Volume 1 • Number 1 • January – March 2014

ORGAN DYSFUNCTION AND MANAGEMENT

Official Journal of
SMT. GULABBEN RASIKLAL DOSHI AND SMT. KAMLABEN MAFATLAL MEHTA
INSTITUTE OF KIDNEY DISEASES AND RESEARCH CENTRE
DR. H.L. TRIVEDI INSTITUTE OF TRANSPLANTATION SCIENCES

Biocon®

NEPHROLOGY protecting life

A New Life..
The Right Start

Biocon's Transplant Business aims to provide the most comprehensive and cost-effective medications for transplant recipients. As one of the largest manufacturers of immunosuppressants in the world, Biocon has the widest range of products for post-transplant management.

We are committed to transform the lives of transplant recipients through quality medications at affordable prices as well as best-in-class patient support initiatives and programs.

www.biocon.com

ORGAN DYSFUNCTION AND MANAGEMENT

Vol. 1, No.1, January – March 2014

EDITORIAL BOARD

Thymoglobuline®

Rabbit anti-human thymocyte immunoglobuli

A Right Start Ensures a Good Finish

More than 25 years of research and clinical experience have confirmed the consistent results and versatility of Thymoglobuline®[1]

Thymoglobuline® has a long history of safe and effective immunosuppression in transplantation[1]

Thymoglobuline® offers the following advantages:

- A reduced risk of rejection[1]
- The ability to minimize post-transplant maintenance immunosuppression[1]

Reference :
1.Gaber AO, Monaco AP, Russell JA, Lebranchu Y, Mohty M. Rabbit antithymocyte globulin (thymoglobulin): 25 years and new frontiers in solid organ transplantation and haematology. Drugs. 2010 Apr 16; 70(6):691-732.
2.Blaise D, Farnault L, Faucher C, et.al. Reduced-intensity conditioning with Fludarabin, oral Busulfan, and thymoglobulin allows long-term disease control and low transplant-related mortality in patients with hematological malignancies. Exp Hematol. 2010 Dec; 38(12):1241-50.

Summary of Product Characteristics

For the use only of a Registered Medical Practitioner or a Hospital or a Laboratory **THYMOGLOBULINE® Rabbit antihuman thymocyte immunoglobulin Injection THERAPEUTIC CATEGORY** Immune Suppressant **COMPOSITION** Powder for concentrate for solution for infusion Rabbit antihuman thymocyte immunoglobulin: 25 mg/5ml **THERAPEUTIC INDICATIONS** Immunosuppression in transplantation: prophylaxis and treatment of graft rejection. Prophylaxis of acute and chronic graft versus host disease in haematopoietic stem cell transplantation. Treatment of steroid-resistant, acute graft versus host disease. Haematology: treatment of aplastic anaemia. **DOSAGE AND ADMINISTRATION** The posology depends on the indication, the administration regimen and the possible combination with other immunosuppressive agents. Recommendations may be used as reference. The treatment may be discontinued without gradual reduction of dose. Administer doses of corticosteroids and antihistamines are required prior to infusion of rabbit anti-human thymocyte immunoglobulin. **SAFETY-RELATED INFORMATION Contraindications:** Acute or chronic infections, which would contraindicate any additional immunosuppression. Hypersensitivity to rabbit proteins or any of the excipients. **Pregnancy and Lactation :** No prescription unless absolutely required. Nursing must be suspended. **Warnings and Precautions:** Must be used in a hospital setting. In the event of an adverse reaction, the infusion rate can be reduced or the infusion discontinued until the symptoms have disappeared. In the event of an anaphylactic shock, the infusion has to be stopped immediately and any further administration must only be carried out after the benefits and the risks have been carefully weighed up. Thrombocytopenia and/or leucopenia have been identified: the blood cell count must be monitored during and after the treatment. The posology has to be adjusted with the blood cell count. Infections have been reported after administration of Thymoglobuline in association with several immunosuppressive agents. The use of immunosuppressive agents, including Thymoglobuline may increase the incidence of malignancies. Within the context of product traceability, it is strongly recommended that the patient's name and the product batch number are recorded. **Interactions:** To be taken into account: with Cyclosporin, tacrolimus, mycophenolate mofetil: risk of over immunosuppression with a risk of lymphoproliferation ; with live attenuated vaccines: risk of systemic infection due to the vaccine which may potentially be fatal. The risk is increased if aplastic anaemia other rabbit immunoglobulins: formation of antibodies which react with other rabbit immunoglobulins. **Adverse Reactions :** Reported during and after infusions: Systemic: chills, fever, hypotension, tachycardia, vomiting and dyspnoea. Local: pain at the infusion site, peripheral thrombophlebitis. Common delayed allergic reactions such as serum sickness (fever, rash combined with joint pain or muscle pain). Immediate serious allergic reactions. Neutropenia, thrombocytopenia and lymphocytopenia are very common. Adverse events associated with over-immunosuppression including infectious complications, rare malignant and solid tumours. Updated: March 2012 **Local: pain at the infusion site, peripheral thrombophlebitis. Common delayed allergic reactions such as serum sickness (fever, rash combined with joint pain or muscle pain). Immediate serious allergic reactions. Neutropenia, thrombocytopenia and lymphocytopenia are very common. Adverse events associated with over-immunosuppression including infectious complications, rare malignant and solid tumours. Updated: March 2012**

IN THY 12.08.30

genzyme
A SANOFI COMPANY

SANOFI

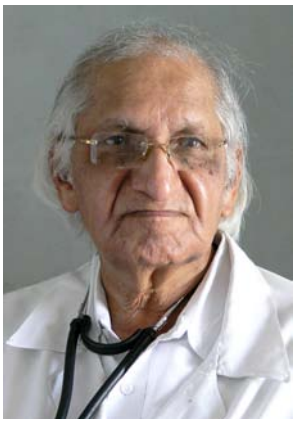
Sanofi Synthelabo (India) Ltd, A Sanofi Group Company
54-A, Sir Mathuradas Vasanji Road, Andheri (E), Mumbai - 400093

ORGAN DYSFUNCTION & MANAGEMENT

Vol. 1, No.1, January – March 2014

CONTENTS

Barefoot Marathon!



The Institute of Kidney Diseases and Research Centre (IKDRC) was established on 7th October, 1981 by the Government of Gujarat as a solution to the rising number of patients requiring care for kidney diseases. On 18th July, 1986 autonomy was granted to allow rapid growth and development of the institute. This creation was started without any planned financial resources! In 1992 the new building with 200 indoor beds was completed and institute moved from Civil Hospital premises to the new premises on the same campus located at its western end. The Institute of Transplantation Sciences was established on 3rd June, 1997 to concentrate better on care pertaining to transplantation. Thus additional 200 beds were added. Under this flag the vision of multi-organ transplantation has also been partially fulfilled. Now the institute has grown in quality and quantity care to become one of the best and the largest institute in the world performing 400 or more kidney transplants per year with a total of 3853 kidney transplants till now. Service, Education and Research have been the essential pillars of the institute. The government of Gujarat has been very kind to extend its support to this project.

The long cherished desire and dream of creating a culture of thinking scientifically with open mind and writing good articles is now being fulfilled! In this inaugural issue my mentor and good friend Prof. John Dossetor has supported by writing on the history of kidney transplantation and attempt to induce tolerance in Toronto general hospital by cross-circulating blood of a liver failure patient with that of a kidney failure patient in 60s followed by transplantation of kidney from dying liver failure patient in to that of kidney failure patient, and that kidney lived till patient lived (in Newfoundland)! My good old friends Professors E.K.M. Smith, Susan Saidman and Peter Knight have been kind enough to give me unstinted support whenever I have asked and for as long as I have requested! Our Indian friends on the campus Professors Sudhaben Multani, V.G.Mavalankar, S.M. Patel, Drupad Chhatrapati, L.B. Trivedi, M. Joshi and many more have supported the cause! The last of the loyal friends who has remained as my friend and advisor for ever, Dr. Veenaben Shah has stood the test of time! Without these kind friends, this day would not have arrived!

I have been fortunate to get support from my dear friends V. Sivakumar from Tirupati, R.K.Sharma from Lucknow, Manisha Sahay from Hyderabad, P. Saunderrajan and N. Gopalakrishnan from Chennai, N.K. Hase from Mumbai, Panduranga Rao from Ann Arbor and of course my own students scattered across the country. This barefoot marathon will prove to be inspiring and encouraging for all of us and I hope to get support from readers across the globe who will join us in various capacities. The title of this journal has been selected in such a way that all branches and sub-specialities can share their experience with us through "Organ Dysfunction and Management"! It brings me a great pleasure to see that the first issue of this journal is being released on the World Kidney Day of year 2014!

H. L. Trivedi, F.R.C.P.(C), D.Sc,
Director, Professor in Nephrology, Clinical Transplantation and
Dept. of Stem Cell Therapy and Regenerative Medicine,
G. R. Doshi and K. M. Mehta Institute Of Kidney Diseases & Research Centre [IKDRC],
Dr. H.L. Trivedi Institute Of Transplantation Sciences [ITS],
Civil Hospital Campus, Asarwa, Ahmedabad- 380016, Gujarat, India.

Review Article

Personalized Immunosuppression : Mix of Tradition and Fashion

M. Sahay

Department of Nephrology, Osmania Medical College and General Hospital,Hyderabad

ABSTRACT

Renal transplantation is the definitive therapy for end stage renal disease. The calcineurin inhibitors, mycophenolate and steroids provide cornerstone immunosuppression after renal transplantation. Many new drugs have been added to the therapeutic armamentarium. There has been a progressive increase in 1-year graft survival over the last decade with triple immunosuppression. However, major challenge in renal transplantation is improvement of long-term graft survival. Renal allografts continue to be lost at a rate of 3–5% per year after the first year of transplantation. Many risk factors are known to contribute to allograft failure, including advanced donor age, poor donor kidney function, delayed graft function, HLA mismatching and acute rejection episodes. A blanket immunosuppression protocol for all these different risk groups may result in inadequate immunosuppression in some while in others it may cause toxicity. Thus immunosuppression needs to be tailored to patients' needs to avoid rejections and minimize toxicity. The "one size fits all" approach needs to be abandoned. Thus personalized immunosuppression is the need of the hour.

KEY WORDS: Immunosuppression, Tacrolimus, Cyclosporine

Tailoring immunosuppression-traditional concepts according to sensitisation level / immunological risk

Based on clinical data and serological tests, the prospective recipients can be divided into the following 3 categories:

Normal or Intermediate Risk: This group includes recipients with unrelated donors like spousal donors or recipients who have related donors with whom they are HLA haplomatched (parents, offspring, siblings) or zero antigen matched donors (siblings or unrelated).

High risk: (a) Very high risk-this group includes sensitized recipients with positive cross match or Panel Reactive Antibody (PRA) > 50%, re-transplantation with a previous history of early (< 1 year) graft loss due to rejection or those with positive donor specific antibodies (DSA) (b)High risk-ABO-incompatible transplantation or certain racial groups like African Americans, delayed graft function or prolonged cold ischemia time.

Low risk: This group includes recipients who have HLA-identical siblings as donors. Among them, those who have an identical twin as their prospective donor are at the lowest risk for rejection.

Recipients who are cross-match negative by complement dependent cytotoxicity (CDC) method but have a positive cross-match by flow cytometry (FCXM) and/or have presence of DSA detected by a solid phase assay (SPA) are at a significantly higher risk of developing both early and late antibody mediated rejection(AMR). These methods need standardization, and appropriate treatment needs to be determined.[1-3]

Normal Immunologic Risk

The KIDNEY DISEASE IMPROVING GLOBAL OUTCOMES (KDIGO) guidelines recommend Basiliximab induction for all patients at normal immunologic risk for rejection.[4] However, the overall graft survival is not significantly different between those who receive Basiliximab induction and those who do not. Also, using induction immunosuppression adds significantly to the cost of transplant. Hence, it may be preferable not to use any induction agents in this group of patients in India. Based on the use of induction, subsequent maintenance immunosuppression is recommended as follows: No Induction: Tacrolimus 0.12 mg/kg/day, target C0 8-10 ng/mL for initial 3 months; 5-8 ng/mL later combined with Mycophenolate mofetil (MMF) 2 gm/day for 1 month followed by 1.5 gm/day subsequently and Prednisolone 20 mg/day initially tapered over 3 month to 5 mg/day.

Address for correspondence : *Professor and Head Department of Nephrology Osmania Medical College and General Hospital,Hyderabad E-mail:drmanishasahay@gmail.com*

Azathioprine (AZA) can be used in place of MMF at a dose of 75-100 mg/day.[4] With Induction: Tacrolimus 0.1 mg/kg/day, target C0 5-8 ng/mL throughout combined with MMF/AZA and Prednisolone as for those without induction.

Tacrolimus or Cycolsporine - which to use?

Tacrolimus in combination with MMF adjunctive therapy showed significantly better graft survival in patients with delayed graft function. There were fewer episodes of corticosteroid-resistant rejection and better renal function at the 3-year follow-up compared with Cyclosporine micro-emulsion plus MMF immunosuppression.[5,6] Annualised chronic rejection rate with Tacrolimus was1.3 % and with Cyclosporine was 3.1%.[7] However in HCV positive patients Cyclosporine is preferred.

MMF or AZA - which is preferred?

MMF combined with Cyclosporine plus corticosteroids has been shown to reduce acute rejection rates or treatment failure compared with a standard triple combination regimen of cylosporine, azathioprine and corticosteroids.[8] Randomized control trials have shown that acute rejection rates with MMF and azathioprine are similar when they are combined with micro-emulsion form of Cyclosporine or with Tacrolimus. Hence, azathioprine may replace MMF in most recipients without adversely affecting outcomes if cost is an issue.[9]

Which triple drug regimen best? TAC+ AZA or TAC+ MMF or CSA +MMF (along with steroids)

In a comparative study of Tacrolimus plus azathioprine versus Tacrolimus plus MMF versus Cyclosporine micro-emulsion plus MMF in primary cadaveric renal transplantation, all patients received the same maintenance corticosteroid regimen and only those with delayed graft function received anti-lymphocyte induction. Overall patient and graft survival rates were equivalent across the three treatment groups at years 1, 2 and 3 post-transplantation. In the Tacrolimus plus MMF treatment group, there was a significant improvement in graft survival in those patients with delayed graft function, fewer episodes of corticosteroid-resistant rejection and better renal function compared with Cyclosporine micro-emulsion plus MMF treatment. Collectively, these findings underscore the importance of tailoring the immune suppressive regimen for individual renal transplant recipients to minimize the risks associated with poor outcome.[10-12] Other randomized trials have also reported favorable results with combined Tacrolimus plus MMF immunosuppression in renal transplantation. In a multi-centre, parallel-group study conducted in 16 European centres, renal transplant recipients were randomized to receive immunosuppressive therapy of Tacrolimus plus corticosteroids either without adjunctive MMF, or with 1 or 2 g/day MMF.[13] At 1-year follow-up, patient survival was 100% in the no MMF group, 97.5% in the MMF 1 g/day group and 97.2% in the MMF 2 g/day group. Corresponding graft survival rates were 90.2%, 92.4% and 93.0%, respectively. The incidence of biopsy-proven acute rejection was lower in the plus MMF groups (15.2% in the MMF 1 g/day group and 5.6% in the MMF 2 g/day group) than in the no MMF group (35.4%). Renal function was comparable in all three treatment groups. Four-year follow-up data for a subset of patients who took part in this study showed patient survival 92% in both the Tacrolimus-corticosteroids group and the Tacrolimus-corticosteroids-MMF (1 g/day) group, and 100% in the Tacrolimus-corticosteroids- MMF (2 g/day) group. Corresponding graft survival rates at 4 years were 72%, 80% and 92%. Good renal function was maintained after 4 years of Tacrolimus-based immunosuppression.

*What is the role of Sirolimus***?**

The 3-month results of a US multicentre study comparing Tacrolimus in combination with either MMF or Sirolimus showed these two treatment regimens to be equivalent in terms of patient and graft survival, delayed graft function, the incidence of biopsy-confirmed acute rejection and renal graft function, although differences were apparent in terms of acute tubular necrosis and hyperlipidaemia. Anti-lymphocyte induction was used in patients with delayed graft function. Sirolimus has also shown promising efficacy in acute rejection prophylaxis when combined with Cyclosporine and corticosteroids.[14]

Minimising CNI-

In Efficacy Limiting Toxicity Elimination (ELiTE)–Symphony study primary kidney transplant recipients were randomized either to a control group treated with standard-dose Cyclosporine, MMF and corticosteroids, or to 1 of 3 experimental groups who received induction antibody therapy with daclizumab, maintenance therapy with MMF and steroids, and either (1) low-dose Cyclosporine, (2) low-dose Tacrolimus, or (3) low-dose Sirolimus (calcineurin inhibitor [CNI] avoidance). After 1 year of follow-up, patients receiving low-dose Tacrolimus had the highest GFR, the lowest rate of biopsy-proven acute rejection and highest rate of graft survival. The worst outcomes occurred in the low-dose Sirolimus, CNI avoidance group.[15] KDIGO recommends continuing CNI life-long rather than withdrawing them (Grade 2B).This regimen was effective in elderly and also expanded criteria donors.

High risk category:

Very high risk-Antithymocyte globulin (ATG) is superior to Basiliximab and is recommended as the induction agent for these patients after desensitization. Desensitization is done by removing preformed antibodies by plasmapheresis, immunoadsorption and prevention of future antibody formation by splenectomy, intravenous immunoglobulin, rituximab, ATG and immunosuppression. The dose of ATG varies in different transplant centers around the world and the optimal dose is not defined.[16] Standard dose of ATG is 1.25 - 2.5 mg/kg/day for up to 7 days and low dose ATG is a cumulative dose of 3.5 mg/kg. However, the risk of cytomegalo virus (CMV), BK polyoma virus (BKV) and post-transplant lymphoproliferative disorder (PTLD) is a concern with higher doses. Subsequently, Tacrolimus is recommended at a dose of 0.14 mg/kg/day titrated to a target trough level of 10-12 ng/mL for initial 1 month, 8-10 ng/mL for the next 2 months and 5-8 ng/ml after 3 months. MMF is recommended at a dose of 2 gm/day for initial 2 months and 1.5 g/day subsequently. Azathioprine can be substituted for MMF at a dose of 75-100 mg/day. Methylprednisolone is recommended at a dose 500 mg on the day of transplant followed by Prednisolone at a dose of 20 mg/day initially tapered to 5 mg/day by 3 months which is to be continued lifelong.

