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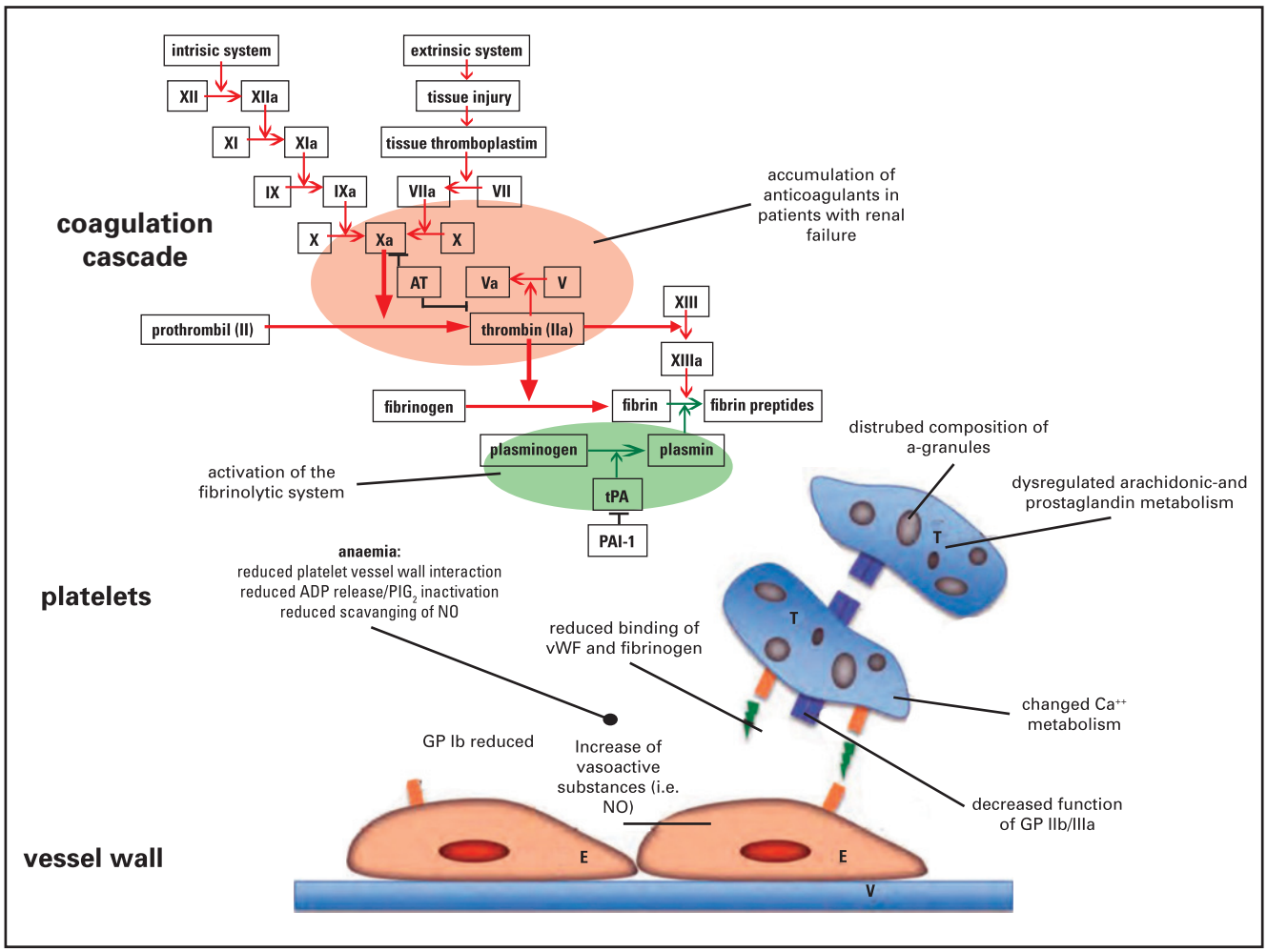
Timely Topics in Transplantation



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Labrad Administration Office
Mr Kokab Mirza
Clinical Laboratories
Department of Pathology and Microbiology
Aga Khan University Hospital
Stadium Road, P. O. Box 3500
Karachi 74800, Pakistan

Tel: 92 21 3486 1551
Fax: 92 21 3493 4294, 3493 2095

hospitals.aku.edu/Karachi/clinical-laboratories

From the Editor's Desk

This issue's goal is to impart transplant related information to health care providers in a readable, understandable format that will not become outdated anytime soon. Focusing on this, we have strived to compile topics that cover the spectrum of basic knowledge, important investigations, clinical conditions and newer techniques in the field of transplantation. Continuing the quest for knowledge while keeping the interest alive, this issue therefore contains broad variety of topics on Haemopoietic stem cell and kidney transplantation.

Transplant medicine is complex and multidisciplinary. Accordingly, the articles in this issue are on pretransplant evaluation, significance of HLA typing, blood product utilization in transplant recipients, monitoring of immunosuppressive therapy and infections in neutropenic patients to name a few. This diversity of articles has been carefully created so that our readers find it appealing and useful to the utmost level.

Dr Natasha Ali
Hematology

Significance of HLA Typing in Transplant Medicine

Nazneen Islam and Afsheen Ibrahim
Molecular Pathology

Introduction

The major Histocompatibility Complex (MHC) is a group of genes located on short arm of Chromosome number six (6p21.3). In humans, the MHC gene products are called human leukocyte antigens (HLAs). This region contains 200 genes, many of which involve in immune responses. The genes encode the HLA Class I (A, B,C) and Class II (DR, DQ, DP) molecule (Figure1). The essential role of the HLA antigens is the initiation and regulation of the immune response(Table 1). The physiologic function of MHC molecules is the presentation of peptide antigen to T lymphocytes and distinguishes "self" from "non-self. The clinical utility of HLA typing in following purposes:

1) Bone Marrow Transplantation
2) Solid organ Transplantation
3) Transfusion Medicine
4) Marker of Autoimmune diseases
5) Population genetics of the HLA system

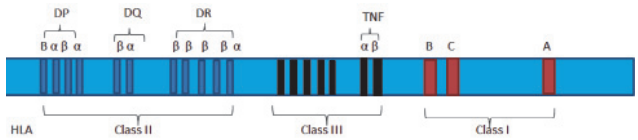


Figure 1. The MHC locus on chromosome 6 covers about 4 Mb of DNA, depending on the individual. Class I genes are 3-6 kb long and class II genes are 4-11 kb in length. TNF-α and TNF-β are not part of the polymorphic HLA system.

Table 1. Description of MHC Class I& Class II locations and function

MHC Region	Gene Products	Tissue Location	Function
Class I	HLA-A, HLA-B, HLA-C	All nucleated cells	Identification and destruction of abnormal or infected cells by cytotoxic T cells
Class II	HLA DR, HLA DQ, HLA DP	B lymphocytes, monocytes, macrophages, dendritic cells, activated T cells, activated endothelial cells, skin	Identification of foreign antigen by helper T cells

HLA and Transplant Immunity

Histocompatibility molecules of one individual act as antigens when introduced into a different individual in organ transplantation. Mismatched HLA antigens can stimulate B cells to produce alloantibodies, which are involved with humoral mechanisms of transplant rejection, Class I antigens controlled by the HLA-A, -B and -C loci are the primary targets of alloantibodies, studies also indicates that antibody reactivity to class II antigens encoded by HLA-DR and HLA-DQ antigens may also result in graft loss.

Table 2. Potential effects of human leukocyte antigen (HLA) on transplant immunity

- **Humoral Immunity**
  - Against Class I HLA antigens
  - Complement-dependent antibodies
  - Complement-independent antibodies
  - Against Class II HLA antigens
- **Cellular Immunity**
  - Direct allorecognition
  - Cytotoxic CD8 T-cells
  - Effector CD4 T-cells
  - Regulatory T-cells
  - Indirect allorecognition
  - Effector CD4 T-cells
- **HLA-Restricted Immune Responses**
  - Antiviral immunity
  - Cytotoxic CD8 T-cells
  - Recurrent autoimmune disease
  - Effector CD4 T-cells

Table 3. HLA matching protocols for kidney transplantation

- **Matching for HLA-A, -B, and -DR Antigens**
- Mismatching for HLA-A, -B, and -DR antigens
  - Broad vs split antigens
  - Acceptable and unacceptable mismatches for highly sensitized
  - Candidates
  - Permissible mismatches with graft outcome similar to on-mismatched transplants
- **DR Matching**
- **CREG Matching**
  - Public and private class I epitopes
- **Structurally Based Matching**
  - Amino acid residue mismatching
  - HLA Match maker

Abbreviations: HLA human leukocyte antigen; CREG cross-reacting groups of antigens

Reference: American Society for Histocompatibility and Immunogenetics, 2004

Clinical Reality of HLA Matching, Graft Survival and Outcome

HLA matching significantly reduces the risk of graft rejection and graft failure after solid-organ transplantation and graft-versus-host disease (GvHD) after hematopoietic stem-cell transplantation.

For example in kidney transplantation, current criteria for HLA matching consider three loci: HLA-A, HLA-B and HLA-DR Many studies have shown the superior results with zero HLA-A, B, DR mismatches which expand longer allograft half-lives (12.5 versus 8.6 years) and increased 10-year survival (52 versus 37 per cent). However in bone marrow and stem cells transplantation the donor's and recipient's HLA tissue types match plays a large part in survival. A match is better when all 5 of the known major HLA antigens are the same as five out of five match.

Table 4. Confounding factors for Kidney Transplant

- **Quality of Donor Kidney**
  - Donor age: expanded criteria donors
  - Extended ischemia time
  - Living donors
- **Recipient Risk Factors**
  - Pediatric recipients
  - Ethnicity
  - Sensitization
  - Immunosuppression

Molecular Analysis of HLA Typing

Various molecular methods have been evolved for HLA DNA typing. PCR SSP i.e. Sequence specific Primer based PCR is one of the reliable method because among them. PCR-SSP methodology is based on the principle that completely matched oligonucleotide primers i.e. HLA-A, B, C, DR and DQ alleles. Primer pairs are designed to be matched with single alleles or group(s) of alleles depending upon the degree of typing resolution required. Completely matched primer pairs allow amplification to occur, i.e. a positive result, whereas mismatched primer pairs doesn't allow amplification to occur, i.e. a negative result (Figure 3). After the PCR process, the amplified DNA fragments are size-separated e.g. by two per cent agarose gel electrophoresis, visualized by staining with ethidium bromide and exposure to ultraviolet light, documented by photography and interpreted. Interpretation of PCR-SSP results is based on the presence or absence of specific PCR product(s) (Figure 4).

