive rising of serum creatinine. .

The evolution and outcome of borderline change

Among 31 cases with borderline change and normal renal function after repeated renal biopsy, 15 cases had no re-

jection, 10 cases still had borderline changes, 6 cases had progressive rising serum creatinine and acute rejection in the second month and fourth month after surgery (4 of them had acute cellular rejection with acute vascular rejection, 2 had acute vascular rejection), 3 cases had chronic allograft nephropathy after six months. The other 15 cases with borderline changes and abnormal renal function were treated with MP intensive treatment, and the serum creatinine of 12 cases reduced to normal level by one month treatment after therapy. And the serum creatinine of the other 3 maintained at 212.6 ± 64.7 μ mol/L, and the renal function of them became normal after 6 months follow-up.

Immunohistochemical changes in borderline change

There were certain amount of CD4+, CD8+ cells and a few PCNA and CD25+ cells in normal transplanted renal tissues. The number of infiltrating cells in borderline changed renal interstitium was higher than that of control group obviously, but it was next only to acute rejection group, and CD4+, CD8+, CD68+, CD25+ cells and PCNA all obviously increased, and CD8+ cells were a little more than CD4+ cells. HLA-DR antigen expression in tubules of borderline changed renal cortex increased dramatically. The number of infiltrating cells in acute rejection renal tissue was obvious higher than that of normal group and borderline change group (Table 2.).

The impact of borderline change on allograft long-term survival

Classify the borderline change cases with intensive therapy as borderline change treatment group (n=15), and the cases without intensive therapy are untreated group (n=31). Compare the renal allograft survival rate of control group and acute rejection group, from the diagram, it can be found that 1 to 2-year survival rate of allograft has no obvious difference among the four groups, however, 3 year survival rate of borderline change untreated group is obvious lower than that of control group and borderline change treatment group (Figure 1.).

DISCUSSION

In this paper, by the control study of the clinical data of 46 renal transplant recipients with borderline changes, the author observed that the borderline changes often happened within three months after renal transplantation, and about the one-third recipients found that when the borderline changes happened, there were no abnormal clinical manifestation of renal function and also no reliable clinical index to indicate its happening. Borderline change of the transplanted kidney can be early observed only by protocol biopsies.

Renal function abnormality would happen or not in the clinic of borderline change, and even with the rise of the serum creatinine, it still did not like the rapid rise of the clinical acute rejection. Banff schema did not pinpoint the significance of borderline change of the renal transplantation [10,11]. From the results of this study, Borderline change also indicated that the acute rejection may happen or would happen, or even lead to early or later allograft nephropathy which would influence the long-term survival of renal allograft [12].

People usually think that the borderline change is a kind of pathology change between normal renal allograft and acute rejection [13], however, the author held different opinions. In this research, the author discovered that the differences between acute rejection and borderline change in renal allograft biopsy specimens are actually the differ-

ences between phenotype and activated state of infiltrating cells in renal allograft. In table 2, we can see CD4+, CD8+, CD68+, CD25+, cells and PCNA clearly increased in borderline change groups, and the HLA-DR antigen expression of renal tubules also increased markedly. This kind of positive cells expression may induce the happening of clinical acute rejection.

The correct analysis and evaluation of renal allograft pathology and immunopathology changes are the precondition to conduct the right anti-rejection treatment and objectively evaluate the immunosuppressant curative effect [14,15]. Whether the borderline change needs the intensive treatment or not, it depends on the original adopted anti-rejection treatment, immunopathological characteristics (such as infiltrating lymphocyte is activate or not) in renal allograft biopsy specimens, the evidence of renal tissue damage, renal allograft functions and so on. In this paper, the author conducted renal allograft biopsy of recipients with abnormal renal function after transplantation, and discovered that the abnormal renal function had nothing to do with other reasons, and it was subclinical rejection, or even just showed borderline change. In addition, the borderline change in previous reports was just the early and slight acute rejection and such borderline change was not treated, as a result, the acute rejection further developed [16,17]. The data showed that borderline change recipients were inclined to anti-rejection treatment, even if the recipients' borderline changes were stable, they also needed to be observed carefully and close followed-up, which had a very important clinical significance of improving the long-term allograft survival.

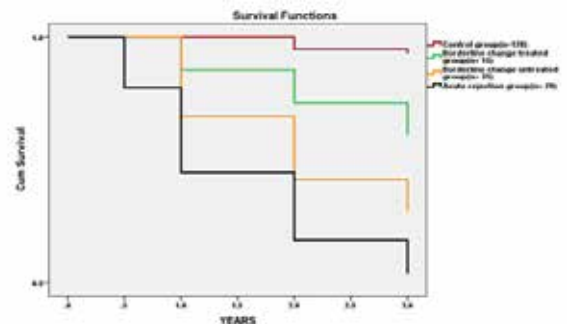
Table 1. Clinical Data

	Control group	Borderline change group	Acute rejection group
Gender (M/F)	97/23	32/14	21/8
Age (years)	36.4±9.4	36.8±7.0	36.3±8.2
Warm ischemia time (min)	7.2±2.0	7.4±1.7	7.4±1.4
Cold ischemia time (h)	15.5±7.2	14.2±6.0	15.0±6.9
CDC (%)	5.0±2.6	4.7±2.3	4.6±2.2
PRA (%)	17.2±2.6	15.3±3.4	15.8±2.9

Table 2. Immunohistochemical change

	Control group (n=120)	Borderline change group (n=46)	Acute rejection group (n=29)
Interstitial positive cell number (mm ²)			
CD4+cell	36.8±8.1	178.4±94.3	264.7±59.5
CD8+cell	33.8±9.6	250.2±155.2	683.3±266.3
CD68+cell	2.7±3.0	21.5±9.6	46.1±23.9
PCNA cell	9.6±4.7	22.8±18.8	167.5±172.6
CD25+cell	2.7±3.0	21.5±9.4	46.1±23.9
Interstitial positive tubule (%)			
HLA-DR	8.9±8.4	48.1±26.3	52.8±22.2

Figure 1. The impact of borderline change on allograft long-term survival



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