High risk-Recipients with poor HLA match, ABO incompatible transplant with no other immunologic risk factors and re-transplant candidates with a prior graft loss due to non-immunologic causes are at a slightly lower risk for rejection than the above group. Basiliximab is recommended for these patients as the induction agent at a dose of 20 mg on day 0 and day 4 post-transplant. For maintenance immunosuppression, Tacrolimus is recommended at a dose of 0.12 mg/kg/day titrated to a target trough level of 8-10 ng/mL for initial 3 months and 5-8 ng/mL later. MMF and steroids are recommended in the same dose as for the sensitized recipients. The timing of initiation of CNI is controversial. The KDIGO guidelines suggest that CNI be initiated either before or at the time of transplant rather than delaying till the onset of graft function. Ekberg at al recommend starting maintenance immunosuppression on day-1 while the Spanish guidelines recommend starting the drugs on day -3.[17]

Low Immunologic Risk

This category of recipients can be further subdivided into 2 subgroups:

Lowest risk group:

Recipients with an identical monozygotic twin as the donor have the lowest risk of rejection. There are no comparative data regarding immunosuppression requirement in this category but most groups consider that some form of immunosuppression is required for a short period since perioperative events like ischemia can predispose patients to rejection even though there is a perfect antigen match. Induction immunosuppression is not required in these recipients. The Spanish Nephrology Society recommends a single perioperative dose of Methylprednisolone, 125 mg iv followed by prednisolone 0.25 mg/kg/day for the next 7 days in combination with MMF 2 g/day for 8-12 weeks which is then tapered and stopped with MMF monotherapy.[18]

Low risk group:

Recipients with HLA identical sibling donors have a low risk of acute rejection. Induction is not required.[19] Three regimens of maintenance immunosuppression have been described in various non-randomized trials: CNI withdrawal: Triple drug immunosuppression is given for 3 months followed by CNI withdrawal at 3 months if no episodes of rejection occurred in the initial 3 months. The Spanish Nephrology Society recommends prednisolone, 0.25 mg/kg/day for 7 days, low dose Tacrolimus targeted to a trough level of 4-8 ng/mL for 6 months and subsequent MMF monotherapy. Steroid avoidance: Withdrawal of steroids within 1 week post-transplant is called steroid avoidance and this approach is recommended over steroid withdrawal which is discontinuing steroids at any time beyond 1 week post-transplant as the latter is associated with increased risk of rejection. Induction is recommended if steroid avoidance is planned. Steroid free/CNI minimization protocol: No induction immunosuppression or perioperative steroids are needed. Tacrolimus can be used for initial 3 months which can be tapered and stopped by 4 months post-transplant if no rejection occurs. Later MMF 2 gm/day and Sirolimus can be continued for 12 months followed by MMF monotherapy. HLA identical donors are induced with ATG for 10 days followed by maintenance therapy with MMF or Sirolimus monotherapy with excellent graft function.[19]

Non-immunological risk categories

Recipients can be categorized as follows:

Increased risk of New onset diabetes mellitus after transplantation (NODAT): This group includes recipients aged > 45 years with strong family history of diabetes, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) on glucose tolerance test, obesity, HCV and CMV infection, poor HLA match and certain ethnic groups like African, Americans and Hispanics. CNI, corticosteroids and mTOR inhibitors can cause NODAT. [20-21] Incidence of NODAT requiring insulin therapy is nearly twice that of

Cyclosporine in the Tacrolimus group. Immunosuppression protocols should aim at CNI and/or steroid minimization in these recipients. Hence, induction immunosuppression is recommended in all recipients. Basiliximab is recommended as the induction agent. Subsequently, there are 3 options for maintenance immunosuppression: High dose Tacrolimus /Steroid avoidance: Tacrolimus 0.14 mg/kg/day and MMF/AZA. Prednisolone is used at a dose of 10 mg/day for initial 7 days only. Low dose Tacrolimus /low dose steroids**:** Tacrolimus 0.10 mg/kg/day, MMF/AZA and Prednisolone 10 mg/day for 1 month followed by 5 mg/day. Cyclosporine/MMF/Steroids**:** CsA in combination with MMF and steroids shows lower incidence of NODAT than Tacrolimus+ MMF and steroid group. In patients at risk for NODAT who are at low immunologic risk Belatacept is combined with MMF and steroids. A regimen of ATG induction followed by Belatacept and MMF for maintenance reduces NODAT with no significant increase in risk of rejection. This approach, which avoids both CNI and steroids, needs further studies before widespread acceptance.

Recipients with marginal donors:

Recipients with donors aged > 60 years or those with donors >50 years with hypertension may be considered to be marginal living donors although no formal definition exists. These donors have a lower GFR as compared to the standard donor and hence reduced graft half life. There is not much data regarding the use of various immunosuppressants in this category. mTOR inhibitors, Sirolimus and Everolimus, have been shown to maintain GFR better than CNI. Results from RCTs which studied conversion from CNI to mTOR inhibitors 4.5 to 6 months post transplant have shown better preservation of GFR with no increased risk of rejection. The Spanish Nephrology society suggests using either Basiliximab or ATG for induction followed by low dose Tacrolimus for 3 months (target C0 4- 7 ng/mL) and subsequent conversion to mTOR inhibitors in addition to MMF and steroids.In children, growth is an important issue. Steroid withdrawal with Daclizumab, Tacrolimus and MMF had same acute rejections as controls at 6 months. There is no evidence to suggest beneficial effects of induction therapy in normal or low risk pediatric recipients who receive triple drug maintenance therapy. Basiliximab or ATG may be considered for children at high immunologic risk or when steroid minimization protocols are planned. Steroids sparing regimens can either be steroid withdrawal i.e. stopping steroids anytime after 1 week, steroid avoidance (steroids withdrawn within the first week), and steroid minimization-low dose alternate day steroids lifelong.[22-24] Results are better with steroid avoidance than with steroid withdrawal after 1 week.

Personalised immunosuppression-recent concepts

TDM- Therapeutic drug monitoring or Pharmacokinetic (PK) monitoring, or dosing according to drug concentrations, has the potential to individualize drug therapy.[25]Immunosuppressants require TDM because of their narrow therapeutic index. In an individual patient this variability can be because of factors like drug-nutrient interactions, drug-disease interactions, renal-insufficiency, inflammation, infection, gender, age, and liver mass. More importantly there is difference in each individual's response to the drug depending on his or her genetic makeup. Genetic polymorphisms in drug targets, drug metabolizing enzymes, and drug transporters have been identified as potential targets for developing a pharmacogenetic strategy to individualize initial drug choice and dose.[26] Drug monitoring thus is helpful for individualizing dosage for Cyclosporine, Tacrolimus, Sirolimus and mycophenolic acid (MPA). Cyclosporine, Tacrolimus, MMF and Sirolimus show inter-individual variability. Sample for assay should be whole blood for CNI and Sirolimus (as these are bound to RBCs) and plasma for MMF. Assays include immunoassays and high performance liquid chromatography (HPLC). Immunoassays give a higher value as they measure metabolites also. HPLC is thought to be more sensitive. The clinical outcomes correlate best with the Area under the Curve (AUC) which is the best measure for drug exposure. However calculating AUC needs a minimum of 3 samples hence it has not become popular for routine use. Instead Cyclosporine level at 2 hours post dose and trough levels of Tacrolimus and Sirolimus dose correlates well with outcome.AUC is recommended for MPA. Individualizing a drug according to drug levels helps in optimal immunosuppression.[27] A new MPA assay based on the enzymatic activity of recombinant IMPDH II (the pharmacological target of MPA) with superb relation with HPLC has been recently produced for the measurement of MPA plasma levels.[28]Measurement of IMPDH activity in CD4+ T-lymphocytes represents a potential refinement of this approach.[29]

Genetic make-up-Pharmacogenomics

Cyclosporine and Tacrolimus are metabolized by CYP450 and glycoprotein P. A marker for CNI exposure is polymorphism in the gene (CYP3A5,cytochrome P450, family 3, subfamily A, polypeptide 5) that encodes the metabolic enzyme cytochrome P450 3A5. Expression is determined by single nucleotide polymorphisms (SNPs) in the CYP3A5 gene. Individuals with at least one wild-type CYP3A5*1 allele are functional expressers and homozygotes for the mutant CYP3A5*3 allele are functional nonexpressers. CYP3A5 expressers take longer to achieve target blood CNI concentrations after transplantation,

require higher doses of CNI, and experience episodes of acute rejection earlier than nonexpressers[30] Other candidate genotypes that have been shown to have a significant influence on CNI pharmacokinetics are ABCB1 (encoding P-gp)[30]CYP3A4*22(31) and P450 oxidoreductase[28-32]There has been recent interest in the pregnane X receptor that regulates the expression of cytochrome P450 and P-gp and its genetic variants as potential predictors of interindividual differences in drug concentrations.[33]Variation in genes coding for the target protein calcineurin or the immunophilins may further explain the observed differences in susceptibility to CNI .The influence of CYP3A5 expression on the pharmacokinetics of Sirolimus is less well studied. However, when prescribed without a CNI, the oral bioavailability of Sirolimus is lower in CYP3A5 expressers[34]and consequently takes longer to achieve target blood concentrations than nonexpressers.[35]There is no significant influence of CYP3A5 genotype on the pharmacokinetics of Everolimus.[36]Genotyping for mutations in the thiopurine-S-methyltransferase gene that metabolizes azathioprine has been widely adopted in some therapeutic areas but not transplantation.[37-38] This may be due to frequent blood count monitoring in transplant recipients following the introduction of azathioprine allowing the avoidance of severe myelotoxicity.UGT1A9 predicts individuals likely to be under-exposed to MPA with increased risk of rejection.[39]

Pharmacodynamic (PD) Monitoring

Monitoring of pharmacokinetics by TDM fails to account for inter- and intra-subject physiological differences in immune reactivity and response to immunosuppressive drugs. Additionally, PK monitoring is unable to evaluate the influence of combination drug therapy or non-drug related factors on the immune system. Thus, although it assists with avoidance of acutely toxic or sub-therapeutic drug levels, it does little to minimize subacute or chronic adverse effects. PD monitoring by direct measurement of immune cell function has the potential to personalize immunosuppression**.** PD monitoring examines the physiological effects of a drug rather than using the surrogate marker of drug concentration. Combining PD with PK monitoring has the potential to improve therapeutic drug dosing, thereby increasing efficacy and safety in an individual patient.

Methods of PD monitoring

Calcineuric phosphatase monitoring-Tacrolimus and Cyclosporine form a complex with their respective binding proteins, immunophilins, which in turn inhibit the phosphatase activity of calcineurin, a key enzyme in the activation of T lymphocytes. Calcineurin activity at trough time points is suggested as a single surrogate predictor for overall calcineurin activity throughout dosing periods. This along with CNI levels might be useful to determine the therapeutic range of Tacrolimus and Cyclosporine concentrations for an individual patient.[40,41] The inhibitory effects on calcineurin activity in peripheral blood mononuclear cells differs between Tacrolimus and Cyclosporine.

Nuclear factor of activated T cells (NFAT) - CNI inhibits activation of NF-ATc, thus preventing its entrance into the nucleus. NFAT regulates cytokine gene expression. Thus decrease in NFAT gene expression to less than 30% reflects adequate CNI dose with minimal rejections and gene expression greater than 40% indicates inadequate immunosuppression and high risk for rejection.

Measuring T lymphocytes and their function - Both B and T lymphocytes have been implicated in the pathogenesis of acute and chronic allograft rejection. However, as T cells are the major targets of most immunosuppressant drugs, and B-cell effector mechanisms depend on T-cell help, T-cell biology has received greater attention as a potential PD marker. T-cell assays can be broadly divided into two major categories: donor antigen specific or non-antigen specific. Donor antigen specific assays involve stimulation of immune cells ex vivo with donor-derived mitogen such as donor lymphocytes. Non-antigen specific assays can be antigen independent (e.g. measurement of lymphocyte subsets), or assess the functional state of T cells following stimulation with a polyclonal stimulant. Although donor-derived stimuli may be more specific in determining immune reactivity to the allograft, the limited availability of donor cells precludes repeat testing in the clinical setting. As such, polygenic stimuli are more applied in routine clinical practice.

Quantification of T- lymphocyte subsets - Fluorescent-activated cell sorting (FACS) analysis is used for sorting and quantification of lymphocyte subsets. A number of studies have shown that standard triple immunosuppressive regimens lead to significant reductions in the CD4+/CD8+ ratio in transplant recipients without effecting total lymphocyte number.[42] One study reported that decreased CD4 helper activity was associated with a lower risk of rejection, however, there was no relationship between the actual pre-transplant T or B-cell subset counts and acute rejection or 1-year graft function. The same study showed a correlation between elevated pre- transplant CD8+ suppressor-effector T-cell subset counts (CD8+CD11b+) and the occurrence of post-transplant infection. Further studies are needed to define the role of lymphocyte subsets in tailoring immunosuppression.

Measurement of T - lymphocyte activation markers and/or proliferation-Surface antigens such as the transferrin receptor (CD71), the alpha chain of the interleukin-2 (IL-2) receptor (CD25), the Fas receptor (CD95) and co-stimulatory and adhesion molecules (CD28, CD154, CD11a, CD54)) are expressed on activated but not resting lymphocytes. Following non- specific mitogen stimulation, these can be measured by FACS analysis. Two studies have shown significantly lower levels of lymphocyte activation in immunosuppressed kidney transplant recipients receiving a CNI, Mycophenolatemofetil and corticosteroids compared with controls (dialysis patients and healthy volunteers).[43] Additionally, reduced expression of the co-stimulatory and adhesion molecules CD28, CD54 and CD154 has been seen following conversion from Cyclosporine to Tacrolimus, this limited data suggests a potential role for this measure in guiding immunosuppressant drug therapy.

Measurement of cytokine production or cytokine mRNA levels -Multiple studies have used ELISA or radioimmunoassay to measure serum cytokine levels in transplant recipients. Results are conflicting, as most studies are on resting or non- activated T lymphocytes. More recent studies have stimulated immune cells with mitogen ex vivo, then measured cytokine production via ELISA, ELISPOT or FACS; or measured cytokine mRNA levels via reverse transcription PCR. Following immune cell stimulation, cytokine concentrations can be measured in culture supernatant using ELISA. A number of studies have shown marked reduction in supernatant cytokine levels (such as IL-2 and IFN-γ) after administration of a CNI.[44]MMF monotherapy has been shown to have little effect on secretion of these cytokines. A significantly lower post-dose IL-2 secretion has been seen in those receiving MMF in combination with a CNI compared with those receiving a CNI alone, suggesting a synergistic effect of the two drugs. There is a significant association of high pre- transplant T-cell IL-10 responses with the occurrence of acute rejection and impaired 1-year graft function. In contrast, there was no association of IL-2, IFN-γ, IL-6, IL-8, (TNFα) and GM-CSF production with these outcomes.

Quantification of cytokine producing cells - Intracytoplasmic cytokines can be measured following mitogen stimulation of immune cells, addition of a monoclonal antibody directed against the cytokine of interest, and then subjecting them to FACS analysis. Positive cells are expressed as percentage of cytokine-producing cells within the T-cell population. [45] Patients treated with a CNI, azathioprine and prednisolone demonstrated significantly lower frequencies of IL-2 secreting CD4+ and CD8+ T cells and IFN-γ and double positive IL-2/IFN-γ secreting CD4+ T cells post-transplantation compared with pre-transplantation. This study also showed that the frequency of IL-2 secreting T cells was more affected by Tacrolimus than Cyclosporine. Thus cytokine producing cell number can be used as target for immunosuppressive therapy. The ELISPOT identifies cytokine-producing cells at the single-cell level and is more sensitive as compared with conventional ELISA and flow cytometry.[46]Peripheral blood mononuclear cells(PBMC) IFN γ/IL5 ratio where IFN-γ is a surrogate for Th1 immunity andIL-5 is a surrogate for Th2 suggests inadequate immunosuppression and has an almost 90% positive predictive power for graft loss within 6 months. Thus cytokine ratios may predict outcomes and may be used to individualize therapy.[47]

Measurement of cytokine mRNA levels-Measurement of cytokine mRNA in PBMC or whole blood of transplant recipients using PCR may help in guiding immunosuppression. Cytokine levels of IL2, IFN -γ and GMCSF mRNA are measured after ex vivo stimulation of T cells. In adequate IS group the levels are lower and there is a delay in peak IL-2 and IL-4 (from 8 to 24 h) and TNF- α (from 4 to 8 h) as compared to the controls. Marked variation in IL2 mRNA expression is seen between patients on Tacrolimus and Cyclosporine, suggesting individually distinct degrees of CNI sensitivity.[48] On the other hand strong suppression of IL-2, IFN-γ and GM-CSF mRNA expression (residual level of transcription after drug intake) was independently associated with the development of infections and malignancy.[49]Most studies measuring cytokine production and mRNA levels have focused on Th1 and Th2 cytokines. There has been no study of Th17 cytokine secretion. The latter is significantly associated with clinical organ rejection

Microarray methodology **-** Overall immune function is mediated by a vast number of genes, hence microarray methodology, which permits the expression of thousands of genes to be assayed simultaneously, holds greater promise. However, there has been only limited published data on the use of microarrays in human transplantation. [50]

Measurement of soluble CD30 concentrations - CD30 is a cell membrane glycoprotein of the tumor necrosis factor receptor family expressed on T and B cells, natural killer cells and some non-lymphoid cells. After activation of CD30+ T cells, a soluble form of CD30 (sCD30) is released into the bloodstream. Unlike other cell surface markers, it can be measured from sera using ELISA technique without ex vivo stimulation of immune cells (commercial assays are now available). A multicentre trial in kidney transplant recipients showed an association between high pre- transplant sCD30 concentrations (>100 U/mL) and the need for anti-rejection

treatment in the first-year post- transplant.[51]The effects of the sCD30 and PRA on graft outcome were similar and additive. Prophylactically anti- lymphocyte antibodies reduce CD30. Hence pretransplant high CD 30 levels may help in guiding decisions regarding induction therapy.sCD30 concentrations on days 3–5 post- transplantation allows differentiation of those who subsequently develop acute rejection from those who have uncomplicated course.1-year sCD30 concentrations can differentiate graft deterioration from chronic allograft nephropathy. Some studies have failed to find an association between sCD30 levels and the development of rejection. [52]

Measurement of intracellular adenosine triphosphate concentrations - Most of the effector functions of immune cells depend on cellular energy supply.[53]Thus, measurement of intracellular adenosine triphosphate (ATP) concentrations in CD4+ cells has been tried as a means of measuring immune response. An assay for ATP quantification (Cylex immune cell function assay, Cylex Inc., Columbia, MD, USA) was approved by the Food and Drug Administration in 2002 for use in immunosuppressed individuals.[54]High pretransplant CD4+ ATP concentrations correlate with rejection and low levels with infection such as polyoma virus. The intersection of the odds ratio curves for infection and rejection was found to occur at an ATP concentration of 280 ng/mL; thus, this value was proposed as a target value when using this test to guide immunosuppressant therapy.

Combined markers - Any single measure of immune function does not fully characterize overall immune status and a panel of tests may be needed. A prospective study that simultaneously evaluated CD4+ count, lymphocyte proliferative response to PHA, neutrophil oxidative burst and phagocytic function and immunoglobulin serum subtype concentrations showed that those with poor leucocyte phenotype and function scores experienced a higher incidence of moderate to severe infection. No difference in malignancy, graft or patient outcomes was seen. International consortia have identified a cross platform biomarker signature for operational tolerance including expansion of peripheral blood B-lymphocytes, absence of donor specific antibodies, donor specific hypo-responsiveness of CD4+ T-lymphocytes, and a high ratio of FoxP3 to gene expression in peripheral blood.[55,56]

Urinary pharmacodynamic markers-High levels in urinary cells of mRNA for FOXP3,the CD8+ cell surface marker, CD103,interferon-inducible protein-10 and the chemokine receptor CXCR360 are associated with acute rejection.[57,58]Thus measurement of urinary gene expression may have potential as a non-invasive means of PD monitoring and tailoring immunosuppression.

Tolerance phenotype and tailoring of immunosuppression- The presence of population of regulatory T cells expressing the CD8+CD28– phenotype has been shown to be inversely related to acute rejection, and associated with successful weaning from immunosuppression. Some of these CD8+CD28− T suppressor cells have CD4+CD25+ Tregs, while others are CD4+ negative. In a study in the patients with tolerant phenotype patients (Group A), mycophenolatemofetil (MMF) was progressively reduced and then stopped. Steroids were subsequently reduced and then interrupted, maintaining an immunosuppressive therapy with low doses of CNI or Sirolimus. In patients without tolerance phenotype (Group B) MMF was reduced and then stopped, while steroids were decreased to 5 mg at alternate days, maintaining CNI or Sirolimus at medium therapeutic dosages (minimized therapy). Patient and graft overall survival in Group A and in Group B were respectively at 100% and 94.7%. Incidence of acute rejection was respectively at 0% in group A and 15.7% in Group B. A careful evaluation of recipient immune reactivity with the presence of T regulatory cells can allow adequate and personalized immunosuppressive regimens, without high risks of acute rejection.[59]

CONCLUSIONS

Immunosuppression is conventionally determined by the immunological risk, age, tendency to develop NODAT, donor type etc. PK monitoring and analysis of PD variability has enormous potential for personalizing immunosuppression, and thus for increasing the efficacy and safety of immunosuppressant drugs. However, there has been no standardized analytical protocol for analyzing the majority of PD markers, hampering comparison of results obtained by different centres. PD is labor intensive and data relating PD parameters to outcomes are extremely limited. As such, outcome studies are vital before these parameters can be used to guide immunosuppressant drug dosing. Thus, while promising data for a number of PK and PD approaches are emerging, large prospective systematic trials providing evidence of superiority of PK/PD guided dosing as compared with current dosing will be required before these techniques can be routinely applied to clinical care. A right mix of clinical and lab parameters will help in optimal immunosuppressive therapy.