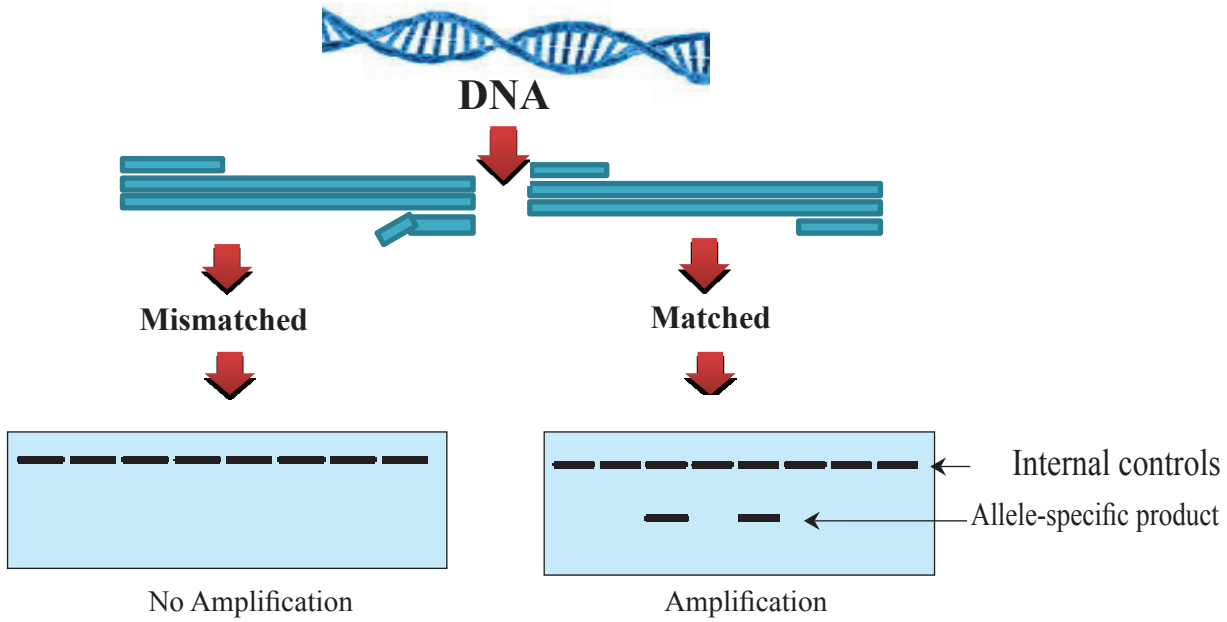
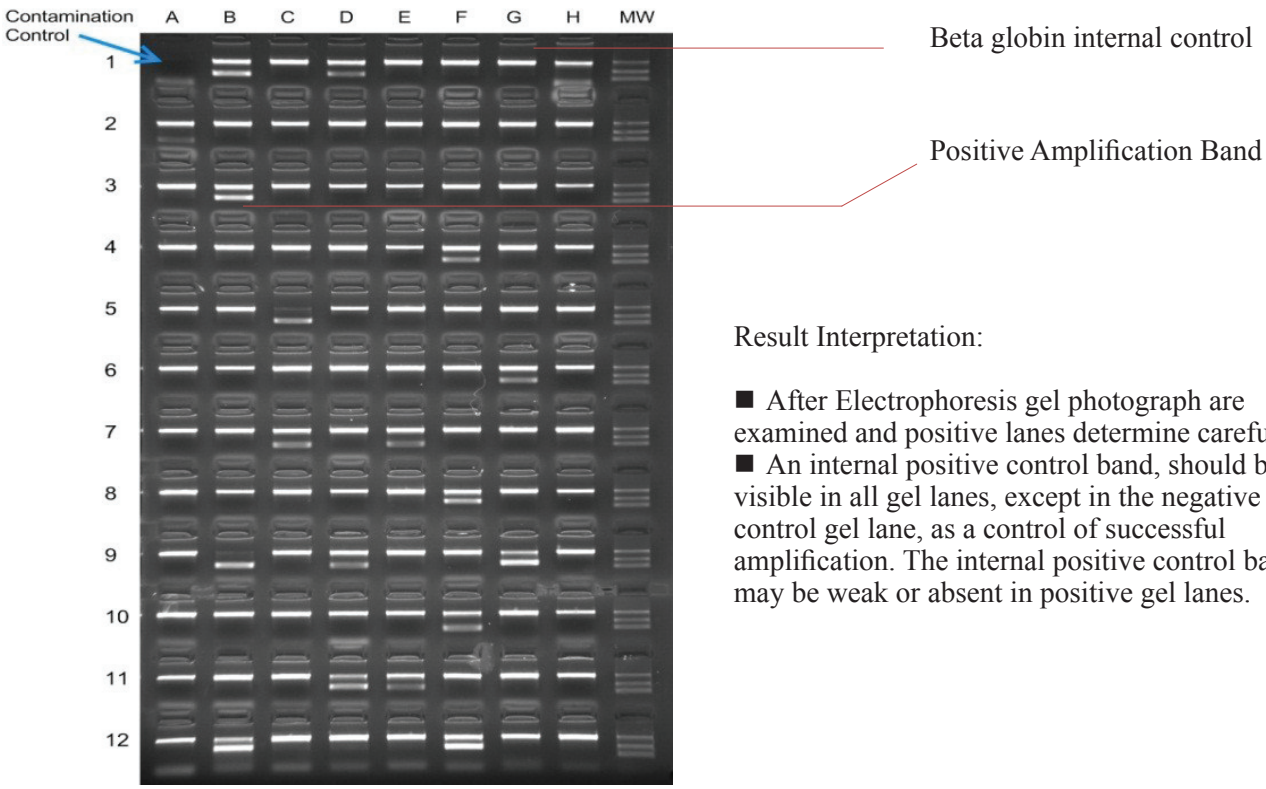


Figure 3. Principle of PCR-SSP. Allele-specific product only from those wells containing primers matching the specimen HLA allele. Wells containing primers that do not match the patient's HLA allele will have a band only from the internal control.



Result Interpretation:

- After Electrophoresis gel photograph are examined and positive lanes determine carefully
- An internal positive control band, should be visible in all gel lanes, except in the negative control gel lane, as a control of successful amplification. The internal positive control band may be weak or absent in positive gel lanes.

Figure 4. Gel electrophoresis of HLA Typing SSP
Ref: Olerup, Micro SSP HLA-A, HLA-B, HLA-C plate

Role of Protocol Renal Biopsies in Transplant Patients

Drs Sidra Arshad and Saroona Haroon
Histopathology

Renal transplantation has become the treatment of choice for patients with end-stage renal disease. Protocol biopsies early after transplantation have detected subclinical disease, which has excited clinical investigators and prompted them to consider this as a potential surrogate marker for evaluating transplantation outcome.

Types of Renal Allograft Biopsies

- Implantation biopsy (donor biopsy): Individual zero time biopsies have been shown to correlate with the graft outcome
- Indication biopsy: Dysfunctional allograft biopsy and allograft biopsy for proteinuria
- Protocol biopsy

Protocol Renal Biopsy

It is performed at pre-determined intervals after transplantation in normal functioning allografts. Aided research and provided insights into the pathogenesis of early and late allograft injury.

Role in Clinical Practice

- Detection of subclinical rejection (SCR)
- Individualization of therapy in high-risk patients and safe reduction of immunosuppression in standard risk individuals becomes possible
- Chronic T cell or antibody-mediated rejection
- Recurrent or de novo glomerulopathies
- Viral nephropathy
- Interstitial fibrosis/ Tubular atrophy(IF/TA)
- Chronic calcineurin inhibitor(CNI) - nephrotoxicity, allowing modifications of therapy to limit ongoing graft injury

When to Perform?

Optimum timing of surveillance biopsies depends on the pathology. Biopsies up to three months from transplant show a higher frequency of SCR with a greater incidence of IF/TA beyond six months and presentation of recurrent and de novo GN, TG and CNI toxicity occurring at various time points from transplantation. Early surveillance biopsy yields the greatest reversible pathology while biopsies beyond one year serve as prognostic indicators of graft survival and help tailor immunosuppression according to the individual.

Preparation of the Biopsy for Evaluation

The biopsy should be processed and prepared according to the renal transplantation guidelines for allograft biopsy handling by an experienced technologists. Allograft biopsies are processed by the rapid method using auto processor and are reported on the same day. According to Banff scheme, it is recommended to prepare at least seven slides(3-4 microns thick), with multiple sections mounted on each slide. Three of these should be stained with hematoxylin and eosin (H&E), three with periodic acid-Schiff reagent (PAS) and one with a Masson's trichrome stain.

Pathological Evaluation

The accurate pathologic evaluation of renal allograft biopsy requires a well-trained renal pathologist with a thorough knowledge of renal transplant pathology, and also of renal and transplant medicine in order to correlate the morphologic abnormalities with the detailed clinical information. The importance of correlation of morphological findings on the renal allograft biopsy with clinical data and a close interaction between the nephrologists and pathologists is no doubt of great significance.

Role of Special Stains and other Ancillary Studies

The PAS and/or silver stains are very useful in delineating tubular basement membranes (TBMs) and in defining the severity of tubulitis, and for evaluating glomerulitis. The PAS stain is also useful in the identification of arteriolar hyalinosis, tubular atrophy and their semi-quantitative scoring. Trichrome stains help in assessing the chronic sclerosing changes in the interstitium and in the arterial intima. C4d antibody is performed to rule out humoral rejection process.

Future Recommendations

While renal biopsy remains the current "gold standard "for investigation and assessment of allograft pathology, development of either supplementary molecular methods or minimally monitoring or noninvasive monitoring has to be expanded. The cost, risk and patient inconvenience of surveillance biopsies must be weighed against potential gains from early interventions guided by the findings. These biopsies have provided marked insights into the subclinical processes affecting the graft with implications for the long term graft outcome.

Umbilical Cord Stem Cells

Dr Natasha Ali
Hematology

In the developed world, haematological malignancies account for 10 percent of new cancer diagnosis. Many of these malignancies can be cured with chemotherapy alone. However, most of the time chemotherapy alone is not enough to achieve a cure and these patients then require haemopoietic stem cell transplantation (HSCT). HSCT provides curative therapy for a variety of diseases. Every year more than 30,000 patients with haematological malignancies receive high dose chemotherapy followed by HSCT. The stem cells used in the procedure can be obtained from bone marrow; G-CSF mobilized peripheral blood and cord blood. Despite this, the availability of a match related donor still remains to be limited.

