SELECTED PROGNOSTIC FACTORS OF LONG-TERM RENAL GRAFT FUNCTION

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Kidney transplantation is a method of choice as a treatement for end-stage renal disease in indicated cases. However, the long graft survival represents only about 50% due to various types of rejection as a leading cause of graft loss in renal transplant recipients.The early dg. of rejection and especially acute rejection, it's adequate management, decreased risk for the future chronic rejection nefropathy. This is a primary goal of the clinician caring for these patients. We use several methods in order to make diagnosis of acute rejection.Urine cytology and urine flow cytometry have been found highly sensitive specific for the early diagnosis of acute rejection, provide us useful information in differentiation from others causes of graft dysfunction.Urine analysis have some advantage over other diagnostic methodes and can facilitate the observation of a graft over time.

Kidney transplantation is method of choice as a treatement for end-stage renal disease in indicated cases. However, the success rate of clinical transplantation in the one year posttransplant period has improved in the past three decades due to number of factors, including better matching of donor-recipient pairs, the use of newer immunosuppressive drugs, organized follow-up care, optimal management of the various post-transplant complications and others,the long graft survival represents only about 50 % due to allograft rejection, which continues to be a major problem and is the leading cause of graft loss in renal transplant recipients.

The renal allograft rejection is classified under four categories that can be distinguished on the basis of their time of onset, clinical and pathologic findings, and the response to antirejection therapy: hyperacute rejection, accelerated acute rejection, acute rejection with acute tubulo-interstitial, vascular or glomerular changes, chronic rejection with chronic tubulo-interstitial, vascular and glomerular, mostly irreversible, changes[1–2,7,13,21].

Hyperacute rejection and accelerated acute rejection are in most cases irreversible and necessitate graftectomy.

It is necessary to differentiate between hyperacute rejection, accelerated accute rejection, acute rejection and acute tubular necrosis as a type of non immunological, ischemic damage of graft in the earliest posttransplant period.

Acute tubulo-interstitial rejection is by far the most common form of rejection observed in organ transplant recipients as early as 5–7 days after renal transplantation, with typical clinical signs including sudden deterioration in graft function, decline of diuresis, oedema, hypertension, rarely fever, graftache. It is primarily mediated by cellular defense mechanisms. Analysis of the cells infiltrating the rejected human renal allografts showes especially T lymphocytes with their subsets, B-lymphocytes, monocytes, macrophages, natural killer cells, neutrophils. Early diagnosis and successful treatment of acute rejection is very important in minimizing graft damage and decrease risk for future chronic rejection nefropathy.

The clinical manifestation of chronic allograft rejection is the classic triad of hypertension, proteinuria (at times in the nephrotic range), and progressive decline in renal function. The progression to end-stage renal failure is from persistent immunologic injury or from nonimmunologic causes or both. This problem can occur as early as 3–6 months after renal transplantation. It is the predominant cause of graft loss in the late posttransplant period[4,12–14,20–21].

The early diagnosis of acute rejection in renal allograft recipients is a primary goal of the clinician caring for these patients.The acute rejection process is more likely to be successfully reversed at an early stage, the longer it progresses, the more permanent damage is done. Rejection must be differentiated from acute tubular necrosis, drug toxicity and infection so as to treat correctly and avoid morbidity and mortality. Recently the connection between acute rejection-especially late acute rejection- and chronic rejection has been appreciated, making the prevention and early treatment of acute rejection even more important[17].

We use several methods in order to make diagnosis: The renal biopsy has been considered the gold standard for the morphological evaluation of renal/graft paren-

chymal disease. However, this diagnostic procedure has inherent limitations. The procedure is invasiv, sometimes painful, expensive, and has potentially dangerous complications[16].

Urine cytology has been recognized as a valuable technique for monitoring renal allograft recipients since the earliest days of transplantation. The finding of immunecompetent cells such as lymphocytes, lymphoblasts and monocytes, as well as renal tubular cells correlates well with acute rejectioin. Lymphocytes are usually absent or present in very low numbers in patients without acute tubular necrosis or acute rejection. They were present in low numbers in most patients with acute tubular necrosis and in significantly higher numbers in most episodes of acute rejection. There was also a 3 to 4 – fold increase in lymphocyte numbers during acute rejection. However lymphocytes comprised only a small percentage of the cells found in the urine during acute rejection. This is because of the large numbers of granulocytes and tubular cells present simultaneously during acute rejection. The masking of lymphocytes by granulocytes and especially tubular cells might account for the faillure of many investigators to detect lymphocytes during AR. Other investigators have reported that lymphocytes accounted for most of the cells present during acute rejection[5–6,17,20].