REFERENCES

1. Hariharan S, Johnson CP, Bresnahan BA, et al. Improved graft survival after renal transplantation in the United States, 1988 to 1996. N Engl J Med 2000; 342: 605-612.
2. Pascual JS, Hernandez DM. Immunosuppression of the living-donor recipient. Nefrologia 2010;30: Suppl 2:80-84.

3. Tait BD, Susal C, Gebel HM, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. Transplantation 2013; 95:19-47.
KDIGO clinical practice guideline for the care of kidney transplant recipients.
4. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group.Am J Transplant. 2009;9 Suppl 3:S1-155.
5. Webster AC, Woodroffe RC, Taylor RC, et al.Tacrolimus versus cyclosporin as primary immunosuppression for kidney transplant recipients: meta-analysis and meta-regression of randomized trial data. BMJ 2005;331: 810.
6. Mayer AD, Dmitrewski J, Squifflet JP, et al. Multicenter randomized trial comparing Tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European tacrolimus multicenter renal study group.Transplantation.1997; 64:436 - 443.
7. Mayer AD. Chronic rejection and graft half-life: five-year follow-up of the European Tacrolimus multicenter renal study. Transplant Proc 2002; 34: 1491-1492.
8. Sollinger HW. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. U.S. renal transplant mycophenolate mofetil study group. Transplantation 1995; 60: 225-232.
9. Clayton PA, McDonald SP, Chapman JR, et al. Mycophenolate versus azathioprine for kidney transplantation: A 15-year follow-up of a randomized trial. Transplantation 2012;94: 152-158.
10. Johnson C, Ahsan N, Gonwa T, et al. Randomized trial of Tacrolimus (Prograf) in combination with azathioprine or mycophenolate mofetil versus Cyclosporine (Neoral) with myco- phenolate mofetil after cadaveric kidney transplantation. Transplantation 2000; 69: 834-841.
11. Ahsan N, Johnson C, Gonwa T,et al. Randomized trial of Tacrolimus plus mycophenolate mofetil or azathioprine versus cyclosporine oral solution (modified) plus mycophenolate mofetil after cadaveric kidney transplantation: results at 2 years. Transplantation 2001; 72: 245-250.
12. Halloran P, Ahsan N, Johnson C, et al. Three year follow up of a randomized multicenter kidney transplant study comparing Tacrolimus (TAC) + azathioprine (AZA) versus Cyclosporine modified (CSA) + Mycophenolate mofetil (MMF) versus TAC+MMF. Am J Transplant 2001; 1: 405.
13. Squifflet JP, Backman L, Claesson K, et al. Dose optimization of mycophenolate mofetil when administered with a low dose of Tacrolimus in cadaveric renal transplant recipients. Transplantation 2001; 72: 63-69.
14. Kaplan B, Meier-Kriesche HU, Napoli KL. The effects of relative timing of Sirolimus and Cyclosporine microemulsion formulation co administration on the pharmacokinetics of each agent. Clin Pharmacol Ther. 1998; 63:48-53.
15. Ekberg H, Bernasconi C, Tedesco-Silva H, et al. Calcineurin inhibitor minimization in the Symphony study: observational results 3 years after transplantation. Am J Transplant. 2009; 9:1876-1885.
16. Gaber OA, Matas AJ, Henry ML, et al. Antithymocyte globulin induction in living donor renal transplant recipients: final report of the TAILOR registry. Transplantation 2012; 94: 331-337.
17. Ekberg H , Qi Z. Practical protocols for living donor kidney transplantation. Wolters Kluwer Health Japan Co., Ltd. 2010.
18. Walker J, Alloway R, Roy-Chaudhury P, et al. A prospective trial of a steroid-free/calcineurin inhibitor minimization regimen in HLA identical live donor renal transplantation. Transplantation 2009; 87: 408.
19. Vincenti F, Schena F, Paraskevas S, et al. A randomized, multicenter study of steroid avoidance, early steroid withdrawal or standard steroid therapy in kidney transplant recipients. Am J Transplant 2008; 8: 307-316.
20. Davidson J, Wilkinson A, Dantal J, et al. New-onset diabetes after transplantation: 2003 international consensus guidelines. Transplantation 2003; 75:10 SS3-SS24.
21. Hecking M, Werzova J, Haidinger M, et al. Novel views on new-onset diabetes after transplantation: development, prevention and treatment. Nephrol Dial Transplant. 2013;28:550-566
22. Pascual J, Van Hooff JP, Salmela K, et al.Three year observational follow-up of a multicenter, randomized trial on Tacrolimus-based therapy with withdrawal of steroids or mycophenolatemofetil after renal transplant. Transplantation 2006,82: 55-61.
23. Rostaing L, Cantarovich D, Mourad G, et al. Corticosteroid-free immunosuppression with Tacrolimus, mycophenolate mofetil, and daclizumab induction in renal transplantation. Transplantation79; 2005.807- 814.
24. Vitko S, Klinger M, Salmela K, et al. Two corticosteroid-free regimens -Tacrolimus monotherapy after basilixima b administration and Tacrolimus/mycophenolate mofetil—in comparison with a standard triple regimen in renal transplantation: Results of the Atlas study.Transplantation 2005;80:1734-1741.

25. Budde K, Glander P. Pharmacokinetic principles of immunosuppressive drugs. Ann Transplant 2008; 13:5-10.
26. Ekbal NJ, Holt DW, Macphee IA. Pharmacogenetics of immunosuppressive drugs: prospect of individual therapy for transplant patients. Pharmacogenomics. 2008;9: 585-596.
27. Belitsky P, Dunn S, Johnston A, et al. Impact of absorption profiling on efficacy and safety of Cyclosporine therapy in transplant recipients. Clin Pharmacokinet 2000; 39:117-125.
28. Blanchet B. Comparison of a new enzymatic assay with a high-performance liquid chromatography/ ultraviolet detection method for therapeutic drug monitoring of mycophenolic acid in adult liver transplant recipients. Liver Transpl 2008; 14:1745-1751.
29. Bremer S, Mandla R, Vethe NT, et al. Expression of IMPDH1 and IMPDH2 after transplantation and initiation of immunosuppression. Transplantation. 2008; 85:55-61.
30. Macphee IA. Use of pharmacogenetics to optimize immunosuppressive therapy. Ther Drug Monit. 2010; 32:261–264.
31. Elens L, Van Schaik RH, Panin N, et al. Effect of a new functional CYP3A4 polymorphism on calcineurin inhibitors dose requirements and trough blood levels in stable renal transplant patients. Pharmacogenomics. 2011; 12:1383-1396.
32. De Jonge H, Metalidis C, Naesens M, et al. The P450 oxidoreductase *28 SNP is associated with low initial Tacrolimus exposure and increased dose requirements in CYP3A5-expressing renal recipients. Pharmacogenomics. 2011;12:1281-1291.
33. Lamba J, Lamba V, Schuetz E. Genetic variants of PXR (NR1I2) and CAR (NR1I3) and their implications in drug metabolism and pharmacogenetics. Curr Drug Metab. 2005;6:369-383.
34. Anglicheau D, Le Corre D, Lechaton S, et al. Consequences of genetic polymorphisms for Sirolimus requirements after renal trans- plant in patients on primary Sirolimus therapy. Am J Transplant. 2005; 5:595-603.
35. Le Meur Y, Djebli N, Szelag JC, et al. CYP3A5*3 influences Sirolimus oral clearance in de novo and stable renal transplant recipients. ClinPharmacolTher. 2006; 80:51–60.
36. Picard N, Rouguieg-Malki K, Kamar N, et al. CYP3A5 genotype does not influence everolimus in vitro metabolism and clinical pharmacokinetics in renal transplant recipients. Transplantation. 2011; 91:652-656.
37. Evans WE. Thiopurine S-methyltransferase: a genetic polymorphism that affects a small number of drugs in a big way. Pharmacogenetics. 2002;12:421-423.
38. MiaoLY, HuangCR, HouJQ,et al. Association study of ABCB1 and CYP3A5 gene polymorphisms with Sirolimus trough concentration and dose requirements in Chinese renal transplant recipients. Biopharm Drug Dispos. 2007; 29:1-5.
39. Van Schaik RH, Van AM, De Fijter JW, et al. UGT1A9-275T.A/- 2152C.T polymorphisms correlate with low MPA exposure and acute rejection in MMF/Tacrolimus-treated kidney transplant patients. Clin Pharmacol Ther. 2009; 86:319-327.
40. Halloran, PF, Helms LM, Kung L, et al. The temporal profile of calcineurin inhibition by Cyclosporine in vivo. Transplantation 1999, 68:1356-1361.
41. Koefoed-Nielsen PB, Gesualdo MB, Poulsen JH, et al. Blood Tacrolimus levels and calcineurin phosphatase activity early after renal transplantation. Am J Transplant 2002; 2: 173-178.
42. Stalder M, Birsan T, Holm B, et al. Quantification of immunosuppression by flow cytometry in stable renal transplant recipients. Ther Drug Monit.2003; 25: 22-27.
43. Bohler T, Canivet C, Nguyen PN,et al. Cytokines correlate with age in healthy volunteers, dialysis patients and kidney-transplant patients. Cytokine 2009; 45: 169-173.
44. Stein CM, Murray JJ, Wood AJ. Inhibition of stimulated interleukin-2 production in whole blood: A practical measure of Cyclosporine effect. Clin Chem. 1999; 45: 1477-1484.
45. Rostaing L, Puyoo O, Tkaczuk J. Differences in Type 1 and Type 2 intracytoplasmic cytokines, detected by flow cytometry, according to immunosuppression (Cyclosporine A vs. Tacrolimus) in stable renal allograft recipients. Clin Transplant 1999; 13:400-409.
46. Schmittel A, Keilholz U, Scheibenbogen C. Evaluation of the interferon-gamma ELISPOT-assay for quantification of peptide specific T lymphocytes from peripheral blood. J. Immunol. Methods 1997; 210: 167-174.
47. Tary-Lehmann M, Hricik DE, Justice AC, et al. Enzyme-linked immunosorbent assay spot detection of interferon-gamma and interleukin 5-producing cells as a predictive marker for renal allograft failure. Transplantation 1998; 66: 219-224.
48. Hartel C, Fricke L, Schumacher N, et al. Delayed cytokine mRNA expression kinetics after T-lymphocyte costimulation: A quantitative measure of the efficacy of Cyclosporine A-based immunosuppression. Clin Chem. 2002; 48: 2225-2231.
49. Sommerer C, Konstandin M, Dengler T, et al. Pharmacodynamic monitoring of Cyclosporine a in renal

allograft recipients shows a quantitative relationship between immunosuppression and the occurrence of recurrent infections and malignancies. Transplantation 2006; 82: 1280-1285.
50. Khatri P, Sarwal MM. Using gene arrays in diagnosis of rejection. Curr. Opin. Organ Transplant. 2009; 14: 34-39.
51. Susal C, Pelzl S, Dohler B, et al. Identification of highly responsive kidney transplant recipients using pretransplant soluble CD30. J Am Soc Nephrol. 2002; 13: 1650–1656.
52. Kim MS, Kim HJ, Kim SI, et al. Pretransplant soluble CD30 level has limited effect on acute rejection, but affects graft function in living donor kidney transplantation. Transplantation 2006; 82: 1602-1605.
53. Kowalski R, Post D, Schneider MC,et al. Immune cell function testing: An adjunct to therapeutic drug monitoring in transplant patient management. Clin Transplant. 2003; 17: 77-88.
54. Barraclough KA, Staatz CE, Isbel NM, et al. Review: pharmacodynamic monitoring of immunosuppression in kidney. Nephrology (Carlton) 2010; 15:522-532.
55. Sagoo P, Perucha E, Sawitzki B, et al. Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. J Clin Invest. 2010; 120:1848-1861.
56. Newell KA, Asare A, Kirk AD, et al. Identification of a B cell signature associated with renal transplant tolerance in humans. J Clin Invest. 2010; 120:1836– 1847.
57. Muthukumar T, Dadhania D, Ding R, et al. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. N Engl J Med. 2005; 353: 2342-2351.
58. Ding R, Li B, Muthukumar T,et al. CD103 mRNA levels in urinary cells predict acute rejection of renal allografts. Transplantation 2003;75: 1307-1312.
59. Pretagostini R , Cinti P, Lai Q, et al. Minimization of immunosuppressive therapy and immunological monitoring of kidney transplant recipients with long-term allograft survival. Transpl Immunol. 2008; 20: 3-5.

Review Article

Novel Non-Invasive Biomarkers in Monitoring Renal Allograft Function

A.V. Vanikar

Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, IKDRC-ITS, Ahmedabad, India

ABSTRACT

Renal transplantation (RT) although a viable therapeutic option for patients suffering from end stage renal disease, has its own risk of rejection and other infection related morbidity /mortality due to life-long immunosuppression requirement. The present review elicits novel non-invasive biomarkers for monitoring RT. With our own experience we have also derived a cost-effective way of monitoring graft function using modalities of solid phase assay for donor specific antibodies and flow-cytometry for T-regulatory cells which are emerging effective tools for monitoring graft function.

KEY WORDS : Novel non-invasive biomarkers, T-regulatory cells, Renal transplantation, Donor specific antibodies, Acute rejection, Stem cell therapy

Renal transplantation (RT) is now a well accepted therapy for end stage renal disease. However the allograft being a major foreign antigen in the recipient immune system, can have dysfunction anytime in its life. As per the timeline, this dysfunction can occur immediately /early/ late after transplantation. Causes for dysfunction are immune/ non-immune like infections/ malignancy or even technical. Routine monitoring of the graft is performed by non-invasive techniques like ultrasonography or Doppler studies and lab tests like urine, serum creatinine (SCr) and blood levels of calcineurin inhibitors. Biopsy still remains the gold-standard for diagnosis of graft dysfunction. However all these diagnostic tools are still inadequate for timely intervention. Hence novel markers like urinary neutrophil gelatinase associated lipocalin (NGAL), β-2 microglobulin, N-acetyl-beta-D-glucosaminidase (NAG), interleukin-18, kidney injury molecule-1 (KIM-1), vascular endothelial growth factor (VEGF), serine protease inhibitor-9 (PI-9) and FoxP3 are being tested to establish acute cellular rejection. Serum has been used for testing acute phase reactants, cytokines, cystatin C, soluble CD154/CD30, and peripheral blood lymphocytes/ monocytes have been used for carrying out mixed lymphocyte reaction, Enzyme-linked immunospot, limited dilutional assays and flow-cytometry for immunophenotyping/ tetramer assays. Although they have shown limited utility on bench, none of them has predicted chronic rejection/ graft dysfunction. Graft biopsy establishes morphologic diagnosis, however it may not always be useful in tracing etiology and therefore in guiding therapy. Similarly perforin and granzyme in urine/ graft have shown limited utility. Measurement of antibodies in serum, and T-regulatory cells ($CD127^{low/-}/CD4^{+}/CD25^{hi}$) in blood are evolving as novel markers for predicting acute and chronic graft injury.

NGAL:

NGAL is a small 25-kD protein, belonging to the "lipocalins" superfamily. It is massively released in blood and urine mainly by activated neutrophils immediately after injury to renal tubular epithelial cells after accumulating in proximal tubules in experimental

Address for correspondence : *Dr. Aruna V Vanikar, MD, Professor and Head Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, G.R. Doshi and K.M. Mehta Institute of Kidney Diseases & Research Centre and Dr. H.L. Trivedi Institute of Transplantation Sciences, Civil Hospital Campus, Asarwa, Ahmedabad, India. E-mail: vanikararuna@yahoo.com*

and human clinical models.[1] NGAL is released before there is rise in SCr. Rise in urinary and serum NGAL has been observed after cardiac surgery, contrast administration, septic shock, and even RT.[2-6] Mori and Nakao proposed a hypothesis of "forest fire theory" explaining the relationship between NGAL and glomerular filtration rate (GFR). They proposed that elevated NGAL in chronic kidney disease (CKD) is an active process resulting from its sustained production by "inflammed" vital tubular cells, whereas the rise in SCr and fall in GFR are the mere passive result of a general loss of functional cells or nephrons.[7] This theory proposes that NGAL would represent a real-time indicator of how much active kidney damage exists within the overall condition of chronic renal impairment. Rostami et al [8] conducted a study on urinary NGAL at 2, 6, 12, 24 and 48 hours after surgery in a cohort of 64 RT patients with majority having received living donor RT to establish the role of NGAL in diagnosis of AKI. They found that NGAL was significantly higher with cut-off point of 204 ng/mL at 2 hours posttransplant in the event of AKI as compared to non-AKI and this value correlated with SCr on 7th postoperative day. However its value rapidly decreased by 2nd postoperative day if graft function picked up. Secondly NGAL does not indicate the reason for AKI. Hence it cannot be used as biomarker for rejection/ immune injury although it is a simple, user-friendly non-invasive technique.

β-2 microglobulin:

It is an 11.8 KD protein forming light chain of major histocompatibility class (MHC)-1 molecule. It is expressed on the surface of all nucleated cells. In the vent of injury, it dissociates from its heavy chain, enters circulation and gets filtered by the glomerulus to be absorbed and catabolized by proximal tubular cells.[9] Thus in event of acute tubular injury due to any cause β-2 microglobulin will be excreted in urine in large quantities. Thus it works as a non-invasive and non-specific marker for AKI/ graft dysfunction and will be elevated 4-5 days before rise in SCr.[10] The other major drawback is that it is highly unstable in urine with its rapid degradation at room temperature and pH < 6.[11]

Cystatin-C

It is a protein which is freely filtered by glomerulus, and reabsorbed and catabolized by tubules. It has been found to be stronger predictor of renal injury than SCr.[12] Herget-Rosenthal et al reported that elevated urinary cystatin -C levels were highly predictive of poor outcome in patients who had non-oliguric AKI.[13] It was suggested by Halawa that combination of plasma NGAL, cystatin C, and urinary NGAL, IL-18 and KIM-1 were useful biomarkers for predicting early graft injury.[14-15] However Slort et al showed in their studies on 16 pediatric transplant patients that cystatin C was not useful for predicting acute graft dysfunction in comparison to SCr.[16]

N-acetyl-beta-D-glucosaminidase (NAG):

It is a lysosomal brush border enzyme of proximal renal tubular cells with a high molecular weight of 130- 140 kd.[17] This enzyme is very stable and hence useful to detect tubular injury. However it is a non-specific biomarker since low excretion of NAG is observed in all cases of tubular injury. Thus it can only predict graft dysfunction however the reason for the same cannot be known by its estimation.

Interleukin-18:

It is a pro-inflammatory cytokine constitutively expressed by every healthy epithelial cell. It has been found to have immunomodulatory role in various inflammatory conditions.[18] IL-18 precursor is enzymatically cleaved by IL-1β-converting enzyme to produce mature 18-kDa, IL-18 protein. [18,19] Renal IL-18 mRNA levels have been shown to be significantly upregulated following ischemia-reperfusion injury along with elevated urinary levels. Protocol graft biopsies also showed presence of IL-18 in proximal tubular cells in event of acute rejection.[19,20]

KIM-1

Kidney injury molecule-1 (KIM-1) is a type I cell membrane glycoprotein containing a unique six-cysteine immunoglobulin-like domain and a mucin domain in its extracellular region.[21] KIM-1 mRNA levels increase more than any other known gene after

kidney injury and is the earliest diagnostic indicator compared with other conventional biomarkers like SCr, proteinuria or urinary NAG levels.[22,23]

VEGF

Vascular endothelial growth factor (VEGF) is an endothelial-specific growth factor that promotes endothelial cell proliferation, differentiation and survival, mediates endothelium-dependent vasodilatation, induces microvascular hyperpermeability and participates in interstitial matrix remodeling. In the kidney, VEGF expression is most prominent in glomerular podocytes and in tubular epithelial cells, while VEGF receptors are mainly found on preglomerular, glomerular, and peritubular endothelial cells.[24] In a study by Pilmore et al, VEGF expression was increased in glomeruli, interstitium and vascular compartment of 17 RT patients with chronic rejection.[25] In another study by Ozdemir et al, VEGF expression in kidney was increased in early phase if injury was to be prevented however subsequent increased expression was found to be associated with poor graft outcome associated with interstitial fibrosis and chronic vasculopathy.[26] However this finding was also noted in event of cyclosporin toxicity. Thus VEGF could be considered as a biomarker for chronic graft dysfunction however its utility to improve the graft outcome is limited.