Umbilical cord blood availability as a prospect for therapeutic use was first reported in Lancet in 1939. It was not until 1970s when the medical brothers Ende published the transplantation of multiple units of umbilical cord blood into an individual3. Although this procedure was not successful due to the multiple disparities of the transplant unit, it did pave a way to investigate cord blood on a more serious level. In 1988 eventually, successful transplant of a patient with Fanconi's anaemia was performed and published in 19894. The possibility to use one of the largest cellular sources available, but normally discarded, was an exciting move which led to umbilical cord blood stem cells being used to treat a variety of malignant and non-malignant haematological disorders. Umbilical cord stem cells present a number of advantages over bone marrow stem cells (Table 1).

Table 1. (Adapted from - The Journal of Perinatal Education, 20(1), 54-60, doi: 10.1891/1058-1243.20.1.54)

Advantages of Umbilical Cord Blood Stem Cells Versus Bone Marrow Stem Cells
• Ease of collection
• No risk for mother or child
• Less time needed for processing (more quickly available for use)
• Less costly than bone marrow collection
• Less risk for transmission of infection
• Less need for stringent antigen typing
• Less rejection

Table 2: (Adapted from - The Journal of Perinatal Education, 20(1), 54-60, doi: 10.1891/1058-1243.20.1.54)

Disadvantages of Umbilical Cord Blood Stem Cells
• Slow engraftment
• Limited cell dose — Small volume of unit — Additional cell doses unavailable
• Autologous donation may have limited benefit owing to hereditary disorders
• Storage issues — Unknown length of long-term storage — Cost related to long-term storage — Quality control

However, like all therapies, it is associated with caveats (Table 2)

These stem cells are stored in stem cell banks after donation. There are three types of umbilical cord blood banks; private, public and direct-donation banks. Private Banks are commercial that promulgate directly to expectant parents. Private Banks charge an initial fee for collection and processing and a yearly fee for storing the specimen. Public umbilical cord blood banks accept altruistic donations. Donated units are processed, antigen typed and stored for future use. Direct donation banks function as an amalgamation of public and private cord blood banks. They collect cord blood without charging fees. In addition, they accept autologous donations and reserve them only for the family.

Umbilical cord blood, once thought of as a waste product of the birthing experience, is now valued for its content of stem cells. Saving cord blood is a worthy undertaking for any family. Obstetricians and haematologists are one of the first resources that an expectant family turns to for knowledge in order to reach an informed decision about collecting umbilical cord blood. Therefore, we as physicians should be well versed on the topic, so that as questions arise, the multiple facets of umbilical cord blood banking can be explored.

Blood Product Utilization in Haematopoietic Stem Cell Transplant Recipients

Dr Hira Qadir
Haematology

Introduction

Hematopoietic stem cell transplant (HSCT) patients often require intensive blood component support. Transfusion may be complicated by transfusion transmitted infection- both viral and bacterial, transfusion-associated (TA)-GvHD, febrile non-hemolytic transfusion reactions (FNHTR) and transfusion-related acute lung injury (TRALI). Alloimmunization to red cell antigens may cause difficulties in selecting compatible blood while alloimmunization to the human leukocyte antigens (HLA) present on platelets may cause refractoriness to subsequent transfusions of randomly-selected platelets.

Leukodepleted Blood Components

Transfused leukocytes cause alloimmunization to HLA Class one antigens (HLA-A1) in a proportion of patients. This may be manifested clinically as FNHTRs, although these may also be caused by antibodies to neutrophils, platelets or plasma proteins and by cytokines such as interleukin (IL)-1, IL-6, IL-8 and tumor necrosis factor which accumulate in stored blood components, especially platelet concentrates. HLA-A1 may cause accelerated destruction of transfused platelets that are HLA incompatible. This is clinically manifest as a failure to achieve a satisfactory increment after platelet transfusion (refractoriness). Donor dendritic cells which are present in red cell and platelet transfusions appear to be responsible for sensitization to HLA. Studies show that removal of leukocytes to less than 5 x 106 per blood component prevents primary HLA-A1 in >97 per cent of patients with hematological malignancies. The use of leukodepleted components also reduces secondary A1 and refractoriness to platelet transfusion.

Indications for Leukodepleted Blood Components

- Pre-HSCT in patients with severe aplastic anemia (SAA) to reduce the likelihood of graft failure
- Pre- and post-HSCT to prevent recurrent FNHTR
- Pre-and post-HSCT to minimize HLA-A1 and platelet refractoriness

Many Blood Services have implemented leukodepletion of a large proportion or, in some cases, all of their blood components. In the UK universal leukodepletion was implemented in 1999 with the aim of minimising the risk of transfusion

associated transmission of the causative agent of variant Creutzfeld-Jakob disease (vCJD); As an alternative to CMV seronegative components.

Gamma-Irradiation of Blood Components and TA-GvHD

HLA incompatible third party leukocytes contained in donated blood components can engraft and initiate an alloreactive response after transfusion. This can cause TA-GvHD, manifest clinically by fever, rash, diarrhea, jaundice and pancytopenia, and this is fatal in >90 per cent of cases, so prevention is essential. Donor leukocytes are inactivated by gamma-irradiation of 2500 cGy and all components for HSCT recipients should be irradiated from the time that conditioning therapy is started and continued until six months post-transplant or until the lymphocyte count is 1 x 109/L in the absence of chronic GvHD. In addition, HLA matched packed red cells should be irradiated, as should those from family members.

Indications for Irradiated Blood Components

Allo-HSCT recipients from time of conditioning therapy for 6 months or until the lymphocyte count is 1 x 109/L in the absence of cGvHD

- Auto-HSC recipients (from 7 days before harvest until 3 months post transplant)
- All donations from HLA-matched donors or 1st or 2nd degree relatives
- All patients with Hodgkin disease at any stage of therapy
- All patients treated with purine analogues e.g. fludarabine
- All patients with congenital immunodeficiency states

Red Cells

Red cells, are transfused to correct anemia due to marrow failure, hemorrhage or hemolysis, aiming to keep the hemoglobin or packed cell volume (PCV) above predefined levels to ensure good tissue oxygenation. Reduced intensity conditioning (RIC) transplants require fewer red cell transfusions. Red cells should be matched for ABO and Rhesus D type. Extended phenotyping may be necessary in patients, e.g. those who have formed red cell alloantibodies after previous transfusions. Red cells should be cross-matched against the patient's serum by standard techniques prior to transfusion.

Platelet Transfusions

Current practice, based on the results of randomized studies, is to transfuse platelet concentrates prophylactically when the platelet count is less than 10 x 109/L. A recent Cochrane Systematic Review concluded that, whilst there is no reason to change current practice, blood products may become scarcer and further trials should be undertaken to compare prophylactic versus therapeutic platelet transfusion.

Conclusion

Transfusion support in HSCT patients requires special consideration and carefully defined policies. The use of high quality blood components which have a high degree of microbiological safety, gamma-irradiated, may be CMV seronegative and leukodepleted provides optimum transfusion support and minimizes the chance of adverse effects.

Use of Magic Marker "C4d" in the Diagnosis of Acute Antibody Mediated Rejection in Renal Transplant Patients

Dr Muhammad Ishaque and Dr Saroona Haroon
Histopathology

Renal transplantation is the treatment of choice for patients with end stage renal disease worldwide. Transplant rejection can be defined as an immune response of the patient that is activated against foreign material i.e. graft, which without treatment leads to the destruction of the graft. There are three different types of rejection when classified according to the time of onset.

1- Hyperacute or accelerated rejection usually occurs during the first hours after transplantation and leads to the loss of the graft. In most cases it can be avoided by the pre transplant cross match test.

2- Acute rejection occurs usually during the first weeks or months after transplantation occurring in 40 per cent to 70 per cent of the cases, if mild rejections are included. It manifests typically as asymptomatic elevation of serum creatinine. The diagnosis is confirmed by renal graft biopsy which shows lymphocytic infiltrate, tubulitis and edema. Acute rejection usually responds well to high dose corticosteroid treatment.

3- Chronic rejection is defined as a gradual but progressive impairment of renal allograft which can occur at any time after the initial post transplantation months. It does not respond well to antirejection treatment usually leading to the loss of graft.

In recent years it has been recognized that about 10% of acute rejection cases generally ensuing in the first three months, are due to donor specific antibodies, not detected before transplantation. In most of these cases antibodies are directed against class I or II HLA antigens, but sometimes they are directed towards different endothelial antigens and cannot be detected even with more advanced tests.

That an antibody mediated process is operating in this type of rejection is testified by the capillary deposition of C4d, which is a degradation product of the classic complement pathway. After an antigen-antibody complex fixes complement, a cascade of events follows, with activation of several complement proteins. The complement protein C4 is split into C4a and C4b. C4b is then converted to C4d. A unique feature of C4d is that it binds covalently to the endothelial and collagen basement membranes, thereby avoiding removal and raising the possibility of serving as an immunologic footprint of complement activation and antibody activity. In 2003 'C4d' was incorporated in the Banff classification.

Together with the serologic evidence of circulating antibodies to donor HLA antigens or to other donor endothelial antigens that were not present at the pre-transplant cross-match, two cardinal morphologic features are needed for the diagnosis:

1) Evidence of acute tissue injury such as:

   (a) acute tubular necrosis;

   (b) neutrophils and/or monocyte-macrophages in peritubular and glomerular capillaries and/or capillary thrombosis or

   (c) fibrinoid necrosis/transmural inflammation of arteries

2) Immunopathologic evidence of an antibody action, such as:

   (a) C4d complement and/or immunoglobulins in peritubular capillaries, or

   (b) immunoglobulin and complement in arteries with transmural fibrinoid necrosis.

If transplantation has occurred greater than one year from the time of the biopsy, the likelihood of recurrence of a native kidney disease is increased. For this reason, a full work-up like that seen for a native kidney biopsy is recommended. This includes light microscopic, immunofluorescent, and electron microscopic analysis plus C4d. C4d by the indirect immunofluorescence approach is a more sensitive than monoclonal antibody staining, it is performed on the frozen section tissue.