Tubular cells are seen in all patients during the first 2 weeks, reflecting ischemic damage to the kidneys. Their presence in urine even in patients with no clinical evidence of acute tubular necrosis and in patients in whom the immediate transplant biopsy showed no features of acute tubular necrosis indicates that mild ischemic damage had nevertheless occurred. The presence of proximal tubular cells in urine after this initial period was related to acute rejection. There is no significant difference in the number of proximal-tubular cells between acute tubular necrosis and acute rejection in that initilal period but after 2 weeks posttransplant the presence of proximal tubular cells could indicate acute rejection. There was also a 4–5 fold increase in the number of tubular cells during acute rejection[20].

Urine cytology was considered to be diagnostic of acute rejection when tubular cells were found in the presence of more than 2 lymphocytes and/or lymphoblasts / high-power field (40x) or lymphocyturia of more than 20 % and concomitant polymorphs of less than 55% of 100 nucleated cells, excluding squamous epithelial cells[17].

Cyclosporine nephrotoxicity is defined as a deterioration of graft function in the absence of acute rejection or chronic rejection, infection, or ischemic damage of the kidney graft with high level of cyclosporine, improvement of graft function after cyclosporine level normalization, with typical morphological signs of tubular cells – small isometric or nonisometric vacuolization of the cytoplasm, intracytoplasmatic basophilic inclusions, megamitochondria, microcalcifications and typical arteriolar changes – some of them detectable by urine sediment analysis too.

Intranuclear inclusions in the tubular cells could be detected in the urine of patients with anti-CMV antibodies of the IgM and IgG class evaluated in context of preliminary urine findings and clinical picture[2,3,13,18–19].

There has been considerable interest in the surface antigens of cells infiltrating allografts undergoing rejection both for identifying cell populations and investigating cell activation[17].

Recently, reports have been published detailing the immunocytology of the urinary sediment of transplant recipients. The technique of urine flow cytometry is utilized to examine the presence of activation markers on the cells in the urine of transplant recipients, using monoclonal antibodies, for example anti-CD3, URO-3 (specific for proximal tubular cells) anti HLA-DR, anti-CD25/IL 2-R/,anti-CD54/ICAM-1/and others. Percentage and total number of positively stained cells are obtained and the flow cytometry considered positive when the mean fluorescence is significantly different from the control with at least 5% of the cells stained with anti-HLA DR or anti-CD3,anti-CD 25, anti-CD 54.The most specific parameter analyzed for the diagnosis of acute rejection was recognized urine flow cytometry with a CD3/URO-3 of more than 1. In normal tissue, expression of class II major histocompatibility antigens / HLA-DR / is confined to macrophages, dendritic cells, B-cells and vascular endothelium. Class I /HLA-A, B, C / antigens are expressed on all nucleated cells. Expression of DR antigens increased considerably on renal tubular cells in patients recently treated for severe rejection. DR antigens are also expressed on cell surface of isolated tubular cells aspirated from transplanted kidneys with acute tubulo-interstitial rejection but nor on tubular cells in normal kidneys or aspirates from kidneys without rejection. Biopsy specimens with increased DR expression in tubules usually have an interstitial T cell infiltrate. Expression of DR antigens on tubular cells is not related to HLA-DR incompatibility between donor and recipient, on the type of immunosuppressive therapy given. The expression of DR antigens on renal tubular cells is considered to be induced by the infiltrating activated T cells or be a consequence of tubular regeneration following rejection or ischaemic damage. We could not find significant DR antigens expression on renal tubular cell membranes in biopsy specimens of normal kidney or of renal tubular cells from fine needle aspirates or biopsy specimens of kidneys not showing rejection[9,15,17,20].