Donor specific antibodies

Human leucocyte antigen (HLA) is a set of glycoproteins present on almost all nucleated cells of the body. Their main function is to identify any foreign protein/ antigen entering the host immune system and take them to the receptors on surface of immune cells like T-cells via antigen presenting cells. This immune recognition for grafted organ is more potent and early than for infective agents like bacteria/ viruses. There are mainly HLA class I and II antibodies directed against HLA class I (A, -B and -C) and HLA class II (DR, -DQ and -DP) antigens. These antibodies are encountered with every immune response and may stay in memory bank formed by B-cells and plasma cells located in lymph nodes, spleen and bone marrow. Eventually these antibodies lead to chronic graft attrition for which there is no answer found till date.

Routine tests to identify these antibodies are lymphocyte cross-match, and now flow-cross-match for T and B-cells. Since 2007 solid phase assay of luminex platform has also proved its utility by detecting about 100 types of HLA class I and II antibodies from small amount of serum (100 microlitre). This testing helps identify the presence of antibodies against the antigens of donor organ present in the serum of recipient, labeled as donor specific antibodies (DSA). This test also elicits other antibodies (non-DSA). All antibodies, especially DSA have been found to be detrimental for graft and patient survival in lung, liver, heart and kidney transplantation by several studies.[27-34] Hence it has been found by some studies, including ours (unpublished data) that regular monitoring of DSA rather than a single study helps in monitoring graft function better.[35]

T-regulatory cells

During the developmental stage at the level of thymic regulation, T-cell receptor (TCR) signaling plays crucial role in duration and functional avidity of T-cells. This regulation decides their further differentiation like CD4 and CD8 subtypes. One of the cell types that get signaling for CD4, CD25 and forkhead P-3 gene are called T-regulatory cells (Tregs). These are low or deficient in expression of CD127 on their surface. T-regs can be induced (i-Tregs) or naturally occurring (n-Tregs). These cells play a role in controlling autoimmune diseases and infections. Muthukumar et al measured urinary cell mRNA levels of Foxp-3 in three sets of patients, 36 patients with acute rejection, 18 with chronic rejection and 29 with normal graft biopsies. They concluded that high levels of FoxP3 along with granzyme were good predictors of acute rejection with successful reversal while low levels were indicative of eventual graft failure after rejection episodes.[36]

Ahmedabad experience

We carried out the present study in a reverse way compared to the existing search for novel biomarkers to identify early immune injury to the renal allograft. We have been using stem cell therapy (SCT) since last 15 years and have now developed a protocol in which we first subject the renal allograft recipients to total

lymphoid irradiation followed by infusion of donor adipose tissue derived mesenchymal stem cells (AD-MSC) and hematopoietic stem cells (HSC) in portal and thymic circulation. RT is then performed after favorable immune response in the form of negative lymphocyte cross-match by CDCC technique, T and B-cell flow-cross-match and single antigen solid phase assay by luminex platform.

We reviewed a set of patients having stable graft function and found that DSA were not always detrimental to graft stability. We further divided our patients in 2 groups, group-1 with HLA class 1/ 2 antibodies (tested by luminex assay) and group-2 with no antibodies. Their demographics, graft function in terms of SCr, rejection episodes, immunosuppression, protocol biopsies and Tregs [$CD127^{low/neg}CD25^{high}CD4^{+}$] in peripheral blood were evaluated. There were 138 patients, 90 (males/ females: 81/9) of group-1 and 48 (males/ females: 46/2) of group-2 with mean age 32.5 and 31.7 years respectively. Mean HLA-match was 2.42 in former and 3.02 in later. Over a mean follow-up of 6 years in the former and 5.76 years in the later, mean SCr (mg/dL) was 1.42 and 1.4 respectively. There were 6.6% rejection episodes in former and 8.3% episodes in the later. Totally 47.8% patients in group-1 vs. 56.3% patients in group-2 were on Prednisone monotherapy, 5-10 mg/day or no immunosuppression. Totally 19 patients in group-1 and 12 in group-2 consented for protocol biopsies; 73.7% in group-1 and 83.3% of group-2 were unremarkable.

The mean Tregs were 3.3% in group-1 and 3.5% in group-2. We further tested Tregs in a cohort of control patients who were subjected to RT without any SCT. Their Tregs were 1.9%. Further while monitoring these patients we found that Tregs remained around 3-3.5% in patients who received SCT and remained < 2% in controls. We studied Foxp3 levels in graft biopsies of patients of all groups. We found that normal biopsies irrespective of group had FoxP3 levels of approximately 0.3%. In event of rejection these levels elevated to about 1% and dropped to previous normal values in event of effective reversal of immune injury. However with chronic changes, the values remained 0.5% to 1.2%. These values inversely correlated with Tregs. With impending injury Tregs dropped to <2% and returned to normal values of about 3% with effective therapy. However with chronic injury they escalated to >3.5%. The pattern of Tregs and FoxP3 in graft biopsies was fairly maintained to normal levels in patients subjected to SCT vs. controls.

Thus we draw the follow conclusions from studies by different groups and our own study.

- Novel biomarkers like NGAL, NAG, IL-18, cystatin, VEGF and KIM-1 indicate acute graft dysfunction before SCr however they do not indicate whether the injury is immune or non-immune. Thus their inclusion in diagnostic kit will be financially prohibitive.
- Single antigen assay although expensive, will definitely indicate the quantity and types of antibodies (both HLA class I/II, and whether they are donor specific or non-DSA). However regular monitoring, at 3 monthly intervals especially in first 2 years of transplantation can help in predicting antibody mediated injury well in advance before SCr is elevated.
- T-regulatory cell estimation in peripheral blood as a single test and at 3 monthly intervals will be useful to predict graft function status.
- In addition, SCT before transplantation can help in minimizing immunosuppression and alleviating immune injury. Secondly in patients subjected to pre-RT SCT, the grafts will be protected from immune injury caused by DSA.
- Tregs will also prove cost-effective, both as therapeutic and diagnostic modality.

REFERENCES

1. Bolignano D, Lacquaniti A, Giuseppe Coppolino G, et al. Neutrophil Gelatinase-Associated Lipocalin (NGAL) and Progression of Chronic Kidney Disease. Clin J Am Soc Nephrol 2009; 4: 337-344.
2. Mishra J, Dent C, Tarabishi R, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005; 365: 1231-1238.
3. Hirsch R, Dent C, Pfriem H, et al. NGAL is an early predictive biomarker of contrast-induced nephropathy in children. Pediatr Nephrol 2007; 22 :2089-2095.

4. Ling W, Zhaohui N, Ben H, et al. Urinary IL-18 and NGAL as early predictive biomarkers in contrast-induced nephropathy after coronary angiography. Nephron Clin Pract 2008; 108 : c176-c181.
5. Wheeler DS, Devarajan P, Ma Q, et al. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. Crit Care Med 2008; 36: 1297-1303.
6. Parikh CR, Jani A, Mishra J, et al. Urine NGAL and IL-18 are predictive biomarkers for delayed graft function following kidney transplantation. Am J Transplant 2006; 6: 1639- 1645.
7. Mori K, Nakao K. Neutrophil gelatinase-associated lipocalin as the real-time indicator of active kidney damage. Kidney Int 2007; 71: 967-970.
8. Rostami Z, Nikpoor M , Einollahi B. Urinary Neutrophil Gelatinase Associated Lipocalin (NGAL) for Early Diagnosis of Acute Kidney Injury in Renal Transplant Recipients. Nephrourol 2013; 5:745-752.
9. Miyata T, Jadoul M, Kurokawa K, et al. Beta-2 microglobulin in renal disease. J. Am. Soc. Nephrol. 1998; 9:1723-1735.
10. Schaub S, Wilkins JA, Antonovici M, et al. Proteomic-based identification of cleaved urinary beta 2-microglobulin as a potential marker for acute tubular injury in renal allografts. Am. J. Transplant. 2005; 5:729-738.
11. Davey PG, Gosling P. Beta 2-microglobulin instability in pathological urine. Clin. Chem. 1982; 28:1330-1333.
12. Dittrich S, Kurschat K, Dahnert I, et al. Renal function after cardiopulmonary bypass surgery in cyanotic congenital heart disease. Int J Cardiol 2000; 73: 173-179.
13. Conti M, Moutereau S, Zater M, et al. Urinary cystatin C as a specific marker of tubular dysfunction. Clin. Chem. Lab. Med. 2006; 44: 288-291.
14. Herget-Rosenthal S, Pietruck F, Volbracht L, et al. Serum cystatin C- a superior marker of rapidly reduced glomerular filtration after uninephrectomy in kidney donors compared to creatinine. Clin. Nephrol. 2005; 64: 41-46.
15. Halawa A. The early diagnosis of acute renal graft dysfunction: a challenge we face. The role of novel biomarkers. Ann Transplant. 2011; 16:90-98.
16. Slort PR, Ozden N, Pape L, et al. Comparing cystatin C and creatinine in the diagnosis of pediatric acute renal allograft dysfunction. Pediatr Nephrol. 2012; 27(5):843-849.
17. Sylva Skálová. The diagnostic role of urinary n-acetyl-β-d-glucosaminidase (NAG) activity in the detection of renal tubular impairment. Acta Medica 2005; 48(2):75-80.
18. Dinarello CA, Novick D, Kim, et al. Interleukin-18 and IL-18 binding protein. Front Immunol. 2013; 4: 289: 1-10.
19. Sugawara I. Interleukin-18 (IL-18) and infectious diseases, with special emphasis on diseases induced by intracellular pathogens. Microbes Infect. 2000; 2:1257-1263.
20. Striz I, Krasna E, Honsova E, et al. Interleukin 18 (IL-18) upregulation in acute rejection of kidney allograft. Immunol. Lett. 2005; 99: 30-35.
21. Vaidya VS, Ferguson MA, Bonventre JV. Biomarkers of Acute Kidney Injury. Annu Rev Pharmacol Toxicol. 2008; 48: 463-493.
22. Vaidya VS, Ramirez V, Ichimura T,et al. Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. Am. J. Physiol. Renal Physiol. 2006; 290: F517-F529.
23. Han WK, Bailly V, Abichandani R, et al. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int. 2002; 62:237-244.
24. D Schrijvers BF, Flyvbjerg A, De Vriese AS. The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. Kidney Int. 2004; 65(6): 2003-2017.
25. Pilmore HL, Eris JM, Painter DM, et al. Vascular endothelial growth factor expression in human chronic renal allograft rejection. Transplantation 1999; 27;67(6):929-933.
26. Ozdemir BH, Ozdemir FN, Haberal N, et al. Vascular endothelial growth factor expression and cyclosporine toxicity in renal allograft rejection. Am J Transplant. 2005; 5:766-774.
27. Colvin RB , Smith RN. Antibody-mediated organ allograft rejection. National Review of Immunology 2003; 5(10): 807-817.
28. Kaczmarek I, Deutsch MA, Kauke T, et al. Donor-specific HLA alloantibodies: long-term impact on cardiac allograft vasculopathy and mortality after heart transplant. Experimental and Clinical Transplantation 2008; 6: 229-235.
29. Terasaki PI. Humoral theory of transplantation. American Journal of Transplantation 2003; 3: 665-673.
30. Lachmann N, Terasaki PI, Budde K, et al. Anti-human leukocyte antigen and donor-specific antibodies detected by Luminex post-transplant serve as biomarkers for chronic rejection of renal allografts. Transplantation 2009; 87(10):1505-1513.
31. Musat AI, Agni RM, Wai PY, et al. The significance of donor-specific HLA antibodies in rejection and ductopenia development in ABO compatible liver transplantation. American Journal of Transplantation 2011; 11(3):500-510.

32. Hachem RR, Yusen RD, Meyers BF, et al. Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. Journal of Heart and Lung Transplantation 2010; 29:973-980.
33. Smith JD, Banner NR, Hamour IM, et al. De novo donor HLA-specific antibodies after heart transplantation are an independent predictor of poor patient survival. American Journal of Transplantation 2011; 11:312-319.
34. O'Leary, Kaneku H, Susskind BM, et al. High mean fluorescence intensity donor-specific anti-HLA antibodies associated with chronic rejection post liver transplant. American Journal of Transplantation 2011; 11: 1868-1876.
35. Kimball PM, Baker MA, Wagner MB, et al. Surveillance of alloantibodies after transplantation identifies the risk of chronic rejection. Kidney Int. 2011; 79: 1131-1137.
36. Muthukumar T, Dadhania D, Ding R, et al. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. N Engl J Med 2005; 353: 2342-2351.

Original Article

Reminiscences of Immunologic Events in Renal Transplants: Partial Tolerance

J.B. Dossetor

In the early days of maintenance hemodialysis – in the era which bloomed following Belding Scribner's truly memorable presentation, in 1962 to the American Society of Clinical Investigation the process of preparation of either the Rotating Drum or Twin Coil type dialysers involved priming with allogeneic blood.[1] Usually 2 units of blood were used each time a patient was dialysed. This meant that, with twice weekly dialysis (the norm at that time), the patient was exposed to over 100 units of allogeneic blood during the course of every 6 months. Most would conjecture, in our present state of immunologic wisdom, that this would cause widespread sensitization of HLA in all patients. [Of course, at the time of which I write the lymphocytic crossmatch, using recipient/ patient's serum, was still in the future. Nor, indeed, was there any HLA typing, at that time].

Nevertheless in 1963, because of the tragedy of deaths from chronic renal failure and the slow spread of long-term maintenance hemodialysis programs, cadaver kidney transplants were carried out in certain transplant centres-- such as the Peter Brent Brigham (Boston), the Cleveland Clinic, and at McGill University (Royal Victoria Hospital, Montreal).[2]

The initial experiences of the McGill group (the first four recipients) were described in 1964.[3] At the time of that report the world experience in renal transplantation had been reviewed in September 1963, only 23 of 96 live related-donor recipients survived 6 months (excluding identical twin transplants,) and only 4 cadaver kidney recipients survived to 6 months.[4] It was decided to continue the program, but not to use living donors. Two of the four recipients had prolonged kidney function of 3 months or more. Clearly there were many perplexing aspects to these early experiences and the question was raised that even the cadaver kidney transplant program might be premature and a moratorium should be called—but this was something which desperate patients cannot accept when there are no alternatives available.

The next publication of the McGill series was in 1967 when 58 cadaver kidney transplants were described. When twenty nine (29) of these were further analysed. And unexpected result emerged "Analysis of the 29 patients, ranked by their evidence of rejection activity in the first three post-transplant months show significant difference in renal function, hippuran renogram pathologic changes in glomerul and vessels, but not interstitial cellular infiltration. There was also a significant difference whereby those with more hemodialysis prior to transplantation showed less evidence of rejection activity."[5]

In discussion of this unexpected finding, not only was a) a correlation shown between evidence of acute tubular necrosis and ischaemic time, but also, b) evidence of an inverse relationship between rejection index and the amount of time on hemodialysis (using the Twin Coil dialyser primed with allogeneic blood for each dialysis). This inverse correlation was true for both the group with high rejection index (closed inverted triangles) and those with low rejection index (open upright triangles). There was also correlation between the number of blood transfusions and improved late continuing function in each group (those with evidence of high rejection activity and those with low rejection activity). But it was also acknowledged that the numbers of transplants were low and also that transferring some of the patients in one group to the other would destroy the significance.

Nevertheless the data convinced us that there was more to HLA sensitization than mere exposure to allogeneic leukocytes. Perhaps it was the route of exposure as well

Address for correspondence : *Professor Emeritus (Medicine/Bioethics, University) Alberta, Canada. E-mail: jdossetor@rogers.com*

as the overwhelming amount of allogeneic blood exposure that caused decreased evidence of late rejection and improved late functioning.

This conviction that large volumes of allogeneic whole blood, delivered intravascularly, might even be beneficial to subsequent renal transplantation, led us to three lines of investigation:

- In live donor situations, the possible benefit of donor-specific blood transfusion prior to transplantation; especially in situations where the recipient now had the added protection of a negative donor lymphocyte serum cross match. (as described by Kissmeyer et al. in 1966; and was rapidly found to be the principal cause of immediate transplant rejection).[6]
- Some experiences in attempting to lessen allograft rejection using donor antigen, in the rat kidney transplant model;[7] and,
- On one rare occasion, evidence of benefit from actual cross-circulation between a prospective donor and a prospective recipient.[8, 9] This very unusual circumstance of donor-specific cross-circulation prior to transplantation will be described in further detail, below.

The occasion which justified the cross-circulation experience occurred when woman in chronic renal failure, in her 30s, with five children, was referred from a remote part of Newfoundland. We told the referring physician that long-term dialysis would not be possible and even cadaver kidney follow-up would be very inadequate, in winter, as her home was really only accessible by coastal boat. However, she had arrived and we were on the point of giving her a period of 3 months on the waiting list, on dialysis, before taking the next step-which would probably be to send her home on peritoneal dialysis if she did not receive a cadaveric transplant.

During this time there was another young woman of the same ABO blood group (there was no HLA-typing available that time) who was in critical condition from advanced liver disease, and likely to die. The similarity of the blood groups was noted by the resident in internal medicine who was looking after both patients. She brought this to my attention and, on discussion, we came up with the possibility of putting into practice a plan where each would agree to cross-circulate with the other, using the function of the organ in the other which each uniquely lacked. To this end a consent agreement was drawn up between both patients and both families to continue with cross-circulation for as long as possible. In this agreement each family agreed, also, that if one of them died, the organ which the other lacked would be available as a cadaveric transplant.

Cross circulation had been used before, prior to the introduction of the membrane oxygenator and consisted of placing silastic tubes in each partner and

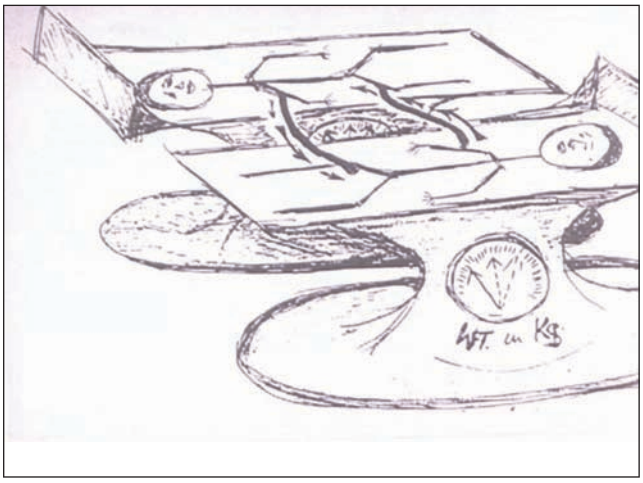


Figure 1 : Diagram of cross circulation

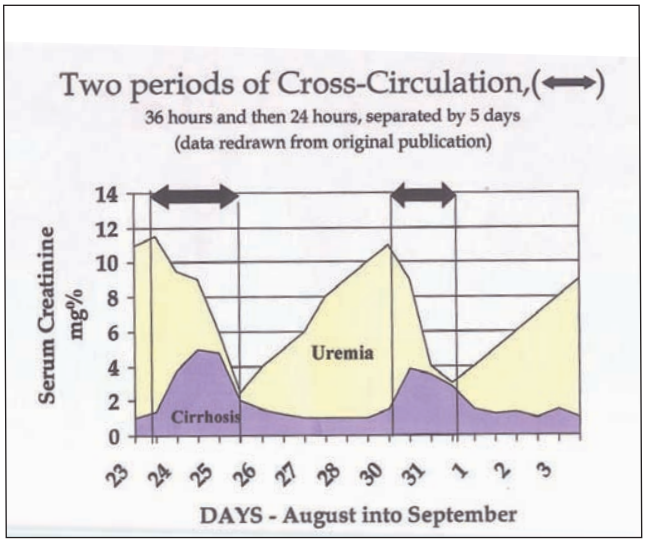


Figure 2 : Biochemical changes which occurred in serum creatinine during the cross-circulation.

connecting them, artery to vein, while both patients lay on bed weigh scales.[10] The volume balance of the two circulations was controlled by adjusting the screw clamps on the linking blood connections in responses to the changes in weight between the patients. (see Figure 1).