C4d is detectable in the glomerular mesangium and at the vascular pole of the normal kidney. This emphasizes that there is constitutive turnover of immune complexes. When the burden of immune complexes increases with immune complex deposition diseases, C4d deposition overflows from the mesangium and vascular pole to the glomerular capillaries. In transplanted kidneys, Donor-specific antibody (DSA) directly engages human leukocyte antigens (HLAs), which are present in the glomerulus as well as the peritubular capillaries. It is known that anti-complement protection in the PTCs is weaker than in the glomerulus (Figure 1). Hence detection of the complement split product C4d in renal live related allograft biopsies is an important adjunctive tool to help understand the alloimmune response and, in particular, to diagnose antibody-mediated rejection (AMR). In patients with this form of rejection, renal insufficiency develops briskly but sometimes insidiously. Although a rescue treatment with new potent immunosuppressive agents and plasma exchange may be successful in some cases, the one year graft-loss ranges from 25 per cent to 50 per cent. C4d is now one of the core diagnostic tools to identify AMR, and is being used in virtually all transplant centers around the world.

The difficulties of interpreting focal staining patterns, the relatively low sensitivity of C4d as a marker for AMR in late renal allograft biopsies, and its lack of utility as a marker for antibody-mediated injury in biopsies of ABO-incompatible allografts suggest that C4d has lost some of its magic during the past decade. However, most experts come to an understanding that if complement targeted therapies will be part of our future treatment options; a marker such as C4d will still be obligatory to identify patients susceptible for those kinds of expensive treatments.

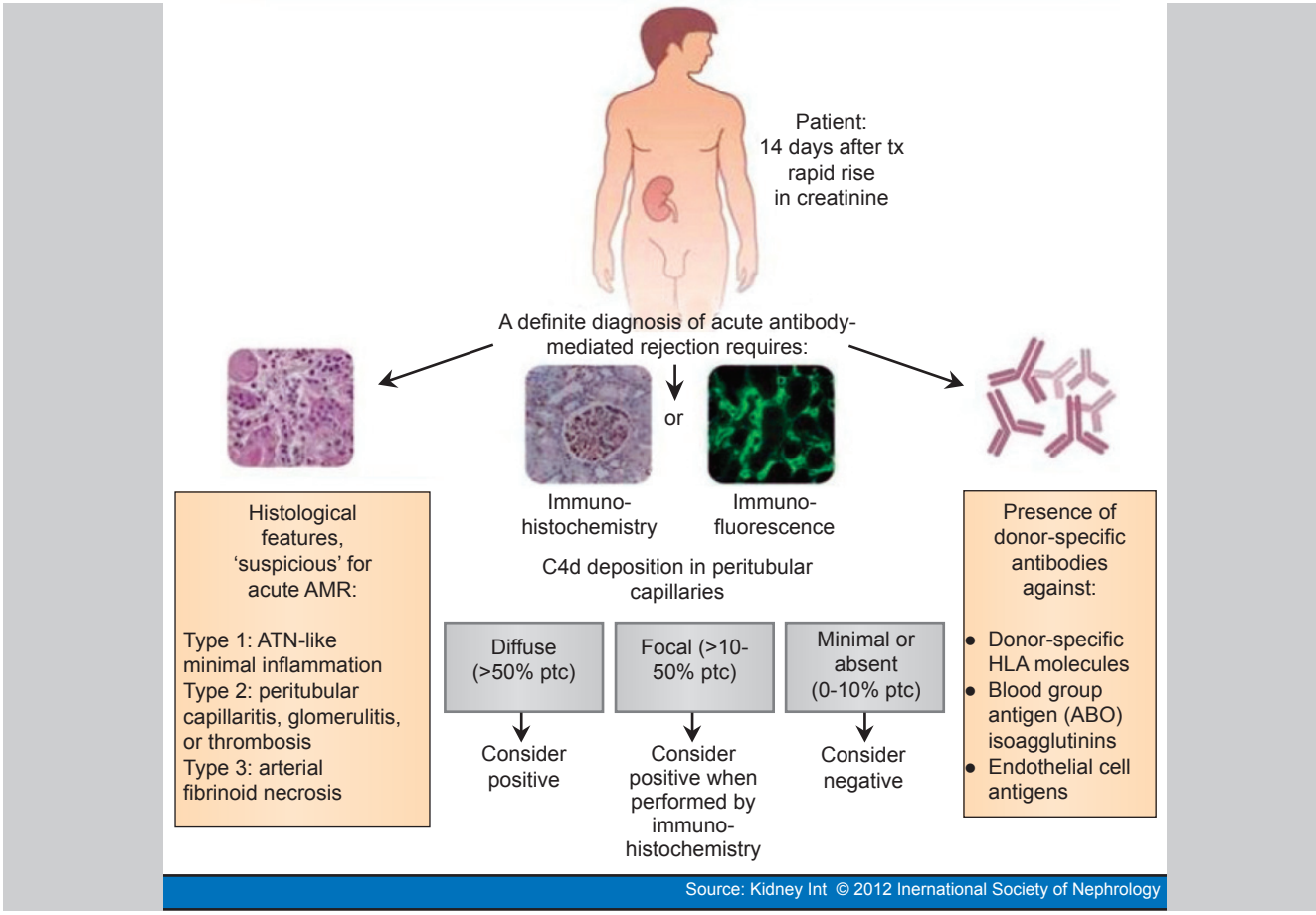


Figure 1. Donor specific antibodies engaging with human leukocyte antigen

Assessing Post Transplant Renal Function

Dr Lena Jafri
Chemical Pathology

Identifying kidney allograft dysfunction as soon as possible will permit timely diagnosis and management. Monitoring allograft function includes direct and indirect measurement of glomerular filtration rate (GFR) and quantitation of urinary protein excretion besides the invasive and various radiological tests available.

Serum Creatinine

The standard test used to monitor graft function is serum creatinine measurement. Creatinine is derived from the metabolism of creatine in skeletal muscle and from dietary meat intake. It is released into the circulation at a relatively constant rate. Creatinine is freely filtered across the glomerulus and is neither reabsorbed nor metabolized by the kidney. However, approximately 10-40 per cent of urinary creatinine is derived from tubular secretion by the organic cation secretory pathways in the proximal tubule. Increased serum creatinine post-kidney transplant that is not explained by dehydration, urinary obstruction, high calcineurin inhibitor levels or other apparent causes is most likely due to an intragraft parenchymal process, such as acute rejection, chronic allograft injury, drug toxicity, recurrent or denovo kidney disease.

Following is the diagnostic criteria for acute kidney injury: an abrupt (within 48 hour) reduction in kidney function currently defined as an absolute increase in serum creatinine of ≥ 0.3 mg/dL, a percentage increase in serum creatinine of ≥ 50 per cent (1.5-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 mL/kg/hour for more than six hour). This criteria includes both an absolute and a percentage change in creatinine to accommodate variations related to age, gender and body mass index, and to reduce the need for a baseline creatinine but do require at least two creatinine values within 48 hour.

The serum creatinine concentration however is an indirect measurement of GFR. At low serum creatinine levels, small changes in creatinine can indicate large changes in function. It is essential for clinical laboratories providing transplant service to keep a strict check on internal quality controls of serum creatinine and to keep its

Table 1. Formulae for Estimating Glomerular Filtration Rate in adults.

Method of GFR calculation	Formulae
CrCl (ml/min/1.73m2)	urinary Cr x volume x 1.73/ serum Cr x1440 x BSA
MDRD (mL/min/ 1.73 m2)	175 x Cr (exp[-1.154]) x Age (exp[-0.203]) x (0.742 if female)
CG (mL/min)	(140 - age) x weight / serum Cr [mg/dL] x 72 x (0.85 for females)
CKD-EPI (mL/min)	If Cr < 0.9 (for male): 141 x (Cr/0:9)0. 411 x 0:993Age If Cr > 0.9 (for male): 141 x (Cr/0:9) -1.209 x 0:993Age If Cr < 0.7 (for female): 144 x (Cr/0.7)-0.329 x 0:993Age If Cr > 0.7 (for female): 144 x (Cr/0:9)-1.209 x 0:993Age
Abbreviations: GFR= glomerular filtration rate; CrCl= creatinine clearance; Cr= creatinine; BSA= body surface area; MDRD= modification of diet in renal disease; exp= exponential; CG= Cockcroft Gault. Serum Cr in mg/dL, weight in kg, height in meters, age in years, and BSA in meter square.	

coefficient of variation within narrow limits (preferably with ±1SD). The clinical laboratories must use a creatinine method that has calibration traceable to an isotope dilution mass spectrometry (IDMS) reference measurement procedure. Additionally, patients with small muscle mass generate less creatinine on a daily basis and normal levels in such patients may mask allograft dysfunction in them. The increasing use of estimated GFR (eGFR) in clinical practice has renewed the interest on the shortcomings of serum creatinine alone.

Formulae that Estimate GFR

The most common methods utilized to estimate the GFR are: measurement of the creatinine clearance; and estimation equations based upon serum creatinine such as the Cockcroft-Gault equation, the Modification of Diet in Renal Disease (MDRD) study equations, and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. However, none have not been validated in our population. Out of these equations which were developed for chronic kidney disease patients, the MDRD has been validated in renal transplant patients.

Table 2. Causes of Proteinuria after Kidney Transplantation.

Allograft Rejection and Drug Toxicity	
Acute rejection	Thrombotic microangiopath
Chronic allograft injury	Transplant glomerulopathy
De Novo and Recurrent Glomerular Disease	
Minimal change disease	IgA glomerulonephritis
Focal segmental glomerulosclerosis	Membranous glomerulonephritis
Membranoproliferative glomerulonephritis	Postinfectious glomerulonephritis
Thrombotic thrombocytopenic purpura	Hemolytic Uremic Syndrome
Vasculitis	Amyloidosis
Light- and heavy-chain deposition disease	Systemic lupus erythematosus
Diabetic nephropathy	

Proteinuria

The rate of protein excretion in healthy adults is <150 mg/day, of which less than 30 mg is albumin while the rest comprises different proteins and glycoproteins originating from tubular epithelial cells. Proteinuria is common after kidney transplantation and typically urine protein levels are below 500 mg/d. However, even these low levels are associated with reduced graft survival. The reason can be the native kidney or the allograft, probably as a consequence of the ischemia-reperfusion injury. Proteinuria falls after successful kidney transplantation within few weeks. The residual or late proteinuria represents graft injury. Most allografts with proteinuria >1500 mg/d have new glomerular pathology. Evidence on proteinuria after renal transplantation shows that microalbuminuria and more significantly proteinuria after transplantation are markers of graft dysfunction, correlated both to graft failure and to cardiovascular risk indicators. In patients with proteinuria one has to exclude recurrent glomerulonephritis (refer to Table 2) in order to avoid unnecessary immunosuppression.