Leukocyte adhesion molecules are critically involved at a number of stages immune and inflammatory response, and their importance in the respons to a renal allograft has been recognized for some years. They are involved in antigen presentation, in the cascade of events leading to extravasation of leukocytes into the allograft, in the subsequent migration of leukocytes through the extracellular matrix, and in the interactions between effector and target cells. Thus the adhesion molecules /for example ICAM-1/ are highly attractive targets for

therapeutic intervention in organ transplantation. Strategies have been explored to exploit the involvement of adhesion molecules in ischemia/reperfusion injury, allograft rejection, and the induction of immunological tolerance. Furthermore, the expression of a number of adhesion molecules is regulated by cytokines /for example IL 2, IL 6/, and elevated levels may be detected both in transplant biopsies and as soluble forms /for example IL 2R, IL 6R/ measured in serum and urine. It has been proposed that these changes in levels might provide useful information in the diagnosis of allograft rejection and differentiation from others causes of graft dysfunction[8,10,22–23].

Both urine cytology and urine flow cytometry are found to be equally highly sensitive in the diagnosis of acute rejection after the ischemic injury period, and to have negative predictive values. Urine flow cytometry is significantly more specific and more accurate than urine cytology for the diagnosis of acute rejection. When both tests are simultaneously positive the sensitivity and specificity for the diagnosis of acute rejection are 87 and 93%, respectively.Both tests are most reliable after the first 5 days following kidney grafting, when urine production and cell excretion are less variable than in the immediate posttransplantation period. There is a good correlation between the severity of urine cytology findings – i.e. counts of lymphocytes and/or lymphoblasts – and the severity of rejection diagnosed by core biopsy results. Cytodiagnostic urinalysis has the advantage over renal biopsy in that it can be repeated as often as necessary and thus can facilitate the observatioin of the development or regression of a renal lession over time. A good agreement was found between the clilnical diagnosis of acute rejection and the results of both urine techniques. Since both tests are highly sensitive for the diagnosis of acute rejection, the absence of signs of rejection by these techniques in phase of failing renal function in a patient should alert the physician to consider other diagnoses. Urine cytology appears to become positive earlier than the clinical diagnosis of acute rejection, and the normalization of the urine-flow cytometry result may be a marker for successfully treated rejection. Also the number of cells in urine cytology progressively declined in rejections that were successfully treated. Monitoring the response to antirejection therapy by these techniques may offer the opportunity to adjust the immunosupressive regimen. It was noted that a higher number of lymphoblasts was seen in association with more severe acute rejection in urine cytology. In addition, urine remained persistently positive in those cases that failed to respond to initial antirejection therapy[5–6,11,17,20].

Our investigation is based on so far published results of urine cytology and urine flow cytometry of transplanted patients. As several other authors we hypothesize that early diagnosis of acute rejection is possible utilizing routine serial urine cytologies, and early treatment may therefore be instituted, with the potential benefit of minimizing graft damage and limiting the total use or regimen change of immunosuppressive drugs. Continuous monitoring of patients with persistently abnormal urine cytology and urine flow cytometry may reveal a subset at increased risk for future acute as well as chronic rejections and alert us to change immunosuppressive strategy in order to asses better long-term graft survival.