Figure 2 shows the biochemical changes which occurred in serum creatinine during the cross-circulation. Creatinine of the uremic patient fell sharply, and this was accompanied by a temporary rise in creatinine in the cirrhotic patient. Similar mirrored inverse changes were seen in serum bilirubin (not plotted in Figure 2) Both patients became alert, coming out of stupor, as a result of these cross circulations which were undertaken for two periods of 36 hours and 24 hours during the first week.

Unfortunately, while preparing for a third episode, on the 10th day, the hepatic patient had an intestinal hemorrhage (presumably from varices) and died. There was considerable delay in implanting her kidney into the uremic patient (it was a holiday long week-end) and there was no diuresis initially. Eventually, renal function returned. No clear cut rejections occurred and she lived for a 9 further years in her remote Newfoundland location, returning for follow-up every year. During this time she successfully gave birth to her sixth (6th) child. Her death (which occurred at her home) was attributed to poorly controlled hypertension, but the transplant had continued to function.

It is difficult not to believe that cross circulation rendered her tolerant in some way to the antigenic constituents of her cross-circulation partner though this cannot be proved, of course.

Evidence later grew to show the hazards of HLA sensitization in transplant recipients and the critical importance of pre-transplant lymphocytoxic cross-matching. But, also, further evidence accumulated in the literature to show the beneficial effect associated with pre-transplant blood transfusions with definitive confirmatory articles by Opelz and Terasaki. [11, 12] In the second of these papers, 1360 kidney grafts were reviewed and the improved correlation of long term function with numbers of pre-transplant blood transfusions had a statistical significance of $P<0.0001$. [13] Other results came from Festenstein et al and others.[13]

This clinical work was backed up by controlled experiments in the rhesus monkeys by Van Es, Balner et al which showed the same beneficial effect.[14]

With improved methods of hemodialysis and its frequency (not less than three times, weekly) and improved immunosuppressive regimens, this beneficial effect has faded into the background of clinical transplantation, though the effect was shown again in a more recent series from a large pediatric renal transplant series from Paris. [15] Here, covering the transfusions with Cyclosporine (short-term) was associated with improved graft survival at 2 and 5 years when compared with those who received blood transfusion without short-term concomitant Cyclosporine coverage. Similar results were reported by Opelz, in 1997, from 14 centres using Cyclosporine as the main immunosuppressive agent so the topic may still have some validity [16.]

The aim of good dialysis care switched to minimal use of blood transfusions- which is a good thing as this era was accompanied by such striking improvements in immuno-suppression. But for those who have had the earlier experiences such as I have described above they were generously referred to by Dr. Trivedi on page 3 of his editorial in the first issue of Transplantation India the dream of inducing partial immunologic tolerance in a donor-specific context never disappeared. It was always there in the background. This is why it was so exciting to hear of the remarkable series of clinical outcomes from Ahmedabad in the last few years.

The mechanisms underlying these remarkable improvements are very complex, indeed and include the concepts of donor chimerism, donor-specific stem cell transplantation, and basic immunologic processes which become increasingly unraveled as the science progresses, But increasing knowledge at the molecular level still does not render unnecessary those bold leaps at the clinical level by which we remember such pioneers as Tom Starzl, Joe Murray, Roy Calne, Christiaan Barnard, and of course, Willem J. Kolff (who pioneered with hemodialysis) and Peter Medawar (who pioneered with the whole concept of immune tolerance).

(I will make no attempt to give a comprehensive list of these pioneers-- it would be too difficult and might even be perilous!)

I, too left the field of possible induction of partial immunologic tolerance at the clinical level and took up research opportunities in:

- histocompatibility, (with studies in two populations who have very restricted HLA antigen profiles as a result of in-breeding the Hutterites in Alberta and the Inuit population of the Canadian Arctic;
- post-transplant immunological monitoring, using recipient serums and lymphocytes with donor spleen cells as targets (preserved frozen donor spleen cells), leading to
- an increasing commitment to study of the ethical issues in the whole field of organ and tissue transplantation.

However, these researches will not be reminisced in this paper as it is dedicated to those who have reopened the whole area of induction of partial immunologic tolerance by means of pre-transplant infusion of donor stem cells and more recently, using donor renal tissue into the recipients thymus as a further adjunct. [17,18]

REFERENCES

1. Scribner, BH. Periodic Hemodialysis for the Rehabilitation of patients with terminal uremia. J Clin Invest. 1962; 41:1398-1399.
2. Murray JE, Merrill JP, Harrison JH, et al. Prolonged survival of human kidney homografts by immunosuppressive drug therapy. New Eng J Med.1963; 268:1315.
3. Dossetor JB, Gault MH, Oliver JA, et al. Cadaver renal homotransplants: Initial experiences. Can Med Assn J. 1964; 91:732-742.
4. National research council conference on human kidney transplants (Washington DC, September 26, 27, 1963) Transplantation 1964; 2: 147.
5. Dossetor JB, MacKinnon KJ, Gault MH, et al. Cadaver renal transplants. Transplantation 1967; 5:844-853.
6. Kissmeyer NF, Olsen S, Petersen VP, et al. Hyperacute rejection of kidney allografts associated with pre-eisting humoral antibodies against donor cells. Lancet 1966; 2:662.
7. Yaguchi Y, MacKinnon KJ, Dossetor JB. Renal modification by donor antigen in the rat. Evidence for significance of this principle in man. In Dausset J. Hamburger J, Mathe G., Eds. Advance in Transplantation, Munksgaard, Copenhagen, p. 393, 1968.
8. (ibid)
9. Dossetor JB. Transplantation: A Canadian career-giving experience.
10. Lilehei CW. A personalized history of extracorporeal circulation. Trans. Am Soc Artif Int Organs 1982; 28:35.
11. Opelz G, Terasaki PI. Prolongation effect of blood transfusions on kidney graft survival. Transplantation 1976; 22: 380-383.
12. Opelz G, Terasaki PI. Improvement of kidney-graft survival with increased number of blood transfusions. New Eng J Med. 1978; 299:799-803.
13. Festenstein H, Sachs JA, Pegrum GD, et al. The influence of HLA matching and blood transfusions on outcome of 502 London Transplant Group renal graft recipients. Lancet 1976; 1: 157-161.
14. Van Es AA, Marquet RL, Van Rood JJ, et al. Blood transfusions induce prolonged kidney allograft survival in Rhesus monkeys. Lancet 1977; 1: 506-509.
15. Niaudet P, Dudley J, Charbit M, et al.Pretransplant blood transfusions with cyclosporine in pediatric renal transplantation. Pediatr Nephrol. 2000;14: 451-456.
16. Opelz G, Vanrenterghem Y, Kirste G, et al. Prospective evaluation of pretransplant blood transfusions in cadaver kidney recipients. Transplantation 1997; 63: 964-967.
17. Trivedi HL, Shah VR, Shah PR, et al. Megadose approach to DBMC infusion induced allograft hyporesponsiveness in living-related allograft recipients. Transplant Proceedings 2001; 33:271-276.
18. Trivedi HL, Vanikar AV, Modi PR, et al. Intrathymic donor antigen inoculation and megadose peripheral hematopoetic stem cell infusion in live related renal allograft transplantation - A Strategy to induce tolerance in clinic. Transplantation India 2002; 1: 32-39.

Original Article

Restless Legs Syndrome in Patients of End Stage Renal Disease on Maintenance Hemodialysis.

V.V. Sainaresh, K. Jagadeesh, SAA Lateef, G.S. Krishna, V. Siva Kumar

Department of Nephrology, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh.

ABSTRACT

Forty seven patients of end stage renal disease on maintenance hemodialysis were studied for prevalence of RLS and it's relationship with anemia, iron profile, calcium phosphate product and dialysis adequacy parameters such as Kt/V and URR. The prevalence of RLS was found to be 6%. The statistical evaluation did not reveal any relation between RLS and anemia, iron profile, calcium phosphate product and dialysis adequacy parameters.

KEY WORDS : Restless legs syndrome, Maintenance hemodialysis, End stage renal disease

INTRODUCTION

Restless legs syndrome (RLS) also known as Ekbom's syndrome,[1] defined in International classification of sleep disorders as "a disorder characterized by disagreeable leg sensations usually prior to sleep onset , that can cause an almost irresistible urge to move the legs. An International Restless Legs Syndrome study group (IRLSSG) gave a consensus on diagnostic criteria of this disorder. According to this group the clinical characteristics of RLS necessary for diagnosis (minimal criteria): 1) the desire to move extremities 2) motor restlessness 3) worsening of symptoms at rest, with at least temporary relief by activity and 4) worsening of symptoms in evening or at night. The diagnosis of RLS was made based on patient's responses to a self administered questionnaire which included minimal criteria provided by IRLSSG. RLS was diagnosed in patients as follows: 1) RLS : satisfying all minimal criteria at time of investigation , 2) Questionable RLS (QRLS): satisfying criteria 1 and 2, but not 3 and / or 4 minimal criteria, 3) Potential RLS (PRLS): not RLS or QRLS but administered drugs with established efficacies for uraemic RLS in placebo-controlled trials (i.e Dopaminergic drugs / Gabapentin and Clonidine), 4) Non RLS: not RLS, QRLS, or PRLS.[2,3]

The prevalence of RLS is estimated to be 2 to 5% in general population and 17 to 57% in uraemic patients as reported in western studies.[2,3,4] In several studies RLS was found to be related to female sex, smoking, long duration of dialysis therapy, iron deficiency, anemia and hyperphosphatemia. Treatment with dopaminergic agents, clonezepam or clonidine has been reported to be effective in uraemic patients. [1,3,4,5]

MATERIAL AND METHODS

We conducted a prospective study on 47 patients of end stage renal disease on MHD in our unit after obtaining informed consent, during the period of January 2007 to January 2008. All the patients who were on dialysis for three or more than three months, weekly thrice for four hours per session were included in the study. The diagnosis and categorization into various groups of RLS were made based on patients responses to a self administered questionnaire, that included minimal criteria, provided by the IRLSSG.[2,3] In addition the demographic information and details of medical history such as information about age, sex, etiology of CKD and presence or absence of diabetes and other co-morbidities were obtained. The laboratory data included serum parameters such as hemoglobin, urea, sugar, albumin, sodium, potassium, calcium, phosphate

Address for correspondence : *Dr. V. Siva Kumar, Professor of Nephrology, Department of Nephrology, SVIMS, Tirupati, Andhra Pradesh, India, E-Mail: sa_vskumar@yahoo.com*

and iron profile (iron, ferritin and transferrin saturation) The adequacy of dialysis was assessed in all the patients using Kt/V and Urea Reduction Ratio (URR). The results were analysed for the prevalence of RLS in our study group and also its relation with various parameters. The statistical significance was assessed using paired student "t" test.

RESULTS

During the period of January 2007 to January 2008, forty seven patients on maintenance hemodialysis were studied. The male: female ratio was 2:1; mean age was 50.04 yrs $\pm$11.10 yrs (Table 1). The etiology of CKD was due to diabetes in 36.17%, hypertension in 31.91%, chronic glomerulonephritis in 14.89%, chronic graft failure in 10.63% and chronic tubulointerstitial disease in 6.38%.Out of forty seven patients, RLS was found in 6%, QRLS in 19%, Non RLS in 74%. Data correlation with regard to anemia, Iron profile, Calcium phosphate product and Dialysis adequacy parameters (Kt/V, URR) between RLS versus QRLS and RLS versus Non RLS did not show a statistical relationship (Table 2).

S. PTH and Vit D estimations were done in all the

Table 1: Demographic details

Type	Age	Number	Male: Female	Diabetic: Non Diabetic CKD	Total duration of Hemodialysis (in months)
RLS	54.66 + 6.02	3 (6.38%)	3:0	2 :1	38
QRLS	53.55 + 6.07	9(19.14%)	7:2	4:5	46.77
Non- RLS	46.94 + 12.18	35(74.44%)	22:13	11:24	30.6

RLS: Restless Legs Syndrome, QRLS: Questionable Restless Legs Syndrome

Table 2a : Comparative analysis between the RLS and QRLS subjects

Type	Hb	S.Cr	S.Ca	S.Alb	$CaXPO_4$	S. Iron	TIBC	Tsat	KT/V	URR
RLS	12	9.1	9.4	4	43.43	90	285	31.5	1.31	63.73
QRLS	9	7.2	9.4	3.7	41.86	90	310	30.2	1.31	63.44
"p"	0.11	0.48	0.34	0.341	0.967	0.303	0.431	0.84	0.63	0.236
Value	3	0	1					8	1	

Table 2b : Comparative analysis between RLS Vs non RLS subjects

Type	Hb	S.Cr	S.Ca	S.Alb	$CaXPO_4$	S. Iron	TIBC	Tsat	KT/V	URR
RLS	11.46	10	9.3	4.0	42.88	70.4	303.66	29.13	1.13	58.56
Non RLS	8.9	7.64	9.54	3.66	41.39	92.97	332.42	28.98	1.19	57.39
"p" value	0.047	0.31	0.52	0.165	0.841	0.162	0.432	0.980	0.706	0.845
		3	9							

Hb: Hemoglobin gms/dl, S.Cr: Pre dialysis serum creatinine mg/dl, S.Alb: Serum albumin gms /dl, Ca X PO4: calcium phosphorus product in mg^2/dl^2,S.Iron: serum Iron µg/dl, TIBC: Total Iron Binding Capacity µg/dl, T sat: Total Transferrin saturation %, URR: Urea Reduction Ratio%

patients of RLS and QRLS. The median values of S.PTH and S.Vit D were 217 pg/ml and 27.6 ng/ml respectively in RLS group. In QRLS group the Median values of S.PTH and S.Vit D were 200.2 pg/ml and 15.4 ng/ml respectively. There was no statistically significant difference noted between the RLS vs QRLS with regard to S.PTH and Vit D status. S. PTH and Vit. D levels of some patients in the Non RLS group were not done so the relationship could not be analyzed in this group

All the patients of RLS were on EPO therapy and parenteral iron supplementation. The mean average dose of EPO was 6000 units per week. As some of the patients of QRLS and Non RLS were not receiving EPO the results were not compared.

DISCUSSION

Restless Legs Syndrome is a movement disorder characterized by an "achy or crawling" paresthesia, usually of the lower extremities. Movement of affected limbs partially relieves such sensation. Classically the sensory and motor aspects are worse at night. [3, 6] Patients frequently experience exacerbation of the symptoms of RLS during dialysis. Thus the restlessness and akathesia lead to symptoms of insomnia, fatigue, reduced concentration, memory loss, decreased motivational drive, depression and anxiety. [1, 3] RLS is the disease of reticulo neuronal population of subcortical region involving the dopaminergic and opiate systems. [3,10]

RLS has been associated commonly with iron deficiency. However iron supplementation even without iron deficiency has been shown to produce improvement in some patients. Thus RLS appears to be a state of brain iron insufficiency. The management of iron in brain is altered in patients with RLS possibly due to blood brain barrier which allows the brain to maintain its iron status independent of blood levels. Iron is involved in a rate limiting step required to convert tyrosine to levodopa which later is decarboxylated to form dopamine. Thus iron deficiency interferes with dopaminergic function. Magnetic resonance imaging estimation of iron in nigrostriatal area correlated with RLS severity. [1, 8,9,10] Serum iron has a marked circadian variation with as much as a 30% – 50% drop in S. iron concentration at night. Low brain iron concentration in patients with RLS may create greater dependence within the brain on serum iron levels. Thus the night time drop in S. iron correlate well with appearance of symptoms of RLS in the nights[1, 10]. Diagnosis of RLS is a clinical decision based on diagnostic criteria of the international RLS study group[3]. Polysomnography may be helpful in diagnosis of resistant RLS and in detection of sleep apnea.[11]

In uremic patients with RLS, the role of hemoglobin or haematocrit, severity of anemia, iron deficiency, phosphate concentrations are debatable in different observations[3]. Similarly the relation between the prevalence and severity of RLS with declining renal function was also different in different studies. Molnar et al[4] found significant relation between RLS and Uremia, where as Winkeleman et al[6] found no relation between RLS and declining renal function.

Drug Therapy of RLS varies according to frequency of symptoms and their association with pain. Dopaminergic agents (Levodopa, Bromocriptine, Pergolide, Pramipexole) other agents such as Opioids, Benzodiazepines, Carbamazepine and Gabapentin were found useful. Iron supplementation helps in resolution of symptoms of RLS. Treatment of renal anemia with erythropoietin and IV Iron reduces the prevalence of severity of RLS. Apart from Pharmacotherapy, clear understanding and counseling regarding sleep hygiene and life style modification helps patients.[1,5]

CONCLUSION

In conclusion, we found the prevalence of RLS was 6% in our study group, common in fifth decade with no predilection to female sex and diabetes as described. The median average blood hemoglobin levels was 11.5 gms/dl and all of them were receiving EPO and parenteral iron supplements indicating that there was no relation between anemia, EPO and parenteral iron supplementation and occurrence of RLS in our group. The median averages of calcium phosphate product, S.PTH, and Vit D levels were in normal range in our patients of RLS, thus indicating no relation between them. Similarly no relation was found between the parameters of adequacy of dialysis such as Kt/V and urea reduction ratio and RLS in our study group.

However in none of the patients the presence of restless legs neither interfered with daily activity nor resulted in psychological stress and it was in a manageable state with counseling on sleep practices and assurance.

REFERENCES

1. Earley CJ. Restless legs syndrome. N Engl J Med 2003;348: 2103-2109.
2. Takaki J, Nishi T, Nangaku M et al. Clinical and Psychological Aspects of Restless legs Syndrome in Uremic Patients on Hemodialysis. Am J Kidney Dis 2003;41:833-839.
3. Seidel VC, Kohnen R, Samheben W, et al. Clinical and biochemical findings in uremic patients with restless legs syndrome. Am J Kidney Dis 1998; 31:324-328.
4. Molnar MZ, Novak M, Ambrus C, et al. Restless legs syndrome in patients after transplantation. Am J Kidney Dis 2005; 45:388-396.
5. Novak M, Mendelssohn D, Shapiro CM, et al. Diagnosis and Management of Sleep Apnea syndrome and Restless legs syndrome in dialysis patients. Seminars in Dialysis 2006; 19:210-216.
6. Winkelman JW, Chertow GM, Lazarus JM. Restless legs syndrome in End stage renal disease. Am J Kidney Dis 1996; 28:372-378.
7. Earley CJ, Connor JR, Beard JH, et al. Abnormalities in CSF concentrations of ferritin and transferin in Restless legs syndrome. Neurology 2000;54: 1698-1700.
8. Allen RP, Barker PB, Wehrl F, et al. MRI Measurements of brain iron in patients with restless legs syndrome. Neurology 2001; 56:263-265.
9. Connor JR, Boyer PJ, Menzies SL, et al. Neuropathological examination suggests impaired brain Iron acquisition in Restless legs syndrome. Neurology 2003; 61: 304-309.
10. Sloand JA, Shelly MA, Feigin A, et al. A double blind, Placebo-controlled trial of intravenous iron dextran therapy in patients with ESRD and restless legs syndrome. Am J Kidney Dis 2004; 43:663-670.
11. Novak M, Mendelssohn D, Shapiro CM, et al. Diagnosis and Management of Sleep Apnea Syndrome and Restless Legs Syndrome in Dialysis Patients. Seminars In Dialysis;19:210–216.

Original Article

Renal Transplantation in Reflux Nephropathy: A Single Centre Experience

K.S.Suthar[1], A.V.Vanikar[1], R.D.Patel[1], K.V.Kanodia[1], S.D. Dave[1], H.L.Trivedi[2], M.R. Gumber[2], H.V. Patel[2], P.R.Modi[3], S.J.Rizvi[3] , S.I. Khemchandani[3]

1. Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, IKDRC-ITS, Ahmedabad, India
2. Department of Nephrology and Transplantation Medicine, IKDRC-ITS, Ahmedabad, India
3. Department of Urology and Transplantation, IKDRC-ITS, Ahmedabad, India

ABSTRACT

Reflux nephropathy leading to end stage renal disease (ESRD) is estimated to affect 7-17% patients, eventually requiring renal transplantation (RTx). However there is increased incidence of posttransplant urinary tract infections (UTI) and rejections in these patients. Stem cell therapy (SCT) holds promises for better outcome. We evaluated RTx outcome in a cohort of reflux nephropathy patients subjected to RTx using SCT and compared them with contemporary RTx controls.