The 2009 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline on the care of the kidney transplant recipient recommended to measure urine protein excretion at least once within the first month after transplantation, every three months during the first year and annually later on. For the new-onset proteinuria or unexplained nephrotic proteinuria renal allograft biopsy should be performed. Proteinuria and albuminuria can be quantified with a 24 hour collection or by using spot urine collections (Albumin-Creatinine Ratio (ACR) or Protein-Creatine Ratio (PCR)). KDIGO suggest that ACR and PCR are reasonable screening tests in the renal transplant recipients. The positive thresholds for proteinuria have been established to be >250 (men) or >355 (women) mg/g by ACR and >200 mg/g by PCR. The KDIGO guidelines recommend monitoring of proteinuria as part of routine transplant follow-up. Any positive screen should be confirmed by a 24 hour urine collection. Twenty-four hour urine protein excretion is the gold standard for quantitative protein assessment. If the 24 hour urine collection is problematic, the urinary protein/creatinine (mg/g) ratio assessed in‘spot’ urine is an excellent surrogate, as it has proved to have an excellent correlation with the protein content of a 24 hour urine collection. The KDIGO also suggest biopsy for unexplained proteinuria >3 g/day.

Coagulopathy in Renal Transplantation

Dr Anila Rashid
Haematology

Chronic renal failure impairs processes of hemostasis in complex ways. Accumulation of protein biodegradation products disturbs platelet production and function, resulting in a hemorrhagic diathesis. The bleeding tendency is increased by vascular defects and deficiency of clotting factors II, V, IX, and X (Figure 1). On the other hand, high concentrations of fibrinogen as well as VII and XIII clotting factors in uremic patient plasma increase the risk of thrombosis. Thrombus formation is not controlled by clotting inhibitors, because of impaired activity of antithrombin III, heparin cofactor II, as well as proteins C and S. Additionally, injury to vascular endothelium stimulates production of factor VIII and von Willebrand factor. Finally, decreased plasma fibrinolytic activity is observed in uremic patients (Figure 2).

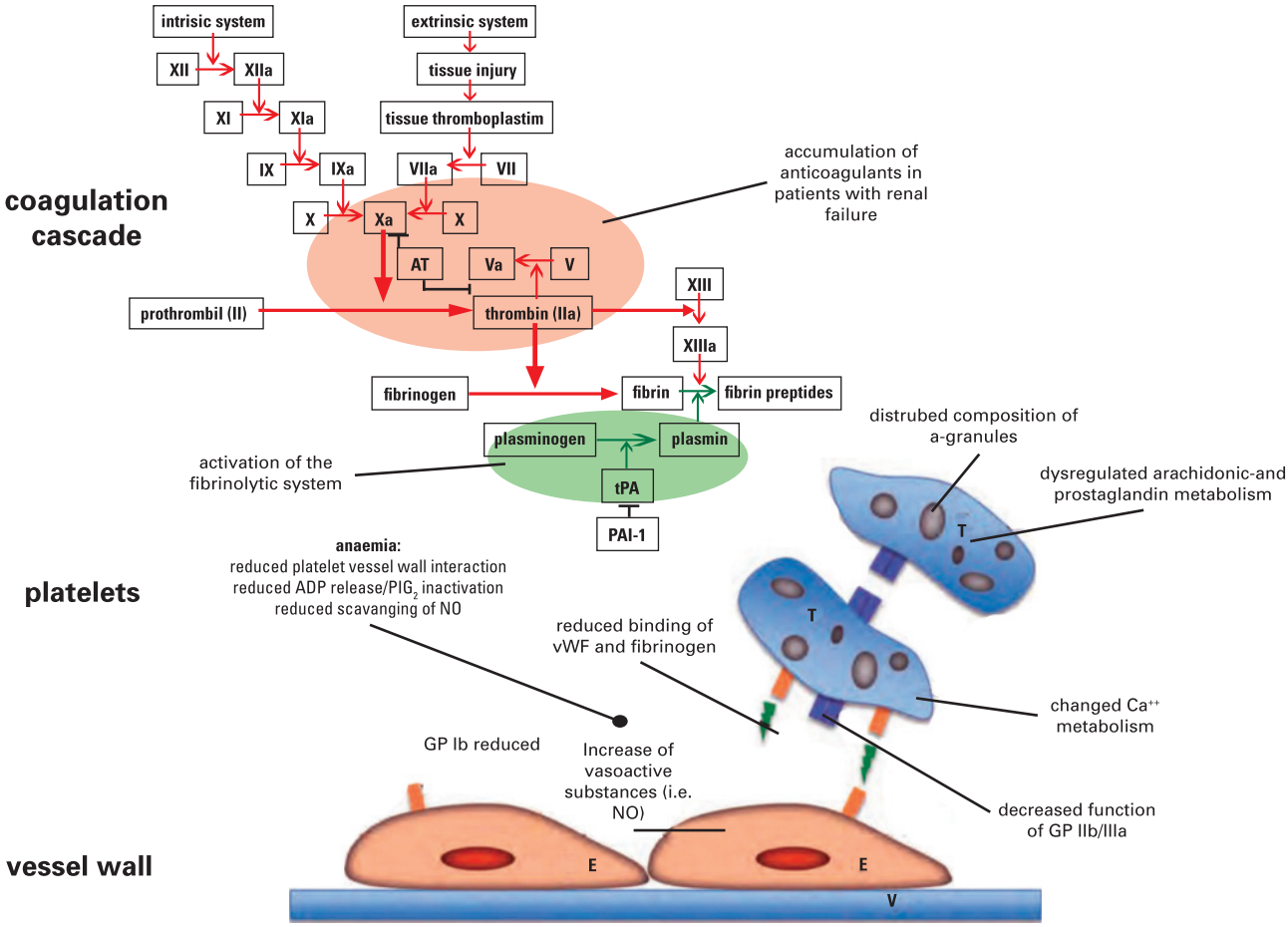


Figure 1. Factors involved in increased risk of bleeding in patients with renal failure and transplant.

Successful kidney transplantation does not result in complete regression of hemostatic disturbances. There are several factors relevant in the peri- and post-transplant periods known to influence the coagulation process, such as the function of the kidney, the immune response, and the immunosuppressive agents used post-transplantation.

Bleeding is the most common early complication after transplant (6.1per cent-8.3 per cent). It concerns mainly platelet function and clotting inhibitor activities.

A hematoma in the kidney graft area is usually caused by bleeding from tiny vessels in the renal hilus that are constricted during the operation, and begin to bleed after improved graft perfusion. The

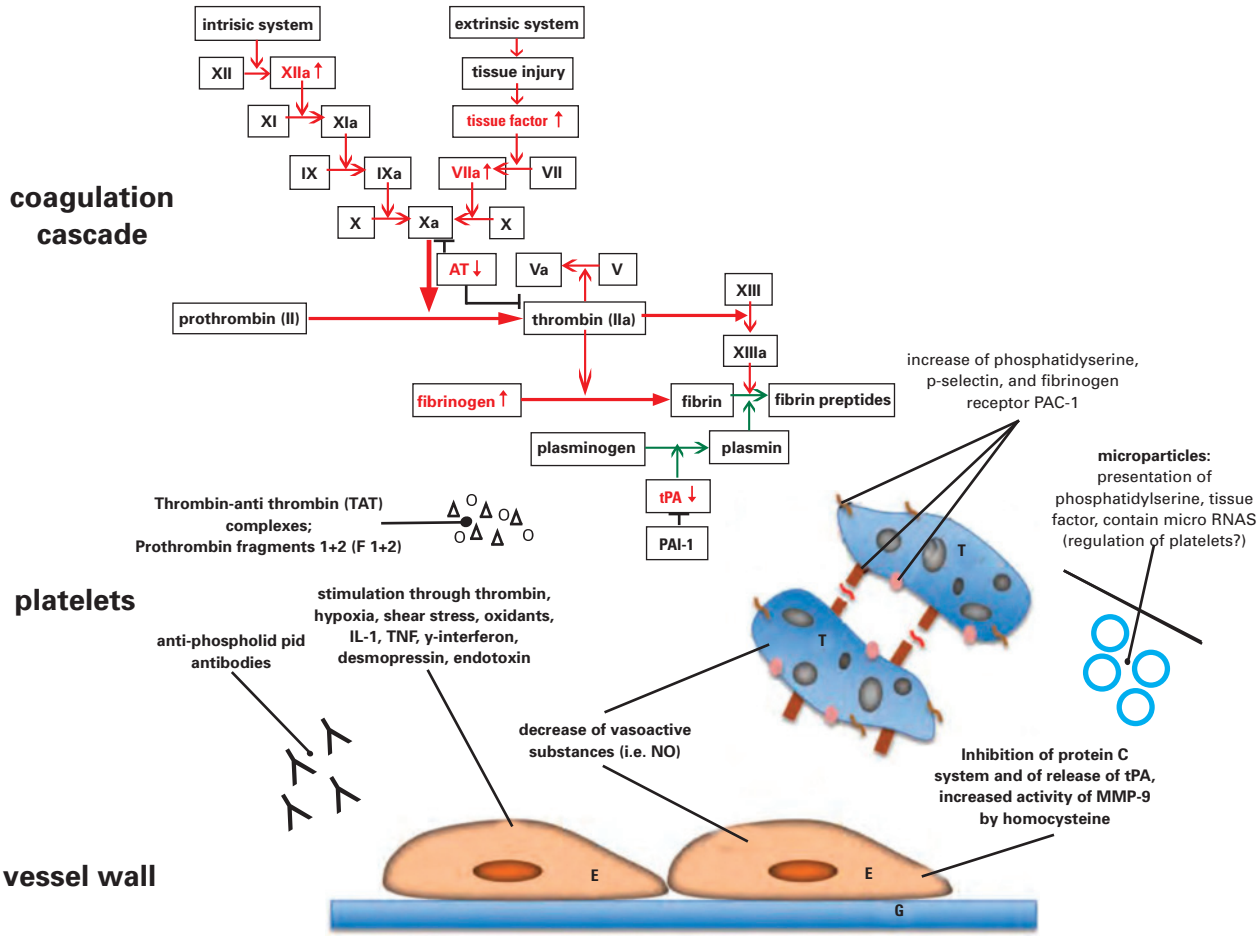


Figure 2. Factors involved in increased risk of thrombosis in patients with renal failure and transplant.
Source: Lutz J et al. Haemostasis in chronic kidney disease. Nephrol Dial Transplant. 2014 Jan;29(1):29-40.

origin of the bleeding can be bladder wall vessels; vascular anastomosis leaks; kidney graft rupture; or a spurious aneurysm due to an infected arterial anastomosis. The risk of bleeding is increased by perioperative antithrombotic and antiplatelet treatment. Hemorrhagic complications requiring surgical intervention significantly increase the risk of transplanted kidney loss.