REFERENCES

1. Aikawa, A., Hadano, T., Ohara, T., Hasegawa, A., Yamashita, M., Shindo, M., Mizuiri, T., Sakai, K., Hirayama, N., Arai, K., Tajima, E. (1998) Relation between ABO blood type antigen and antibody and acute vascular rejection in ABO incompatible kidney transplantation. Transplant. Proc. *30*, 3507–3509.
2. Almond, P. S., Matas, A., Gillingham, K., Dunn, D. L., Payne, W. D., Gores, P., Gruessner, R., Najarin, J. S. (1993) Risk factors for chronic rejection in renal allograft recipients. Transplantation *55*, 752–757.
3. Beik, A. I., Morris, A. G., Higgins, R. M., Lam, F. T. (1998) Serial flow cytometric analysis of T-cell surface markers can be useful in differential diagnosis of renal allograft dysfunction. Clin. Transplant. *12*, 24–29.
4. Christiaans, M. H., Overhof-de Roos, R., Nieman, F., van den Berg-Loonen, E. M. (1998) Donor-specific antibodies after transplantation by flow cytometry: relative change in fluorescence ratio most sensitive risk factor for graft survival. Transplantation *65*, 427–433.
5. Corey, H. E., Alfonso, F., Hamele-Bena, D., Greenstein, S. M., Schechner, R., Tellis, V., Geva, P., Koss, L. G. (1997) Urine cytology and the diagnosis of renal allograft rejection. I Acta Cytol. *41*, 1732–1741.
6. Corey, H. E., Alfonso, F., Hamele-Bena, D., Greenstein, S. M., Schechner, R., Tellis, V., Geva, P., Koss, L. G. (1997) Urine cytology and the diagnosis of renal allograft rejection. II. Acta Cytol. *41*, 1742–1746.
7. Cosio, F. G., Pelletier, R. P., Falkenhain, M. E., Henry, M. L., Elkhammas, E. A., Davies, E. A., Bumgardner, G. L., Ferguson, R. M. Impact of acute rejection and early allograft function on renal allograft survival. Transplantation *63*, 1611–1615.
8. Fuggle, S. V., Koo, D. D. (1998) Cell adhesion molecules in clinical renal transplantation. Transplantation *65*, 763–769.
9. Hall, B. M., Duggin, G. G, Philips, J., Bishop, G. A., Horvath, J. S., Tiller, D. J. (1984) Increased expression of HLA-DR antigens on renal tubular cells in renal transplants: relevance to the rejection response. The Lancet *8*, 247–251.
10. Issekutz, A. C., Rowter, D., Springer, T. A. (1999) Role of ICAM-1 and ICAM-2 and alternate CD11/CD18 ligands in neutrophil transendothelial migration. J. Leukoc. Biol. *65*, 117–126.
11. Karakayali, H., Moray, G., Demirag, A., Akkoc, H., Haberal, M. (1998) Results of various immunosuppressive regimens at our transplantation center. Transplant. Proc. *30*, 3101–3102.
12. Lee, C. M., Scandling, J. D., Pavlakis, M., Markezich, A. J., Dafoe, D. C., Alfrey, E. J. (1998) A review of the kidneys that nobody wanted: determinants of optimal outcome. Transplantation *65*, 213–219.
13. Leggat, J. E., Ojo, A. O., Leichtman, A. B., Port, F. K., Wolfe, R. A., Turenne, M. N., Held, P. J. (1997) Long-term renal allograft survival. Transplantation *63*, 1268–1272.
14. Lindholm, A., Ohlman, S., Albrechtsen, D., Tufveson, G., Persson, H., Persson, N. (1993) The impact of acute rejection episodes on long-term graft function and outcome in 1347 primary renal transplants treated by 3 cyclosporine regimens. Transplantation *56*, 307–315.
15. Mannon, R. B., Kotzin, B. L., Nataraj, C., Ferri, K., Roper, E., Kurlander, R. J., Coffman, T. M. (1998) Downregulation of T cell receptor expression by CD8(+) lymphocytes in kidney allografts. Clin. Invest. (United States) *101*, 2517–2527.

16. Matoušovic, K., Martínek, V., Špatenka, J., Kolský, A., Janda, J., Rossmann, P., Chadimová, M. (1999) Význam biopsie ledviny v současné nefrologii. Praktický lékař *79*, 194–198.
17. Roberti, I., Reisman, L., Burrows, L., Lieberman, K. V. (1995) Urine cytology and urine flow cytometry in renal transplantation – a prospective double blind study. Transplantation *59*, 495–500.
18. Rush, D. N., Henry, S. F., Jeffery, J. R., Schroeder, T. J., Gough, J. (1994) Histological findings in early routine biopsies of stable renal allograft recipients. Transplantation *57*, 208–211.
19. Rush, D. N., Jeffery, J. R., Gough, J. (1995) Sequential protocol biopsies in renal transplant patients. Transplantation *59*, 511–514.
20. Segasothy, M., Birch, D. F., Fairley, K. F., Kincaid-Smith, P. (1989) Urine cytologic profile in renal allograft recipients determined by monoclonal antibodies. Transplantation *47*, 482–487.
21. Vanrenterghem, Y. F. Ch. (1995) Acute rejection and renal allograft outcome. Nephrol. Dial. Transplant. *10*, 29–31.
22. Weber, C., Springer, T. A. (1998) Interaction of very late antigen-4 with VCAM-1 supports transendothelial chemotaxis of monocytes by facilitating lateral migration. Immunol. *161*, 6825–6834.
23. Wever, P. C., Boonstra, J. G., Laterveer, J. C., Hack, C. E., Woude, F. J., Daha, M. R., Berge, I. J. M. (1998) Mechanisms of lymphocyte-mediated cytotoxicity in acute renal allograft rejection. Transplantation *66*, 259–264.