Thirty-three patients transplanted between 2007-'10, belonging to two groups; group-1 (n= 24) who underwent living-donor (LD) RTx using SCT and group-2 with 9 contemporary controls. Patient and graft survival, serum creatinine (SCr), rejection episodes, infections and immunosuppression requirement were compared.

Demographics were comparable in both groups. Mean patient survival at 1 and 5 years in group-1 was 100% and 95.8% vs. 100% and 43.7% in group-2. Death-censored graft survival for the same time intervals was 95.8% in group-1, vs. 100% and 50% respectively in group-2. Mean SCr(mg/dL) was 1.57and 1.34 in group-1 vs. 1.33 and 1.99 in group-2. Incidence and intensity of rejections was lesser in group-1 vs. group-2. There was 4.17% patient loss in group-1 vs. 22.2% in group-2. Recurrent UTI occurred in 37.5% patients in former and 55.5% in later group. There was no difference in UTI incidence with respect to native nephrectomy in both the groups.

RTx using SCT gives better results in reflux nephropathy than contemporary RTx in terms of patient and graft survival and graft function.

KEYWORDS : Vesico-ureteral reflux, Urinary tract infection, Kidney transplant

INTRODUCTION

Reflux nephropathy leading to end stage renal disease (ESRD) is estimated to affect 7-17% patients.[1,2,3] Refluxing systems cause recurrent urinary tract infection (UTI) and scarring ultimately leading to ESRD.[4,5,6] UTI in renal transplant patients pose major health concerns with clinical signs and symptoms ranging from asymptomatic bacteriuria to graft abscess or urosepsis.[7] UTI is the most frequent infection after renal transplantation with an incidence between 10% and 98% and the risk of associated bacteremia close to 12%.[8] Renal transplantation (RTx) is reported to be a good therapeutic option for such patients. Nevertheless, the rates of serious posttransplantation complications that are associated with UTI, such as bacterial septicemia, remain high for patients even in the modern era.[8] There is no clear correlation between graft function with post-transplant UTI. There is inadequate literature regarding status of posttransplant

Address for correspondence : *Dr. Kamlesh S. Suthar, M.D., PDCC, Asst. Prof., Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, G.R. Doshi and K.M. Mehta Institute of Kidney Diseases & Research Centre and Dr. H.L. Trivedi Institute of Transplantation Sciences, Civil Hospital Campus, Asarwa, Ahmedabad, India. E-mail: drksuthar@gmail.com*

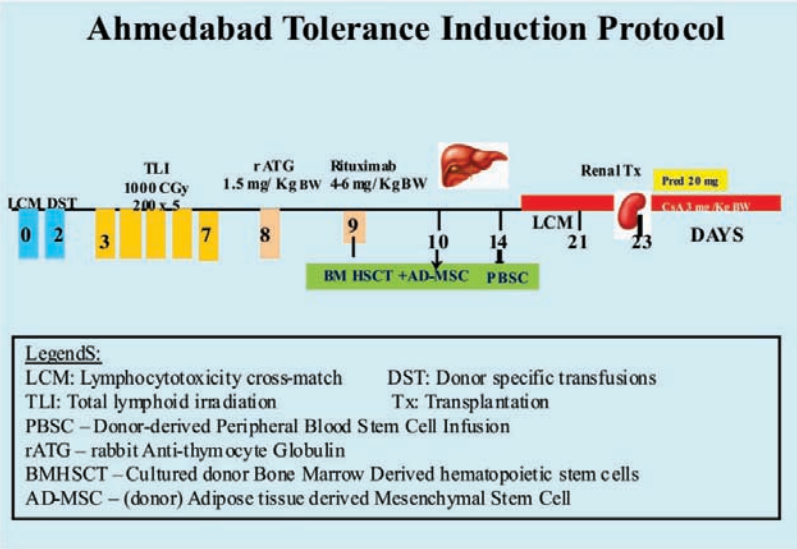


Figure 1: Ahmedabad Tolerance Induction Protocol

reflux.[1] So we carried out retrospective study to evaluate outcome of RTx in a cohort of our patients with reflux nephropathy. We also looked for the association of persistent native refluxing systems with post-transplant UTI in these patients.

MATERIAL AND METHODS

We studied 33 patients transplanted between 2007 and 2010, for ESRD due to the original disease of reflux nephropathy belonging to two groups; group 1 of 24 patients who underwent living-donor (LD) RTx under tolerance induction protocol (TIP) using stem cell therapy (SCT) under non-myeloablative conditioning (Figure-1) and group 2 with 9 contemporary controls who opted out of TIP. Both groups had induction therapy of methyl prednisone (500 mg/day for 3 days) with or without rabbit ATG (rATG) (1.5 mg/kg single dose). TIP patients were administered calcineurin inhibitor (CNI) [cyclosporine 2-3 mg/kg/day, or tacrolimus 0.08 mg/kg/day] and prednisone (10

Table 1: Patient demographics and results

	Group-1 (n=24)	Group-2 (n=9)
Patient Mean Age (Years)	26.4	24.4
Gender (M:F)	20:4	8:1
Donor Mean Age (Years)	48.17	46.7
Donor Gender (M:F)	6:18	7:2
HLA match /6	2.87 ± 0.81	3 ± 0*
Mean Follow-up (Years)	4.01 ± 0.86	3.63 ± 1.42
Present S.Cr (mg/dl)	1.45 ± 0.33	1.3 ± 0.35
UTI	9 (37.5%)	5 (55.6%)
Graft biopsy diagnosis		
ag1t1v1i2	4(16.6%)	0
>ag1t1v1i2	0	4 (44.4%)
ATIN	6 (25%)	2(22%)
cg1t1v1i2	2(8.3%)	0
>cg1t1v1i2	0	1 (11.1%)

*Remarks: * HLA matching was not available in 5 deceased donors.*

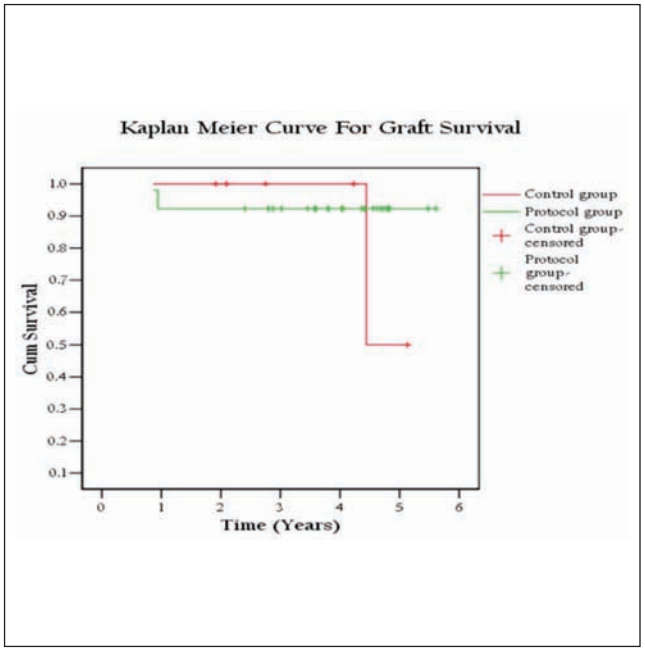


Figure 2: Kaplan Meier curve for patient survival

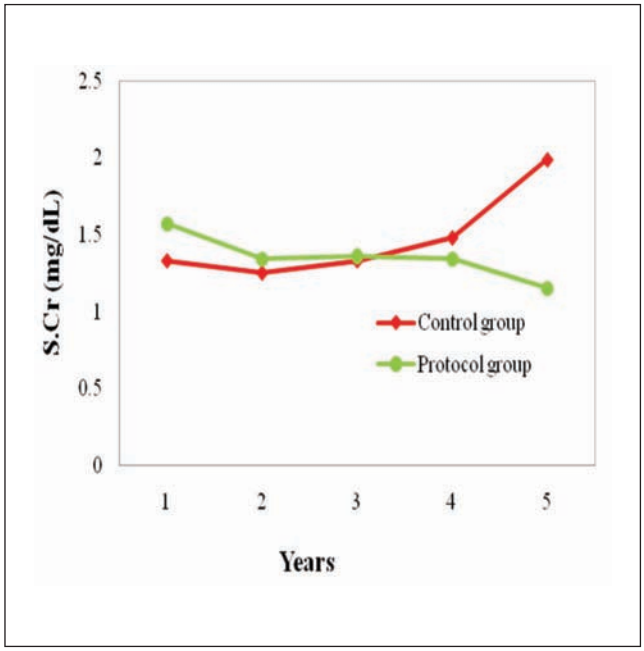


Figure 3: Graft survival in terms of serum creatinine

mg/day) with or without Mycofenolate mofetil (MMF) (0.75 mg/day) or Azathioprine (0.5-1 mg/kg/day). Controls were given our standard triple immunosuppression with little higher dosage as compared to TIP including calcineurin inhibitor (CNI) [cyclosporine 3-5 mg/kg/day, or tacrolimus 0.08 mg/kg/day], prednisone (20 mg/day) and Mycofenolate mofetil (MMF) (1.5-2 mg/day) or Azathioprine (0.5-1 mg/kg/day). Their graft survival, function in terms of serum creatinine (SCr), rejection episodes, infections and immunosuppression requirement were evaluated (Table 1). Patients with symptoms like fever, flank/abdominal pain, dysuria, urinary frequency/urgency/incontinence, malodorous urine, or malaise were investigated for UTI through culture. These UTI were correlated with post-transplant UTI rates, concomitant nephrectomy, or nephroureterecto my with refluxing remnants.

RESULTS

Mean patient age was 26.4 (range:7-48) years in group-1 and 24.4 (range:12-46) years in controls, with 20 (83.3%) males in former and 8 (88.8%) in later. Mean donor age was 48.17 (range:26-65) years with 6 (25%) males in group-1, and 46.7 (range:17-53) years with 7 (77.7%) males in the later. Group-2 also had 55.5% deceased donors. HLA match was comparable in both group. Native nephrectomy was performed in 15 (62.5%) group-1 and 6 (66.7%) group-2 patients. Mean patient survival at 1, 3 and 5 years in group-1 was 100%, 95.8% and 95.8% respectively vs. 100%, 87.5% and 43.7% respectively in group-2 (Figure-2). Death-

Table 2: Post renal transplant UTI incidence

	Group-1 (n=24)		Group-2 (n=9)	
	Total cases	UTI	Total cases	UTI
Native Nephrectomy	15 (62.5%)	5 (33.3%)	6 (66.7%)	5 (83.3%)
No Native Nephrectomy	9 (37.5%)	4 (44.4%)	3 (33.3%)	2(66.7%)

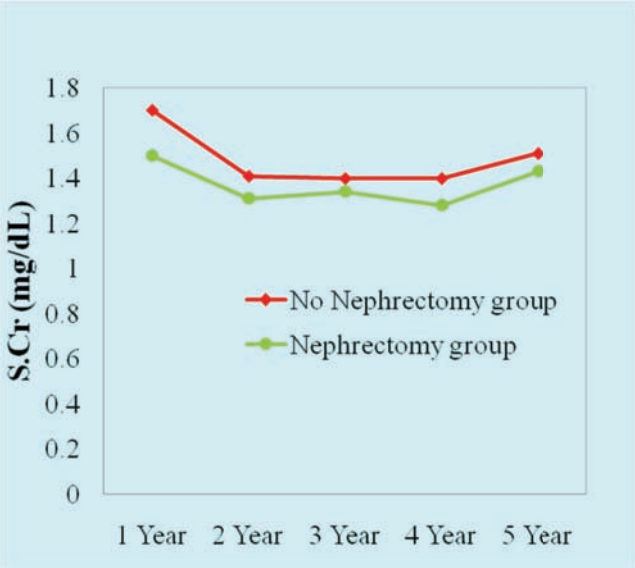


Figure 4: Comparative graft survival in terms of serum creatinine of native nephrectomy Vs non native nephrectomy in Group-1

censored graft survival for the same time intervals was 95.8% in group-1 vs. 100% at 1 and 3 years, and 50% at 5 years respectively in group-2. Mean SCr (mg/dL) for 1, 3 and 5 years were 1.57, 1.36 and 1.34 in group-1 vs. 1.33, 1.33 and 1.99 in group-2(Figure-3). Recurrent UTI rate of 37.5% was reported in group 1 which was lower as compared to 55.5% in group-2, which subsequently responded to antibiotics (Table-2). There was no significant difference in incidence of UTI with respect to native nephrectomy. In native nephrectomy patients, UTI rate was quite higher, 83.3% in group 2 than 33.3% in group 1 and also same in non native nephrectomy patients as it was 66.7% in group 2 compared to 44.4% in group 1. The graft function was better in patients with lower rate of UTI in both groups(Figure-4). Acute Rejections (AR) affected 16.6% in group-1 with AR grade ⩽ ag1at1av1ai2 vs. 44.4% in group-2 patients showing AR grade ⩾ ag1at1av1ai2. Chronic rejections (CR) grade ⩽ cg1 ct1 cv1 ci1 of 8.3% was noted in group-1 vs. 11.1% in group-2 with CR grade ⩾ cg1 ct1 cv1 ci1. We lost 4.17% (1 patient) from group-1 due to septicemia in 2nd year post transplant and 22.2% (2 patients) in group-2 due to septicemia; 1 in 2nd year and 1in the 5th year of transplantation.

DISCUSSION

Rtx in reflux nephropathy is equally safe and effective as in patients with non-urological renal failure. UTI is the most common infection seen after kidney transplantation, although different studies report widely varying incidence rates .[9,10,11] The 58.3% incidence of UTI in this specific renal transplant population is consistent with previously published occurrence rates .[12,13, 14] It is observed that incidence of UTI was lower in group-1 compared to group-2 probably due to lesser use of immunosuppression due to TIP. The effect of early or late UTI after kidney transplantation on graft life and patient mortality has been controversial, with some studies reporting adverse effects of UTI on both parameters whereas other studies did not.[10,14,15] Risk factors for developing UTI after kidney transplantation also have varied among studies. It has been seen that native nephrectomy is not much effective in reducing the UTI contrarily to some other study.[13] However, the observation that most of the infections occurred in patients with persistent refluxing systems has never been previously documented. Further investigations involving a larger cohort or a multicenter analysis may reveal a more statistically significant correlation between refluxing systems and UTI in renal transplant patients. So urinary tract infections must be detected and treated early. Recurrent infections may require long course of antibiotics and even removal of the native kidney with ureter. Detailed urodynamic studies are prerequisite to avoid post Tx reflux and its complications.

CONCLUSION

Transplantation is a viable option for reflux nephropathy however recurrent UTI is not well-controlled after transplantation in such patients in spite of native nephrectomy. TIP with SCT gives better results in these patients in terms of decreased incidence of UTI, rejections and graft/ patient loss and hence should be encouraged.

REFERENCES

1. Fenton S, Desmeules M, Copleston P, et al. Renal replacement therapy in Canada: A report from the Canadian Organ Replacement Register. Am J Kidney Dis. 1995;25:134-150.
2. Disney APS. Reflux nephropathy in Australia and New Zealand: prevalence, incidence and management – 1975-1989. In: Bailey RR, ed. Proceedings of the Second C. J. Hodson Symposium on Reflux Nephropathy, Christchurch, New Zealand, 1991;53-56.
3. Wing AJ. Causes of end-stage renal failure. In: Cameron S, Davison AM, Gru¨nfeld J-P, Kerr D, Ritz E, eds. Oxford Textbook of Clinical Nephrology. Oxford, UK: Oxford University Press, 1992;1227-1236.
4. Ransley PG, Risdon RA. Reflux and renal scarring. Br J Radiol: 14 (Suppl.):1978;1.
5. Roberts JA. Pathogenesis of pyelonephritis. J Urol: 1983;129:1102-1106.
6. Bailey RR. The relationship of vesico-ureteric reflux to urinary tract infection and chronic pyelonephritis-reflux nephropathy. Clin Nephrol. 1973;1: 132-141.
7. Arnow PM. Prevention of bacterial infection in the transplant recipient. The role of selective bowel decontamination (Review, 63 refs). Infect Dis Clin North Am:1995;9: 849-862.
8. Abbott KC, Napier MG, Agodoa LY. Hospitalizations for bacterial septicemia in patients with end stage renal disease due to diabetes on the renal transplant waiting list. J Nephrol 2002;15:248-254.
9. Abbott KC, Swanson SJ, Richter ER, et.al. Late urinary tract infection after renal transplantation in the United States. *Am J Kidney Dis* .2004;44:353-362.
10. Takai K, Tollemar J, Wilczek HE, et al. Urinary tract infections following renal transplantation. *Clin Transplant.* 1998; 12: 19-23.
11. Goya N, Tanabe K, Iguchi Y, et.al. Prevalence of urinary tract infection during outpatient follow-up after renal transplantation. *Infection.* 1997;25:101-105.
12. Burgos-Revilla FJ, Pascual-Santos J, Marcen-Letosa R, et al. Stenosis in kidney transplantation. Actas Urol Esp: 1999; 23: 95-104.
13. Casale P, Grady RW, Mitchell ME, et al. Recurrent urinary tract infection in the post-transplant reflux nephropathy patient: is reflux in the native ureter the culprit? Pediatr Transplantation. 2005;9:324-327.
14. Muller V, Becker G, Delfs M, et al. Do urinary tract infections trigger chronic kidney transplant rejection in man? J Urol. 1998;159:1826-1829.
15. Giral M, Pascuariello G, Karam G, et.al. Acute graft pyelonephritis and long-term kidney allograft outcome. Kidney Int. 2002; 61:1880-1886.

Case Report

Emphysematous Pyelonephritis with Human Immunodeficiency Virus Infection

K.V. Kanodia[1], A.V. Vanikar[1], S.P. Gandhi[2], P. R. Modi[3], H. L. Trivedi[4], R. D. Patel[1], K. S. Suthar[1], L. K. Nigam[1]

1. Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, IKDRC-ITS, Ahmedabad, India
2. Department of Radiology, IKDRC-ITS, Ahmedabad, India
3. Department of Urology and Transplantation, IKDRC-ITS, Ahmedabad, India
4. Department of Nephrology and Transplantation Medicine, IKDRC-ITS, Ahmedabad, India

ABSTRACT

Emphysematous pyelonephritis is a rare life-threatening necrotizing infection of renal parenchyma and perirenal tissue associated with gas-forming bacteria, usually occurring in diabetic patients. We report a successfully treated case of emphysematous pyelonephritis in a 42 year old woman with human immunodeficiency virus infection. This case highlights the importance of considering the diagnosis in people with diabetes in the general population who are not routinely investigated for HIV infection especially since they have a higher risk of developing emphysematous pyelonephritis in addition to other co-morbid infections.

KEYWORDS

Diabetes mellitus, Emphysematous pyelonephritis, HIV infection

INTRODUCTION

Emphysematous pyelonephritis (EPN) is a rare life-threatening necrotizing infection of renal parenchyma and perirenal tissue with gas-forming bacteria, usually occurring in patients with diabetes.[1-7] We report a rare case of successfully treated EPN in a patient with human immunodeficiency virus (HIV) which has been rarely reported.