Arterial thrombosis develops in 0.5 per cent to 3 per cent of transplanted kidneys. Renal vein thrombosis is observed in 0.5 per cent to 4per cent patients.

The reason for thrombosis in the early postoperative period is usually a technical failure: torsion or kinking of the vessel, an overly too narrow anastomosis, or injury to the endothelium. Other reasons can be cardiac arrest, persistent hypotension, acute rejection, diabetes mellitus, thrombophilia, and later, glomerulonephritis, post-transplant erythrocytosis or the side effects of an immunosuppressive drug, particularly cyclosporine. Since the transplanted kidney does not possess a collateral circulation, arterial or venous thrombosis usually results in graft loss despite immediate surgical intervention.

Routine hematologic assessment of all transplant candidates includes a complete blood count, measurement of partial thromboplastin time, prothrombin time, International Normalized Ratio (INR), fibrinogen and D-dimer concentration. Patients with recurrent miscarriage, arterial/venous thrombosis, hemodialysis graft or fistula thrombosis, or prior unexplained graft thrombosis should be evaluated for underlying hypercoagulable state like lupus anticoagulant, acquired inhibitors, inherited thrombophilia and may require anticoagulation therapy in the perioperative period. Patients with bleeding tendencies should have additional coagulation studies like bleeding time and platelet function test.

Transplant physician along with hematologist should search for the risk factors both in pre- and post-transplant period in order to detect high risk patients and apply suitable prophylactic measures.

Post Renal Transplant Erythrocytosis

Dr Maria Shafiq
Haematology

Post-transplant erythrocytosis (PTE) is defined as persistently elevated hemoglobin and hematocrit levels that occur following renal transplantation and persist for more than six months in the absence of thrombocytosis, leukocytosis, or another potential cause of erythrocytosis.

The threshold hematocrit used to define PTE is variable and ranges between 51 to 54 per cent; most clinicians use 51 per cent (corresponding to a hemoglobin concentration of approximately 17 g/dL).

The following hormonal systems and growth factors have been implicated in the pathogenesis of PTE:

- Erythropoietin
- Hematopoietic growth factors such as insulin-like growth factor-1 and its binding proteins and serum-soluble stem cell factor
- Renin-angiotensin system
- Endogenous androgens

Even among PTE patients with serum erythropoietin concentrations within normal range, the concentration may be inappropriately elevated for given haemoglobin.

Diagnostic evaluation includes the exclusion of renovascular hypertension or underlying malignancy and, in selected patients, an evaluation for chronic obstructive pulmonary disease (COPD).
The preferred initial treatment for patients with PTE who have a hemoglobin concentration <18.5 g/dL is an angiotensin receptor blocker (ARB) or angiotensin converting enzyme (ACE) inhibitor, since these agents are effective in the majority of patients, are reasonably safe, and, among many patients, provide a necessary antihypertensive effect. Theophylline may be effective in some patients who cannot take ACE inhibitors or ARBs and for whom repeated phlebotomy is unacceptable. Alteration of the immunosuppressive regimen to include antiproliferative agents may be effective in treating PTE but is rarely done in the absence of other indications.

For patients who present with hemoglobin >18.5 g/dL, therapeutic phlebotomy is recommended in addition to an ARB or ACE inhibitor. For patients who do not respond to ARBs or ACE inhibitors, intermittent phlebotomy is suggested rather than other therapies.

Therapeutic Drug Monitoring of Cyclosporine

Dr Sibtain Ahmed
Chemical Pathology

Cyclosporine is acyclic peptide composed of 11aminoacids. Discovered in the lab of Sandoz in Switzerland in 1972, cyclosporine has since revolutionized transplant medicine. It is a selective immunosuppressant agent. In the cytoplasm, cyclosporine binds to its immunophilin, cyclophylin forming a complex. The cyclosporine-cyclophylin complex binds and blocks the function of the enzyme a calcineurin, which has a serine/threonine phosphatase activity. As a result, calcineurin fails to dephosphorylate the cytoplasmic component of the nuclear factor of activated T cells, and thereby the transport of activated T cells to the nucleus. Consequently, T cells do not produce IL-2, which is necessary for full T-cell activation. Therefore it inhibits T cell activation.

Cyclosporine is indicated for the prophylaxis of organ rejection in kidney, liver, heart and bone marrow transplants. The drug may also be used in the treatment of chronic rejection in patients previously treated with other immunosuppressive agents.
It is also used for the treatment of autoimmune conditions, such as rheumatoid arthritis and psoriasis to ease symptoms. Cyclosporine presents some pharmacokinetic features listed below that make therapeutic drug monitoring imperative:

- Narrow therapeutic index
- Extremely high pharmacokinetic variability
- Differences in bioavailability between different pharmaceutical products on the market
- High risk of interactions cytochrome P450 3A4 and 3A5 dependent with direct and immediate consequences (over/under dosing)
- Nephrotoxicity that is dose dependent

The drug exhibits very poor solubility in water, and, as a consequence, suspension and emulsion forms of the drug have been developed for oral administration and for injection. Cyclosporine formulations are not bioequivalent and cannot be used interchangeably. Formulations are described as modified (Neoral) and non-modified forms (Sandimune). Peak concentrations are reached in two - six hours after oral administration with non-modified formulations and 1 to 2 hours after the modified forms. Elimination of cyclosporine is biphasic and is primarily biliary. Terminal half-life is variable with formulation and patient, ranging from five to 18 hours for the modified forms and from 10 to 27 hours for the non-modified forms.

Patients treated with the cyclosporine are at high risk of developing renal injury. Calcineurin inhibitor nephrotoxicity is manifested either as acute azotemia, which is largely reversible after reducing the dose, or as chronic progressive renal disease, which is usually irreversible. Other renal effects of cyclosporine include tubular dysfunction and, rarely, a hemolytic uremic syndrome that can lead to acute graft loss. Metabolic abnormalities resulting from alterations in tubular function noted with cyclosporine therapy include hyperkalemia, hyperuricemia, metabolic acidosis, hypophosphatemia, hypomagnesemia, and hypercalciuria. Acute cyclosporine nephrotoxicity is usually reversible with cessation of therapy as both the plasma creatinine concentration and systemic blood pressure fall toward baseline values for that patient . The important clinical problem is to differentiate cyclosporine-induced renal dysfunction from acute rejection. The only definitive diagnostic test is biopsy of the renal allograft.

Monitoring should take into account the blood level of cyclosporine and the therapeutic interval (different for renal, liver and heart transplantation) and the correlation that exists between this interval and acute graft rejection, on one hand, and nephrotoxicity, on the other hand.With eighty percent of cyclosporine sequestered in erythrocytes, whole blood is the preferred specimen for analysis. Peak concentrations (C2) are defined as samples collected two hours post-dose. High levels of cyclosporine in peak samples are correlated with reduced rejection rates, especially in the first year after transplant surgery.Therapeutic ranges usually are based on specimens drawn at trough or C0 (i.e. immediately before the next scheduled dose). Recommended therapeutic ranges for cyclosporine for various transplants are described in Table 1 and Table 2.

Table 1. Recommended Therapeutic ranges for Cyclosporine (C0 levels)

Post Renal Transplant	Cyclosporine Levels
Up to 2 months	200-300 ng/ml
3 months	200 ng/ml
4 - 12 months	80-180 ng/ml
Post Liver Transplant	**Cyclosporine Levels**
Less than or equal to 1 month	350-450 ng/ml
2-6 months	250-350 ng/ml
More than 6 months	170-240 ng/ml

Post Cardiac Transplant	Cyclosporine Levels
Less than 6 weeks	300-420 ng/ml
6-12 weeks	180-300 ng/ml
More than 12 weeks	120-180 ng/ml

Table 2. Recommended Therapeutic ranges for Cyclosporine (C2 levels)

Post Renal Transplant	Cyclosporine Levels
Up to 1 month	1700 ng/ml *
2 months	1500 ng/ml *
3-4 months	1200 ng/ml *
5-6 months	1000 ng/ml *
7-12 months	800 ng/ml *

* (+/- 20%)

The purpose of monitoring is to prevent rejection (graft survival) and improved tolerance (avoidance of adverse reactions, particularly nephrotoxicity and too high immuno-suppression). Trough (C0) residual concentration is directly correlated with nephrotoxicity, but it is not a useful marker for prediction of acute rejection. Both nephrotoxicity and acute rejection are better correlated with the area under the concentration-time curve measured between 0 - 4 hour or 0 -12 hour (AUC0-4, AUC0-12). These values can be better estimated using the value of C2 than the residual concentration (C0).

Tacrolimus for Prevention of Transplant Rejection

Dr Shabnam Dildar
Chemical Pathology

Tacrolimus (previously known as FK506) is a 23-membered macrolide lactone antibiotic isolated from the fermentation broth of a fungus *Streptomyces tsukubaensis*. It is an immunosuppressive agent belonging to the calcineurin inhibitor group. It emerged as a valuable therapeutic alternative to cyclosporine following solid organ transplantation, was granted approval by the U.S. Food and Drug Administration (FDA) for preventing rejection after liver (1994), kidney (1997), and heart transplants (2006). It is also used for the therapy of autoimmune diseases and to reduce inflammation in inflammatory bowel disease, severe steroid-refractory or steroid-dependent ulcerative colitis, and Crohn's disease.

Mechanism of Action

After entry into the cell, tacrolimus binds to the FK-binding protein (FKBP) which is an intracellular cytoplasmic immunophilin. The drug-immunophilin complex binds and inhibits the activity of the phosphatase activity of calcineurin. The calcium/calmodulin-dependent protein phosphatase interrupts the calcium-dependent signal transduction pathway in T-cells. Inhibition of calcineurin by tacrolimus leads to interference with translocation to the nucleus of various nuclear factors involved in the transcription of cytokine genes, such as the cytosolic subunit of the nuclear factor of activated T-cells. Thus preventing its entrance into the nucleus. Therefore leads to

the inhibition of T-cell activation, resulting in immunosuppression. Although this activity is similar to that of cyclosporin, studies have shown that the incidence of acute rejection is reduced by tacrolimus use over cyclosporin. Although short-term immunosuppression concerning patient and graft survival is found to be similar between the two drugs, tacrolimus results in a more favorable lipid profile, and this may have important long-term implications given the prognostic influence of rejection on graft survival.

Tacrolimus Metabolism

Tacrolimus is highly lipophilic and is excreted from the body after receiving extensive metabolism. Tacrolimus is metabolized by Cytochrome P450 3A4 (CYP3A4), thus its concentrations are affected by drugs that inhibit (calcium channel blockers, antifungal agents, some antibiotics, grapefruit juice) or induce (anticonvulsants, rifampin) this enzyme. Target steady-state concentrations vary depending on clinical protocol, the presence or risk of rejection, time from transplant, type of allograft, concomitant immunosuppression, and side effects (mainly nephrotoxicity). Optimal trough blood concentrations are generally between 5.0 and 20.0 ng/mL. Adverse reactions tend to occur the most frequently in the first few months after transplantation and decrease with time possibly in line with reductions in its concentration. Adverse reactions include nephrotoxicity, neurotoxicity, diabetogenesis, gastrointestinal disturbances, hypertension, infections and malignant complications.

Therapeutic Drug Monitoring of Tacrolimus

Tacrolimus has a low therapeutic index with a high inter-and intra-pharmacokinetic variability, and possesses several side effects and drug interactions. All these factors requires individualized monitoring of the drug levels in blood. Therapeutic monitoring of tacrolimus is useful for guiding dosage adjustments to achieve optimal immunosuppression while minimizing dose-dependent toxicity. Therapeutic drug monitoring of tacrolimus is usually initiated on the second or third day of therapy. It is continued for the first two weeks, three - seven times per week; with a gradual reduction in the monitoring frequency. Therapeutic Drug Monitoring of tacrolimus is important for monitoring whole blood tacrolimus concentration during therapy, particularly in individuals who are on CYP3A4 substrates, inhibitors, or inducers. Is also essential to monitor tacrolimus levels when adjusting dose to optimize immunosuppression while minimizing toxicity and also for evaluating patient compliance.

Some key points to note while monitoring tacrolimus are:

- Peak tacrolimus levels in whole blood are achieved about one - two hours following oral administration
- The cornerstone of tacrolimus evaluation is the trough level obtained 12 hours after the administration of the drug. This measurement provides a good indication of the total drug exposure.
- There is not a good correlation, as with some other medications, between the dose of tacrolimus given and level of drug in the blood
- Absorption and metabolism of oral doses of tacrolimus can vary greatly between people and even in the same person depending on the time of the dose and what, if any, food has been eaten
- Tacrolimus can cause kidney damage (nephrotoxicity), especially in high doses. Measuring levels in patients who have had a kidney transplant may help to distinguish between kidney damage due to rejection (because drug level is low) and kidney damage due to tacrolimus toxicity (drug level is high)

Specimen Type and Therapeutic Range

Since 90 per cent of tacrolimus is in the cellular components of blood, especially erythrocytes, whole blood is the preferred specimen for analysis of trough concentrations.

Target range for trough whole-blood sample is 5-20 ng/ml. The recommended therapeutic range applies to trough specimens drawn just before the next dose (i.e. immediately before a scheduled dose). Blood drawn at other times will yield higher results. Higher levels are often sought immediately after transplant, but as organ function stabilizes at about 4 weeks from transplant, doses are generally reduced in stable patients for most solid organ transplants. Trough concentrations should be maintained below 20 ng/mL. Preferred therapeutic ranges may vary by transplant type, protocol, and co-medications.

An Approach to Diagnosis of Infections in Febrile Neutropenic Patients

Dr Faryal Saleem
Microbiology

Febrile neutropenia is defined as 'single oral temperature of 38.5°C or more or a temperature of 38°C recorded on three different occasions within a 24 hour period at least four hours apart in a neutropenic patient'. Here, neutropenia maybe defined as an absolute neutrophil count (polymorphnuclear cells) of 500/ml or less.

In as many as 50 per cent of the cases, infections are the main cause of febrile neutropenia. The infections maybe bacterial, fungal or viral in origin but many a times typical signs and symptoms of these infections are absent due to the altered host immune responses. Fever might be the only manifestation thus it is important for physicians to do stringent monitoring of patients especially when they are undergoing a neutropenic phase during their chemotherapy.

It is imperative that all measures are taken to diagnose and treat any infections that may be a cause of febrile neutropenia in patients. Antibiotic therapy must be started within one hour of the patient reaching the hospital. Apart from complete blood count, including differential leukocyte counts and other biochemical markers of infections, there are various tests that are used in a laboratory to diagnose infections, with blood cultures being the primary diagnostic modality. For blood cultures to yield valuable results it is important that they are taken with standard protocol of aseptic techniques. At least two sets of blood cultures from two separate venipuncture sites are recommended. If indicated, cultures from any other clinically suspicious site may also be taken. Table 1 shows common organisms which are isolated from different clinical specimens through routine culture and molecular methods.

Positive blood cultures are gram stained and preliminary information is conveyed to the clinician so that any modification in empirical therapy

Table 1. Common Organisms isolated in various specimen cultures of Febrile Neutropenia

Causative Agent	Organisms	Specimens	Diagnostic Modalities
Bacteria	*Staphylococcus aureus* Streptococci *Pseudomonas species*	Blood, pus, body fluids, swabs from wounds etc.	Culture and sensitivities
	Enterococci *Escherichia coli* *Klebsiella species*	Blood, urine, pus, body fluids, swabs from wounds etc.	Culture and sensitivities
	Mycobacterium species	Sputum, bronchoalveolar lavage fluid, pus etc.	Culture and sensitivities
Fungi	*Candida species*	Blood, urine, body fluids, tissue culture etc.	Gram staining, culture and sensitivities, PCR
	Mucormycosis	Tissue culture, skin scrapings, etc.	Gram staining, **fungal smear and culture** and sensitivities, PCR
	Aspergillus spp	Tissue culture, skin scrapings, etc.	fungal smear and culture and sensitivities, PCR
Viral	Cytomegalovirus Herpes simplex virus	Bronchoalveolar lavage fluid, vesicular/pustular fluid etc.	PCR

may be instituted. Several rapid diagnostics and susceptibility methods have been developed from positive blood culture vials. Direct disc diffusion from positive vials is currently in use in several laboratories including ours. Molecular identification using multiplex PCR, matrix assisted laser desorption ionization- time of flight (MALDI-TOF), fluorescent in-situ hybridization (FISH) are just a few examples of advancements in rapid identification techniques which are expected to improve patient management. Similarly susceptibility testing methods employing detection of resistance associated gene mutations can provide additional benefit in guiding empirical therapy.

Thus, it is essential for the clinicians to evaluate the patients suffering from febrile neutropenia carefully and send all the relevant investigations without any delay to find out the etiology of fever as quickly as possible. In most of the cases a positive blood culture with proper identification and susceptibility profile of the causative agent is enough to warrant an early diagnosis of infection. In these cases, timely use of appropriate antimicrobials may prove to be lifesaving for the patient.

Significance of CMV Antigenemia Assay In Renal Transplant Patients

Waqas Khan and Saher Faisal
Molecular Pathology

Introduction

Pp65 CMV antigenemia test allows the indirect immunofluorescence detection of internal matrix phosphoprotein (protein kinase) 65-68 KD (pp65) of Human Cytomegalovirus (HCMV) in peripheral blood leukocytes. The pp65 detection in peripheral blood leukocytes allows the diagnosis of acute or reactivated infection. The HCMV pp65 antigenemia is a rapid, quantitative, simple, sensitive easy for the diagnostic of active Cytomegalovirus infection. Antigenemia is more sensitive than viral isolation and rapid culture serology. In all cases, the sensitivity is 100% in symptomatic patients. Antigenemia can be detected from several days to one week before the onset of symptoms. Antigenemia is successfully used in the diagnostic and follow-up of HCMV infections in heart, heart-lung liver, kidney, and bone-marrow graft recipients and in AIDS patients.

Principle of the Assay

This method uses a monoclonal antibodies pool which recognizes two epitopes of the protein (pp65) expressed in the nucleus of peripheral blood HCMV infected polymorph nuclear and mononuclear cells, during blood dissemination. Fluorescein isothiocyanate (FITC) conjugated antibody will bind to the antigen-antibody complex. Unbound conjugate is removed by washing with PBS. FITC exhibits an apple green fluorescence when excited by ultraviolet light allowing visualization of the complex by fluorescence microscopy. Nuclear fluorescence indicates a positive specimen. Uninfected cells counter-stain dull red due to the presence of Evans blue in the FITC conjugated antibody reagent.

Utility of the Assay

HCMV antigenemia test is recommended for the diagnosis of patients infected with CMV virus as this test is used to detect the antigen of cytomegalovirus (CMV). This test may also be used when a CMV infection is suspected in patients with HIV or organ transplant recipients.

Results Interpretation

The HCMV isolation from leukocytes of peripheral blood (viremia) and/or pp65 antigenemia are

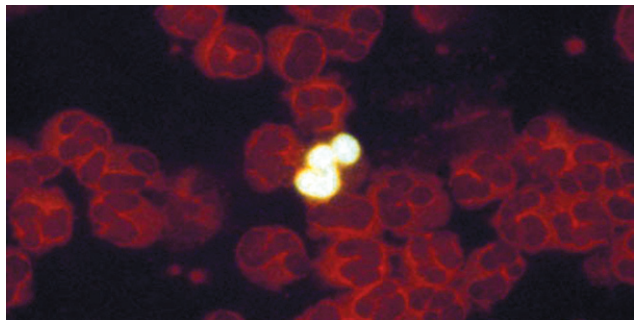


Figure 1. Antigenemia Signal from a CMV Positive Patient
Source: virology-online.com/viruses/CMV4.htm

evidence for active systemic infection. A single stained cell indicates a positive antigenemia.

Conclusion

Antigenemia is more sensitive than viral isolation, rapid culture and serology. Antigenemia can be detected from several days to one week before the onset of symptoms, and from two to 25 days before rapid culture. Antigenemia is also positive for a longer period than viremia demonstrated by co-culture. This test can be even performed by laboratories where the culture are not available, and only requires as specific apparatus a simple cytocentrifuge.

Pretransplant Serological Evaluation

Dr Hafsa Majid
Chemical Pathology

The success of solid organ transplantation requires careful selection of transplant recipient through a process of medical evaluation and screening. Two major complications occurring due to long term immunosuppression after transplant are infections and malignancy. That is why screening a potential transplant recipient for infectious diseases is an important pre-transplant component. Such screening may lead to the discovery and treatment of occult active infection improving patient outcomes, may help determine post-transplant prophylactic strategies, or may disqualify the recipient from receiving a transplant.