CASE REPORT

A 42-year-old female, known case of type 2 diabetic on oral hypoglycemic treatment for last 2 years, presented in emergency with acute left flank pain of 4 days duration. She had a history of low-grade fever for 5 days. On examination, her blood pressure was 130/90 mmHg and pulse rate was 100/minute with tenderness in her left flank region. On investigation, her hemoglobin was 9.1 gm/dL; total leukocyte count, 1.86×10^3/μL with 87% neutrophils; platelet count, 2.36×10^5/μL, random blood sugar 545 mg/dL; serum acetone was absent, serum creatinine, 1.86 mg/dL and serum potassium 3.6 mEq/L. She was reactive for HIV-1 by enzyme-linked immunosorbent assay and Western blot. A routine urine examination by dipstick showed trace albumin; microscopic examination revealed 40-42 pus cells/high power field and no organism was isolated on culture. Her blood culture was negative. Ultrasonography of abdomen showed features of left kidney (LK) EPN. Computed tomography (CT) (Figure 1) showed an enlarged and edematous LK with multiple pockets of air in the pelvi-calyceal system, renal parenchyma, perinephric region and ureter, suggestive of EPN Class 3A. She was treated with intravenous antibiotics, insulin and percutaneous nephrostomy (PCN). However, PCN did not relieve her symptoms and an emergency nephrectomy of her LK

Address for correspondence : *Dr. Kamal V. Kanodia, M.D. (Path & Bact.), P.D.C.C.(Renal and Transplant Path), Professor, Dept. of Pathology, Lab Medicine, Transfusion Services and Immunohematology, G.R. Doshi and K.M. Mehta Institute of Kidney Diseases & Research Centre and Dr. H.L. Trivedi Institute of Transplantation Sciences, Civil Hospital Campus, Asarwa, Ahmedabad, India. E-mail : kamalkanodia@yahoo.com*

was carried out the next day. The specimen was sent for histopathological examination.

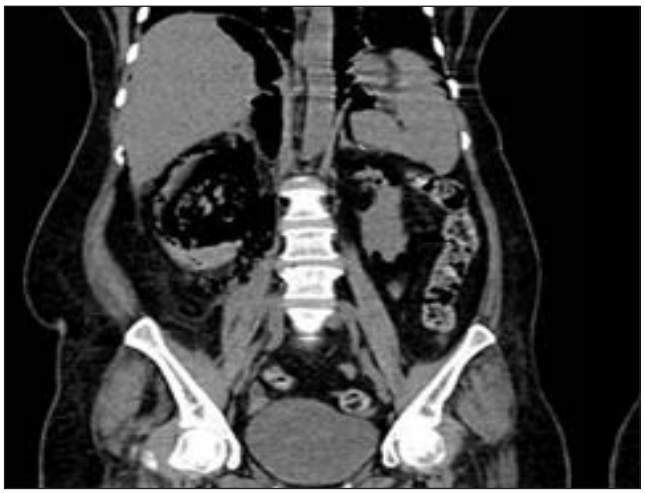


Figure 1: Multiple air collections in renal parenchyma and perinephric region suggestive of emphysematous pyelonephritis (computed tomography).

On gross examination LK weighed 480 gram and measured 14×9×7 ccm. Outer surface showed large areas of hemorrhage and necrosis. Cut surface showed patchy areas of hemorrhage and necrosis in the cortex and medulla. Microscopy showed extensive corticomedullary necrosis, focal hemorrhage and overlying marked infiltration with neutrophils, cell debris, few foamy macrophages and lymphocytes in the parenchyma (Figure 2). There were very few viable glomeruli that showed mild mesangial prominence with fairly open capillary lumina lined by membranes of normal thickness. Bowman capsules were unremarkable. Tubules revelead mild degenerative changes. Interstitium was mildly prominent for focal edema and leucocytic infiltration. Blood vessels were unremarkable.

She was discharged 2 weeks after surgery. On follow-up of 3 months she had no complaints and is on anti-retroviral therapy.

DISCUSSION

First described by Kelly and Mac Cullum in 1898, EPN is a necrotizing parenchymal and perirenal infection commonly due to Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas, Enterobacter, and rarely due to yeasts.[2] It is commonly

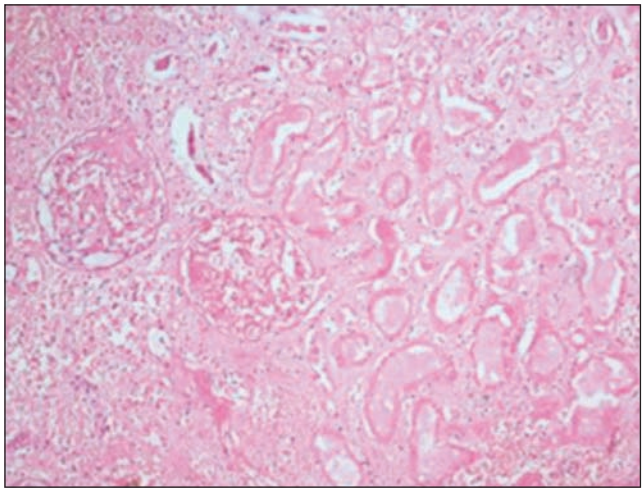


Figure 2: Cortical necrosis, focal hemorrhage and overlying marked infiltration with neutrophils, cell debris and lymphocytes (H&E, x100).

seen in patients with diabetes, especially with poor glycemic control. Other predisposing factors are urinary tract obstruction, polycystic kidneys, end stage renal disease and immunosuppression. Women are affected twice as often as men. Patients usually present with chills, fever, flank pain, lethargy, confusion or septic shock. CT is the investigation of choice for diagnosis, staging and in assessing the response to therapy.[1,8]

Huang and Tseng categorized EPN in four classes based on CT-findings: Class 1– Gas confined to the collecting system; Class 2 – Gas confined to the renal parenchyma alone ; Class 3A – Perinephric extension of gas or abscess ; Class 3B – Extension of gas to pararenal space (beyond the Gerota fascia) ; Class 4 – Bilateral EPN or EPN in solitary kidney ; Class 1/2 EPN usually respond to PCN and antibiotics. In EPN class 3/4 without sign of organ dysfunction, antibiotics with percutaneous drainage is recommended. Nephrectomy should be done in patients with bilateral EPN and in class 3/4 EPN with $\geqslant$ 2 risk factors like thrombocytopenia, acute renal failure, altered sensorium or shock. Huang and T Seng analyzed the composition of in situ gas and showed that it comprised of hydrogen (12.8%), carbon dioxide (14.4%), nitrogen (63.5%) and oxygen (6.3%).[1]

EPN has been reported in renal transplant recipients and in patients with polycystic kidney disease.[9-15] However it has been rarely reported in coexisting HIV

infections.[16] The age, sex, site of infection, serum urea nitrogen level, and blood glucose level were not the prognostic factors, and the best combination of characteristics of EPN with favorable outcome was that of a patient with non-obstructive unilateral disease receiving combined medical and surgical treatment within a short interval of symptom onset.[17] Our patient had poor prognosticators including CT class 3A, diabetes and HIV, yet recovered fully. We would like to emphasize that patients presenting with EPN should be considered for screening for HIV for timely surgical management.

CONCLUSIONS

This case highlights that EPN, HIV infection and diabetes could exist simultaneously and early nephrectomy is advisable in such cases. As extensive EPN is life-threatening, early diagnosis and aggressive management of such patients can improve their long-term prognosis.

Acknowledgements

We acknowledge Miss Yogita for her contribution in writing the manuscript and technician Mr Babubhai and his team for preparation of histopathological slides.

REFERENCES

1. Huang JJ, Tseng CC. Emphysematous pyelonephritis: Clinicoradiological classification, management, prognosis, and pathogenesis. Arch Intern Med 2000; 160:797-805.
2. Kelly HA, Mac CullumWG. Pneumaturia. JAMA 1898; 31:375-381.
3. Hui L, Tokeshi J. Emphysematous pyelonephritis. Hawaii Med J 2000; 59:336-337.
4. Roy C, Pfleger DD, Tuchmann CM, et al. Emphysematous pyelitis: findings in five patients. Radiology 2001; 218:647-650.
5. Gupta P, Gupta R, Jandial K, et al. Emphysematous pyelonephritis in the setting of diabetes mellitus. J Assoc Physicians India 2011; 59:119-120.
6. Shokeir AA, El-Azab M, Mohsen T, et al. Emphysematous pyelonephritis: a 15- year experience with 20 cases. Urology 1997; 49:343-346.
7. Chen KW, Huang JJ, Wu MH. Gas in hepatic veins: a rare and critical presentation of emphysematous pyelonephritis. J Urol 1994; 151:125-126.
8. Tseng CC, Wu JJ, Wang MC, et al. Host and bacterial virulence factors predisposing to emphysematous pyelonephritis. Am J Kidney Dis 2005; 46:432-439.
9. Ortiz A, Petkov V, Urbano J, et al. Emphysematous pyelonephritis in dialysis patient after embolization of failed allograft. Urology 2007; 70:372. e17-19.
10. Baliga KV, Narula AS, Sharma A, et al. Successful medical treatment of emphysematous pyelonephritis in a renal allograft recipient. Ren Fail 2007; 29:755-758.
11. Chuang YW, Chen CH, Cheng CH, et al. Severe emphysematous pyelonephritis in a renal allograft: successful treatment with percutaneous drainage and antibiotics. Clin Nephrol 2007; 68:42-46.
12. Arai S, Makino T, Okugi H, et al. A case of emphysematous pyelonephritis in a renal allograft. Transplantation 2006;81:296-297.
13. Kolla PK, Madhav D, Reddy S, et al. Clinical profile and outcome of conservatively managed emphysematous pyelonephritis. ISRN Urol. 2012; 2012:931982.
14. Mohsin N, Budruddin M, Lala S, et al. Emphysematous pyelonephritis: a case report series of four patients with review of literature. Ren Fail 2009; 31:597-601.
15. Lakshminarayana G, Mathew A, Rajesh R, et al. Bilateral emphysematous pyelonephritis in autosomal dominant polycystic kidney disease. Indian J Nephrol 2012; 22(2):136-138.
16. Mohamed Ashif PA, Sandeep P, Sasidharan PK. Emphysematous pyelonephritis in a patient infected with the human immunodeficiency virus. Saudi J Kidney Dis Transpl 2012; 23:1046-1050.
17. Ahmad M, Dakshinamurty KV. Emphysematous renal tract disease due to Aspergillus fumigatus. J Assoc Physicians India 2004; 52:495-497.

Letter to Editor

Misplacement of Hemodialysis Permcatheter into Anterior Mediastinum Following Brachiocephalic Vein Perforation

V.B. Kute[1], D.K.Yadav[1], P.R. Shah[1], M. R. Gumber[1], H.V. Patel[1], A.V. Vanikar[2], S.Gandhi[3], H.L.Trivedi[1]

1. Department of Nephrology and Transplantation Medicine, IKDRC-ITS, Ahmedabad, India
2. Department of Pathology, Laboratory Medicine, Transfusion Services and Immunohematology, IKDRC-ITS, Ahmedabad, India
3. Department of Radiology, IKDRC-ITS, Ahmedabad, India

KEYWORDS : Hemodialysis, Permcatheter, Complication

Dear Sir,

Hemodialysis Permcatheter is one of the commonly used procedures done in nephrology practice. All should be aware of possible vascular complications during catheter insertion for early diagnosis and treatment to prevent morbidity and mortality.

A 42-year-old woman with end-stage renal disease secondary to hypertensive nephropathy was on regular hemodialysis thrice per week initially via right internal jugular vein (IJV) catheter and there-after via left arteriovenous(AV) fistula for last six months. She presented to us with AV-fistula failure and was prepared for permcath insertion. Doppler evaluation of her neck vessels was suggestive of partial thrombosis of right IJV and right subclavian veins.

She was undertaken for placement of a cuffed MAHURKAR™* MAXID™ 14.5Fr. Dual Lumen Permcath in left IJV. For the placement of the Permcath, the left IJV was punctured using doppler guidance and the guidewire was then introduced. There was no resistance while inserting the guidewire and permcath was then positioned using the modified Seldinger technique. There was no complication during procedure.

There was mild resistance on aspiration of blood and injection of saline via venous port with normal flow in arterial ports after placement. Post procedure Chest X-ray was performed to confirm the position of the catheter (Figure 1). She was undertaken for haemodialysis six hours after procedure via this new catheter. However, at this time, blood could not be aspirated from either port of the catheter and on injection of saline through the catheter, she had pain at right shoulder. CT scan of the thorax was performed which showed permcatheter tip outside the vascular lumen as a result of puncture of left brachiocephalic vein with subsequent migration into the anterior mediastinum (Figure 2A & 2B). The permcatheter was removed by pulling out uneventfully with expert help from Department of Cardiothoracic and Vascular surgery. There was no complication post procedure and a new MAHURKAR™* Dual Lumen Catheters inserted uneventfully, with real-time ultrasound guidance 1 week later and new AV-fistula was made.

Central veins cannulation especially that of neck for placement of haemodialysis catheters is a common procedure in the management of patients with end-stage renal failure. The right IJV is the preferred site for central venous catheter placement because of lowest complication rate.[1] Cannulating the left IJV has a higher chance of complication because the left

Address for correspondence : *Dr. Vivek Kute, M.D., DM Nephrology (Gold Medalist), FASN, Assistant Professor, Department of Nephrology and Clinical Transplantation, G.R. Doshi and K.M. Mehta Institute of Kidney Diseases & Research Centre and Dr. H.L. Trivedi Institute of Transplantation Sciences, Civil Hospital Campus, Asarwa, Ahmedabad, India. E-mail: drvivekkute@rediffmail.com*

brachiocephalic vein lies more transverse, thus making the catheter more prone to angulation and perforation. We had to place permcath on left side because there was partial thrombosis of right IJV.[1]

Accidental puncture and perforation of blood vessels or injury to nearby structures is a common complication during catheter placement. Denys and Uretsky et al reported rate of carotid artery puncture around 8% in patients undergoing internal jugular vein cannulation.[2]

Ultrasound guidance is helpful in correct central venous catheter placement with success rate for the IJV catheterization procedure of 96.7% compared to 82% without ultrasound guidance.[3]

Schummer et al.[4] reported a case of perforation of superior vena cava (SVC) following hemodialysis catheter placement into left IJV.

Our patient presented with right shoulder pain possibly due to irritation of phrenic nerve by dialysis catheter because of the close proximity of the right phrenic nerve to SVC.[5]

We would recommend that following placement of any central venous catheter, straight chest radiographs should be performed in all patients to ensure correct catheter positioning. If there are radiographic findings of note, catheter dysfunction and/or chest , back or shoulder pain after central venous infusion, a CT

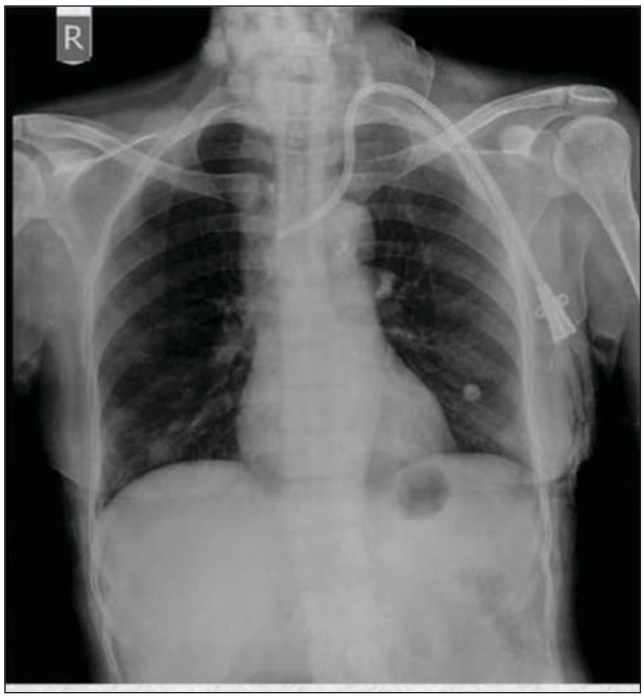


Figure 1: Post procedure Chest x-ray performed to confirm the position of the catheter

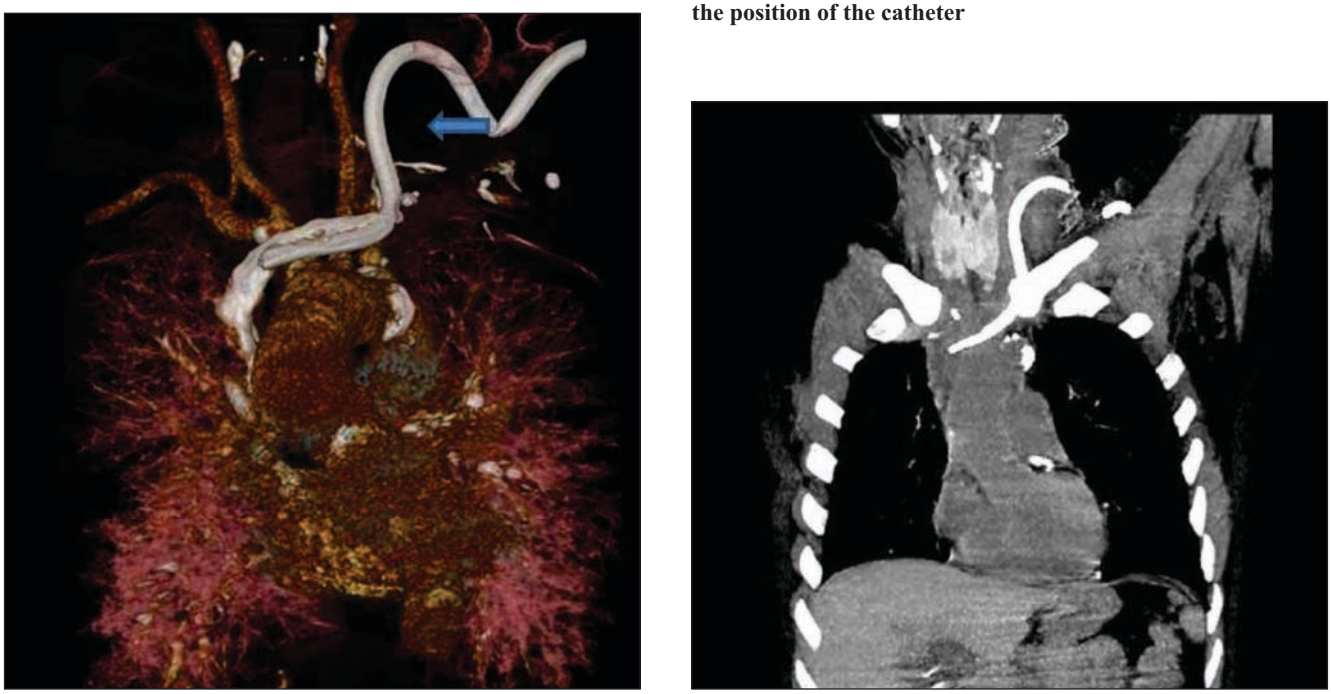


Figure 2A and 2B: CT scan of the thorax was performed that showed permcatheter tip outside the vascular lumen as a result of puncture of left brachiocephalic vein with subsequent migration into the anterior mediastinum

scan of the thorax or venography should be performed to assess the position of the catheter..

Fluoroscopic guidance during intravenous haemodialysis catheter placement can assess the position of the catheter, any vascular abnormalities or stenosis and therefore prevent misplacement and should be used specially in difficult case like catheter placement on left IJV. Early diagnosis and treatment is crucial to prevent morbidity and mortality.

Declaration of interest: The authors report no conflicts of interest.

REFERENCES

1. Schillinger F, Schillinger D, Montagnac R, et al. Post catheterisation vein stenosis in haemodialysis: comparative angiographic study of 50 subclavian and 50 internal jugular accesses. Nephrol Dial Transplant 1991; 6:722-724.
2. Denys BG, Uretsky BF, Reddy PS. Ultrasound-assisted cannulation of the internal jugular vein. A prospective comparison to the external landmark-guided technique. Circulation 1993; 87:1557-1562.
3. Farrell J, Gellens M. Ultrasound-guided cannulation versus the landmark-guided technique for acute haemodialysis access. Nephrol Dial Transplant 1997; 12:1234-1237.
4. Schummer W, Schummer C, Fritz H. Perforation of the superior vena cava due to unrecognized stenosis. Case report of a lethal complication of central venous catheterization. Anaesthesist 2001; 50:772-777.
5. Mir S, Serdaroglu E. An elevated hemidiaphragm 3 months after internal jugular vein hemodialysis catheter placement. Semin Dial 2003; 16:281-283.

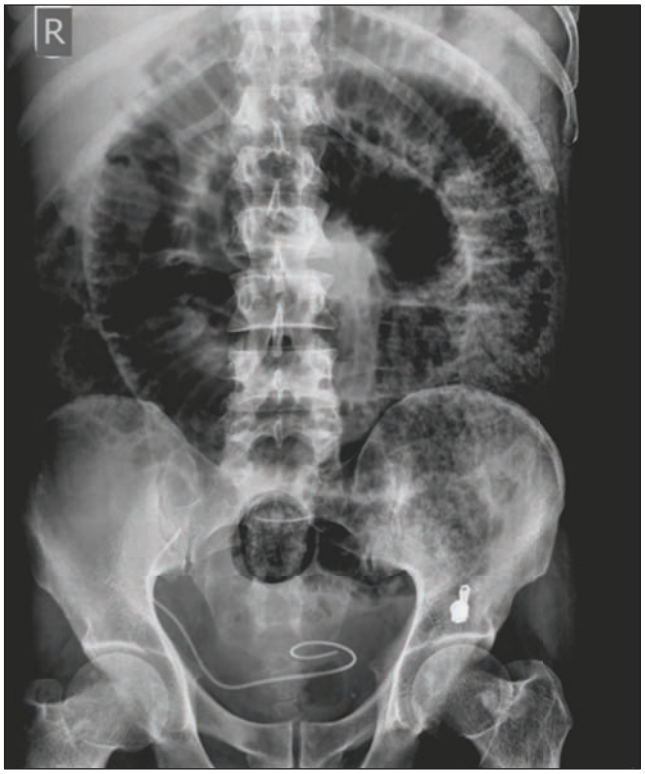
Image

Under-Recognized Adverse Effect of Mycophenolate Mofetil

M. P. Patel[1], V.B. Kute[1], S. Gandhi[2], M.R. Gumber[1]

1. Department of Nephrology and Transplantation Medicine, IKDRC-ITS, Ahmedabad, India
2. Department of Radiology, IKDRC-ITS, Ahmedabad, India

A 42-year-old male with end stage renal disease underwent successful live donor renal transplantation. His serum creatinine was 0.9 mg/dL on 4th post operative day (POD). His immunosuppressive regimen consisted of oral prednisone, tacrolimus and mycophenolate mofetil (MMF) (1.5 gram/day). Oral feeds were started on 2nd POD and he regained normal bowel habits by 4th POD. However he developed diffuse pain and distention of abdomen followed by one episode of vomiting on 10th POD. He was afebrile, but anxious having tachycardia and hypertension. On physical examination, there was absence of peristalsis with generalized abdominal distention and diffuse tenderness but no signs of peritoneal irritation. The investigations were unremarkable (complete blood count, renal function tests, serum electrolytes, Liver function tests). Plain abdominal radiography showed severely dilated loops of small bowel (Figure 1) and ultrasonography of abdomen revealed dilated bowel loops with absence of peristalsis. No other abnormality was reported. So MMF was stopped considering it as a culprit for paralytic ileus. He responded well to conservative management with nasogastric suction and decompression of bowel with intravenous fluid support. After regaining normal peristalsis in next 24 hours, oral feeds were started and MMF was switched over to Azathioprine.



Though paralytic ileus is a common post-operative complication, in this patient it developed after regaining bowel movements in the post-operative period, so it's unlikely to be related to surgery. After excluding the possibility of mechanical obstruction, MMF administration is the most likely etiology for paralytic ileus in view of significant gastrointestinal side effect profile.[1] Various gastrointestinal complications of MMF like vomiting, diarrhea, abdominal pain, dyspepsia and gastritis have been described but presentation of paralytic ileus with acute abdomen has not been reported. Our case highlights the unusual gastrointestinal complication of MMF and warrants vigilance in renal transplant recipient.

REFERENCE

1. Behrend M. Adverse gastrointestinal effects of mycophenolate mofetil: aetiology, incidence and management. Drug Saf. 2001; 24: 645-663.

Address for correspondence : *Dr. Vivek Kute, M.D., DM Nephrology (Gold Medalist), FASN, Assistant Professor, Department of Nephrology and Clinical Transplantation, G.R. Doshi and K.M. Mehta Institute of Kidney Diseases & Research Centre and Dr. H.L. Trivedi Institute of Transplantation Sciences, Civil Hospital Campus, Asarwa, Ahmedabad, India. E-mail: drvivekkute@rediffmail.com*

In Memorium : CARL GROTH (1933- 2014)

We lost Carl Groth, Professor Emeritus of Transplantation Surgery at Karolinska Institutet, Stockholm, Sweden on the late night of 16th February, 2014.

Carl Groth was born in Helsinki, Finland in 1933. He graduated from Medical School at the Karolinska Institutet where he received his M.D. in 1961 and Ph.D. in 1965. He was trained in liver transplantation under Prof. T.E. Starzl from 1967 to 1972 at Denver. While in Denver, Groth also pioneered a splenic transplantation in a patient with haemophilia in 1969. Subsequently, working in a dog model in 1974, he established that transplantation of any lymphatic tissue can correct haemophilia. In 1973 Groth became the Chief of Transplantation Surgery at Huddinge Hospital in Stockholm and Professor of Transplantation Surgery at the Karolinska Institutet in 1984. He retired in 2000. His lifetime work has focused on clinical kidney, pancreas, liver, and islet transplantation, and on research in xenotransplantation. The first pancreas (1974), bone marrow (1975), liver (1984), and islet cell transplantation (1998) in Sweden were carried out by Groth and his co-workers.

Groth was particularly interested in immunosuppressive agents, and he was the chief investigator for several early, multinational patient studies with cyclosporine, tacrolimus, mycophenolate mofetil and sirolimus. Being interested in transplantation for metabolic diseases, Groth showed that transplantation of isolated hepatocytes could correct metabolic deficiencies in rats in 1976. In the clinical settings, he found that Gaucher's disease could be cured by bone marrow transplantation in 1985.

Groth's group has been engaged in pioneering work regarding xenotransplantation. In the early 1990-ies the group performed a pilot trial with pig-to-man islet transplantation, which resulted in a number of important observations, including the first finding of surviving pig cells in the human body in 1994.

Groth was the President of the Transplantation Society from 2001 to 2002. He was the founding President of the Scandinavian Transplant Society in 1983, the International Pancreas and Islet Transplant Association in 1993, and the International Xenotransplantation Association in 1998. H e was an Honorary Fellow of the Royal College of Surgeons of England (1989), the American Surgical Association (1992), the American Society of Transplant Surgeons (1997), and the International Xenotransplantation Association (2005). He was a member of the American Philosophical Society (2004), the oldest scientific Society in the USA (founded 1743) and member of the jury of Maharshi Sushruta Gnyaanpeeth Sanmaan (the highest honor given by an IKDRC-ITS in India from 2007 till his demise. Groth was a member of the Nobel Assembly at the Karolinska Institutet from 1986 to 1999 and Chairman of the Assembly in 1998. In 1998 he was awarded the King's Medal for “eminent accomplishment in transplantation surgery, internationally and in Sweden”. He was the recipient of Reuterskiöld Award 1995 (the foremost surgical Award in Sweden), the Medawar Prize 2006 (the foremost international Award in Transplantation), and the ASTS-

Roche Pioneer award (the highest honour bestowed by the American Society of Transplant Surgeons, 2008). He was honored with Maharshi Sushruta Gnyaanpeeth Sanmaan given by IKDRC-ITS, India in 2006.

Groth was a Visiting Professor at the University of Pittsburgh Medical School, (2005-2006), Central South University, (2006-2010) in Changsha, China for transplantation of pig islets for diabetes. He was a Member of the WHO Expert Advisory Panel on Human Cell, Tissue and Organ Transplantation (2005-2012). Since 2007 he was the Editor-in-Chief of the journal Xenotransplantation.

His bibliography includes more than 700 scientific articles and some 40 books chapters. He was given invited lectures on at least 250 occasions in 39 different countries.

His association with us started in 2006 when he was awarded with Maharshi Sushruta Gnyaanpeeth Sanmaan in Ahmedabad. In the following year in 2007, he inaugurated the new building of transplantation at the institute. He was one of the key contributors in the development of liver transplantation at IKDRC-ITS, Ahmedabad. Today the institute is proud to proclaim that we have performed 76 liver transplants so far!

We have lost a caring friend who will be remembered by generations of transplanters and patients alike from this part of the world.

RESPONSE FROM MRS. GROTH

Dear Professor Trivedi,

Thank you so much for your very kind mail. My husband was very impressed by your work and he often talked about your achievements. Thank you for the photos - some good friends can be seen.

My husband and myself had a good life for 55 years, 3 children and 9 grandchildren, so we were rich!

With best wishes,

Birgit Groth

Manuscript Submission Guidelines

Scope of the Journal:

This journal plans to accept manuscripts related to any branch/ subspecialty of Medicine which throws light on understanding of etiopathogenesis of a disease/ disorder including immune dysregulation and will cover the management including medical/ paramedical aspects and recent developments like transplantation/ replacement/ cell therapy.

Manuscripts submitted should not have been published earlier or be under simultaneous consideration for publication by any other journal. Violation may lead to retraction of the published article by the Journal and other actions as deemed necessary by the Editorial Board. All articles (including invited articles) will be peer-reviewed, and accepted articles will be edited as per the Journal's style. Accepted manuscripts will become the permanent property of the Journal and may not be reproduced, in whole or in part, without the written permission of the Editor.

Studies involving intervention in human subjects or animals should have received the approval of the institutional ethics committee. A statement to this effect and that an informed consent was obtained from participating human subjects must be included in the manuscript text. If the institution has no formal ethics committee, a statement by the authors that the terms of the Helsinki Agreement were adhered to will be necessary.

Manuscripts must conform to the instructions given below:

Submission of Manuscripts:

All manuscripts must be submitted to ?Web site. Authors are requested to submit the text, tables, and figures in electronic form to this address.

By submitting a manuscript, the author agrees to the following:

1. The work is original and free from plagiarism.
2. It has not been submitted for publication/is not under consideration for publication in any other Journal.
3. All authors are aware of the order of authorship. The corresponding author shall be solely responsible in case disputes arise.
4. Once published, copyright of manuscript shall stand transferred to the Journal.
5. Conflict of interest' if any, must be explicitly stated at the end of the manuscript.

General Information:

Original Articles/ Research: Full-length papers that address research issues with exhaustive study design and methodology. The entire manuscript should not exceed 3000 words and 50 references. Abstract should not exceed 250 words.

Brief Communications should not contain more than 2000 words (including abstract and references). In addition, 2 figures and/or tables will be allowed.

Review articles will be solicited by the editors. The length of review articles should not exceed 3500 words, 4 images/ figures and 4 tables. References will have no limit.

Case reports should be limited to 1500 words, should not have more than 4 authors and should not contain over 2 illustrations and 10 references.

Letters to the editor containing interesting observations and comments on the articles published in the journal in previous issues of the same year / immediate preceding issue are welcome for publication. Due care should be taken in the formation of the letter to avoid any misunderstanding. The replies to such letters will also be published as and when received from the authors. The letter and the reply

should not exceed 500 words and should not contain more than 5 references.

Images: Any interesting image (maximum 2 figures) including pathology, radiology, skin lesions, surgical techniques or any interesting clinical image will be welcome. No abstract/ reference is required. Image should be accompanied by brief relevant description not exceeding 150 words.

The manuscripts will be forwarded to an associate editor and two reviewers. Manuscripts that are outside the range of interest of readership or that fail to satisfy the technical requirements will be rejected and promptly returned to authors without further review. Manuscripts returned to authors for modification should be returned to the editorial office as early as possible, but not later than 2 months, failing which the article will not considered for publication. Subsequently fresh submission will be required. The editorial board reserves the right to assess suitability of the language, modify the letters and text, and improve the photographs and illustrations to enhance clarity of expression and presentation without affecting the message and meaning being conveyed by the article.

Manuscript Preparation: The manuscript be typed using 'Times New Roman' fonts, size 11, in double space throughout.

Title page: This should include (i) complete manuscript title (ii) all authors' full names listed as first name, middle name, last name with their highest academic degrees and affiliations (iii) name and address of the corresponding author along with fax, telephone number and e mail address (iv) Financial disclosures. The title should not exceed 150 characters including spaces between words and abbreviated title of not more than 50 characters. Do not use abbreviations in the title.

Abstract: It should be structured as background, material and methods, results and conclusions and should not exceed 250 words and 3 to 5 key words may be included.

Manuscript body: Order may be followed as:

- Introduction
- Methods
- Results
- Discussion
- Conclusion
- Acknowledgements
- References
-Tables
- Figure legends.

Abbreviations should be defined at the first mention in the text, each table and figures.

Tables: Type double-spaced on separate sheets; each table should have a title and be numbered in the order of appearance in the text. Use superscript letters to indicate footnotes typed at the bottom of the table.

Figures: High-resolution photographs of all figures including graphs, black and white light and electron micrographs and color photographs, must be submitted. The use of color illustrations is encouraged, but authors should contact the editor prior to their preparation for advice and assistance. All figures should be clearly labeled. Photomicrographs should be sized to fit one column (8cm) or two columns (17cm); the maximum plate size is 17 x 22 cm. Figures and tables must be cited in parenthesis at appropriate places.

Legends: Legends should state degree of magnification or scale bars to be used on the photograph. Graphs must be of professional quality. Computer generated graphs should be of laser quality. High contrast prints of Xrays/ CT/MRI/ PET scans / photographs and electron micrographs are essential.

All weights and measures must be given in metric units. Avoid use of full stops in the middle of abbreviations (ECG, not E.C.G). Use generic names of drugs. Define unusual abbreviations with the first use in the body of the manuscript. Text footnotes should be typed on a separate page.

References: References should be typed on a separate page and numbered in the order of appearance in the

text. Reference number in the text should be typed as superscript and not in the brackets. For references, the names of first 3 authors should be mentioned followed by using "et al" for more than 3 authors. Citations of unpublished observations or personal communications should be placed in the text in parenthesis only and should not be referenced. These may be mentioned as shown in the example below:

Trivedi H, Shah VR, Vanikar AV, et al. High-dose peripheral blood stem cell infusion: a strategy to induce donor-specific hyporesponsiveness to allografts in pediatric renal transplant recipients. Pediatric Transplantation 2002; 6:63-68.

For references of chapters in Book(s): This may be : Author(s), chapter title, book title, edition, editor(s), city of publication and publishers, year, page.

Example: Broune WE. The immunobiology of different types of renal allograft rejection, in Contemporary issues in Nephrology, Renal transplantation, ed. Milford EL, Brenner BM, Stein JH, New York. Churchill, 1989 p 45-96.

Checklist for the Manuscript

1. Include the original typed manuscript
2. Send high-resolution photographs of figures (300 DPI JPG/ TIF scanned image of the figures; low resolution files of figures i.e. less than 300 DPI are not good for printing) to be included along with each manuscript.
3. Include a cover letter containing a copyright transfer statement.
4. Include all authors personal signatures on the statement that each author has approved the final version of the manuscript and takes public responsibility for the work, including an undertaking that the manuscript has not been submitted for publication in any other journal and if accepted for publication in "Organ dysfunction and Management" it will not be published in whole or in part without the editor's written permission. The declaration by authors should also state that all clinical investigations described in the manuscript have been undertaken in accordance with the guidelines proposed in the Declaration of Helsinki.
5. Designate a corresponding author and provide a telephone number, fax number, email and mailing address.

Address for Correspondence and Manuscript Submission

SMT. GULABBEN RASIKLAL DOSHI AND SMT. KAMLABEN MAFATLAL MEHTA
INSTITUTE OF KIDNEY DISEASES AND RESEARCH CENTRE
DR. H.L. TRIVEDI INSTITUTE OF TRANSPLANTATION SCIENCES
CIVIL HOSPITAL CAMPUS, ASARWA, AHMEDABAD -380 016
e-mail :ikdrcad1@sancharnet.in • www.ikdrc-its.org

Declaration & Copyright Transfer Form

TO BE SIGNED BY ALL AUTHORS

I/We, the undersigned author(s) of the manuscript entitled

_____

_____

_____

_____

hereby declare that

(a) the above manuscript which is submitted for publication in the Organ Dysfunction and Management ("ODM") is NOT under consideration elsewhere.

(b) the manuscript is NOT published already in part or whole (except in the form of abstract) in any journal or magazine for private or public circulation.

(c) I/we give consent for publication in the ODM in any media (print, electronic, or any other), and assign copyright to the ODM in the event of its publication in the ODM.

(d) I/We affirm that the manuscript does not violate the intellectual property rights of any third party. I/ We agree to indemnify and hold harmless the ODM in respect of any claim on account of violation of intellectual property rights.

(e) I/we do not have any conflict of interest (financial or other) other than those declared*.

(f) I/we have read the final version of the manuscript and are responsible for what is said in it.

(g) the work described in the manuscript is my/our own and my/our individual contribution to this work is significant enough to qualify authorship.

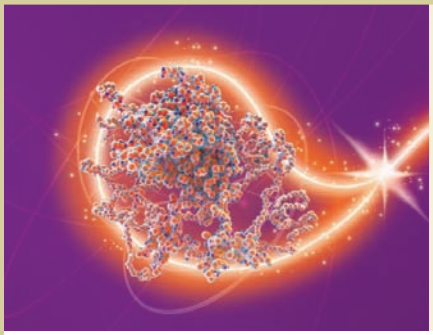
(h) no one who has contributed significantly to the work has been denied authorship and those who helped have been duly acknowledged.

(i) I/we also agree to the authorship of the article in the following sequence:

Authors Name (s)	Signature (s)	Date
1. ----------	----------	---------
2. ----------	----------	---------
3. ----------	----------	---------
4. ----------	----------	---------
5. ----------	----------	---------
6. ----------	----------	---------

NOTE:

1. All authors are required to sign this form.
2. No addition, deletion or change in the sequence of authors is allowed at the later stage without valid reasons.
3. If the authorship is contested at any stage, the manuscript will be either returned or kept in abeyance till the issue is resolved.
4. This form may be photocopied and used.

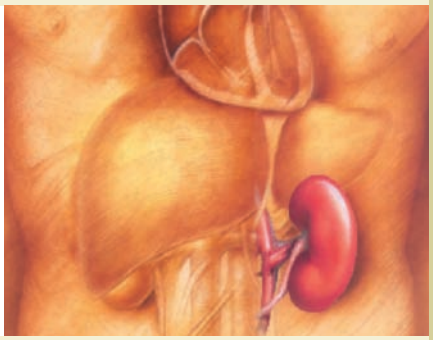


MIRCERA®

methoxy polyethylene glycol-epoetin beta

CellCept®

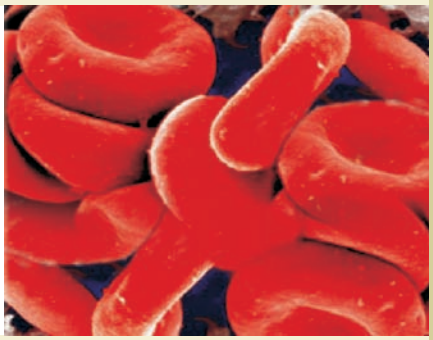
(mycophenolate mofetil) Caps. 250 mg Tabs. 500 mg



Valcyte®

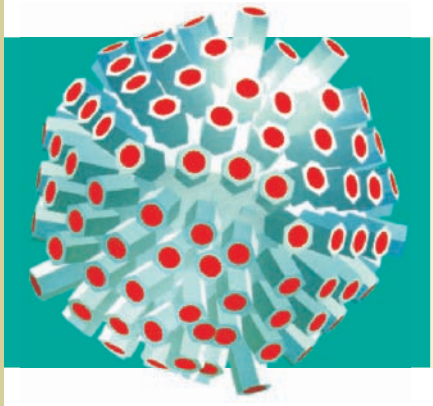
valganciclovir

simply potent



NeoRecormon®

Epoetin Beta



Cymevene®

ganciclovir

Marketed in India by :

Roche Products (India) Pvt. Ltd.

"The View", 2nd Floor, 165, Dr. Annie Besant Road,
Worli, Mumbai - 400 018. INDIA.
Tel.: +91-22-24941414 Fax : +91-22-24949500

For scientific information write to : india.medinfo@roche.com

Roche

KB (02/13)

A dream realized through tireless efforts of a few so that many may live longer and better !