At the time a patient is identified as a potential transplant recipient, an initial evaluation including a detailed history and physical examination is required to assess the candidate's suitability for transplantation. This assessment includes laboratory screening tests and serological evaluation (Table 1) to establish the candidate's prior exposure to common organisms that may reactivate after organ transplantation . The United Network for Organ Sharing (USA) has issued this list of required infectious disease screening tests for both organ donors and potential recipients. Once a patient has cleared the initial evaluation, they may be listed for solid organ transplant.

Recommended hepatitis B (HBV) screening tests include hepatitis B surface antigen (HBsAg) and antibody (HBsAb) and hepatitis B core antibody (HBcAb). HBsAg positive or patients with any evidence of prior hepatitis B infection (HBsAg negative and HBcAb positive) can be further assessed by HBV DNA and treated if appropriate. Even patients with no evidence of viremia and isolated HBcAb positivity may experience HBV reactivation post-transplant occasionally leading to fulminant hepatitis. Patients with isolated HBsAb and a history of prior vaccination have demonstrated immunity to infection with hepatitis B and do not require HBV immunization pre-transplant. Screening for the presence of antibody to hepatitis C virus (HCV) in all candidates is necessary and further testing with HBV RNA if positive. Transplantation of non-hepatic organs into an HCV seropositive recipient may result in accelerated hepatitis and liver failure. Screening for viral pathogens such as HIV is a required part of the pre-transplant evaluation. Though not an absolute contraindication to transplant, HIV

Table 1. Pre-Transplant Serology Recommended for All Potential Organ Recipients and Donors

Pre-Transplant Serology Includes
Hepatitis B serology (HBsAg, HBcAb, HBsAb)
Hepatitis C Ab
HIV serology
CMV serology (IgM and IgG)
EBV serology
Toxoplasma IgG
Interferon gamma release assay or PPD skin test for *Mycobacterium tuberculosis*
Rapid plasma reagin (RPR) or specific treponemal testing

infection must be documented and viral replication controlled prior to surgery.

Screening for cytomegalovirus (CMV), a common post-transplant infection is also recommended as it is potentially transmissible through transplantation and carry a predictable disease risk. The seronegative recipient for CMV Ab receiving a seropositive donor's organ has the greatest risk of disease. Post-transplant cytomegalovirus may occur in any recipient positive (R+) case as well as in recipient negative patients exposed to the virus through blood transfusion or other close contact. Epstein-Barr virus (EBV) infection is a risk factor for post-transplant lymphoproliferative disease, particularly in seronegative patients who develop primary EBV infection due to seropositive donor. Screening for EBV can be done by testing Epstein Barr Nuclear Antigen (EBNA) IgG, EBV-Viral Capsid Antigen (VCA) IgM and EBV-VCA IgG. Primary infection is indicated by positive EBV-VCA IgM, EBV-VCA IgG and negative EBNA IgG. While past infection is presence of positive EBNA IgG, EBV-VCA-IgG and negative EBV-VCA IgM.

Although antibiotics given pretransplant provide coverage for infection with Toxoplasma, but screening for exposure to the parasite, *Toxoplasma gondii* is routinely performed. Because the organism can form cysts in organs especially myocardial tissue, so seronegative recipients of seropositive heart donors are at greatest risk for reactivation post-transplant. Reactivation disease in other seronegative organ transplant recipients can result in a nonspecific syndrome of multiorgan failure post-transplant that is often fatal if not recognized.

Latent bacterial infection with syphilis can be assessed using Rapid plasma reagin testing. Positive tests can be further confirmed with specific treponemal assays and treated. While latent infection with *Mycobacterium tuberculosis* can be detected using Purified protein derivative skin testing or interferon-gamma release assays for *Mycobacterium tuberculosis*. False negative results may occur in patients with organ failure as little is known about the efficacy of such testing in patients who may be immunocompromised as a result of pre-transplant organ dysfunction. Patients who test positive for latent tuberculosis should be evaluated clinically and radiologically for active disease and can be treated for latent tuberculosis where indicated.

Further to these tests all transplant candidates should also be tested for active infection with bacterial pathogens as clinically indicated. Active infections should be fully treated prior to transplant whenever possible. A thorough assessment of potential recipients prior to organ implantation will improve graft and patient survival, lower costs, and reduce infectious complications post-transplant.

Significance of Panel Reactive Antibody Testing in Renal Transplant Patients

Maheen Hassan, Sheeba Parveen, Abdul Jabbar and Tariq Moatter
Molecular Pathology

Introduction

The immune system makes abundance of proteins called antibodies. When an antibody recognizes the foreign proteins in case of infectious agent, organ transplant, blood transfusion and/or as a result of pregnancy, it recruits other proteins and cells to fight these foreign bodies off.

In case of an organ transplant patient, when one of the organs of the patient has failed to work due to illness or injury, antibody screening is essential. Before organ transplant, doctors must match if the recipient has pre-existing panel of antibodies that may react against donor's organ in order to reduce the chances of graft rejection.

Panel Reactive Antibody (PRA) is an immunological test routinely performed on the blood of people awaiting organ transplantation. The PRA score is expressed as a percentage between 0 per cent and 99 per cent that represents the likelihood of

the recipient's blood having an antibody against a particular donor. It represents the proportion of the population to which the person being tested will react via pre-existing antibodies. These antibodies target the Human Leukocyte Antigen (HLA), a protein found on most cells of the body.

A high PRA usually means that the individual is primed to react immunologically against a large proportion of the population. Individuals with a high PRA are often termed "sensitized", which indicates that they have been exposed to "foreign" (or "non-self") proteins in the past and have developed antibodies to them. Transplanting organs into recipients who are "sensitized" to the organs significantly increases the risk of rejection, resulting in higher immunosuppressant requirement and shorter transplant survival. People with high PRA therefore spend longer waiting for an organ to which they have no pre-existing antibodies.

Anti HLA antibodies are usually referred to as panel reactive antibodies (PRA) test that sometimes as referred to as percent reactive antibody, since the result is expressed as percentage.

Utility of the Assay

PRA is a very useful marker in renal transplantation. Monitoring panel reactive antibody levels is important with kidney transplant candidates. High PRA levels mean that doctors have more difficulty locating a matching donor, which can present a problem if an immediate transplant is necessary. This means that patients with a higher PRA count may have to wait much longer for a matching donor kidney. Patients with elevated PRA levels are also more likely to experience organ rejection and must be given a more advanced regimen of anti-rejection medications.

Principle of the Assay

Panel Reactive antibody testing utilizes a panel of color-coded beads, which are coated with purified HLA antigens. Test serum is first incubated with beads, any HLA antibodies present in the test serum bind to the antigens and then are labeled with R-Phycoerythrin (PE)-conjugated Goat anti-human IgG. The flow analyzer detects the fluorescent emission of PE from each bead, allowing almost real-time data acquisition.

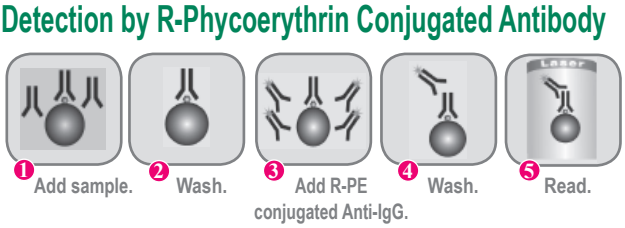


Figure 1. Principle of Panel reactive Antibody Testing
Source: www.szabo-scandic.com/uploads/media/OLI_ProductCatalog2013-14_web.pdf

Conclusion

Till date, PRA is the sole established quantitative indicator of pre-transplantation immunologic responsiveness. The exact incidence of humoral alloimmune responses after kidney transplantation is still uncertain, as routinely post-transplantation monitoring of antibodies is not performed. An allograft is the most important cause for development of antibodies, but transfusions, infections, and pregnancy can also stimulate antibody formation; the degree of sensitization is stronger and more prolonged when different causes act together in the same person.

Importance of Monitoring Cytomegalovirus (CMV) and BKV (Polyomavirus) Infection in Renal Transplant Patients

Dr Mahesh Kumar
Molecular Pathology

Kidney transplantation is indicated in patients with end stage renal disease, patients who have estimated renal function <20 per cent of normal on two sequential determinant or dialysis dependent or significant signs and symptoms of uremia such as nausea, loss of appetite, insomnia or chronic fatigue

or acid-base or electrolyte irregularities unresponsive to oral treatment. Viral infections i.e. CMV and BKV are major problem in these allograft recipients, most commonly one - six months after transplantation, results from reactivation of "latent" viral infection in the host or from the graft. Therefore, monitoring for reactivation of these viral infections should be routine.

Viral Latency and Reactivation

The nature of viral latency varies with the specific virus, the tissue infected, and the nature of the host immune response. Some latent viruses are metabolically inactive, whereas others are constantly replicating at low levels determined by the effectiveness of the host's immune response. Multiple factors contribute to viral activation after transplantation, including immune suppression (especially reduction of cytotoxic immunity), graft rejection and therapy, inflammation (cytokines), and tissue injury. Cellular pathways activated after transplantation are involved in the control of viral replication, including nuclear factor KB, IKB, and JAK-STAT (the Janus family of protein tyrosine kinases [JAKS] and signal transducers and activators of transcription [STAT]proteins) . Treatment of rejection can also result in a significant release of proinflammatory cytokines, including TNF-alpha and IL-1B, which may increase viral replication. Viral latency may be interrupted periodically, leading to reactivation and spread of infectious virus with recurrent disease. For CMV, viral genomes can be found in CD14+ monocytes and CD34+ progenitor cells, but the primary reservoir for latent cytomegalovirus and the mechanisms by which latent CMV infection is maintained are unknown. Subclinical activation of CMV is common. CMV reactivation has been extensively studied. Allogeneic immune responses and fever (via TNF alpha) have been shown in vitro to up-regulate both CMV promoter activity and viral replication. Immune suppression is not essential for the reactivation of latent CMV but serves to perpetuate such infections once activated. For other viruses (e.g. BK polyomavirus), specific types of tissue damage (warm ischemia, reperfusion injury, but not cold ischemia) may precipitate viral activation. Warm ischemia and reperfusion have been linked to an inflammatory state in grafts (via activation of TNF-alpha, nuclear factorKB, neutrophil infiltration, and nitric oxide synthesis), tubular-cell injury, and enhanced expression of cell-surface molecules. These changes contribute to viral activation. Thus, immune injury, inflammatory cytokines, and ischemia-reperfusion injury stimulate viral replication and alterations in the expression of virus specific cell-surface receptors. The host response is also less effective because of the mismatch in major histocompatibility antigens between the organ donor and host, which reduces the efficacy of direct pathway antiviral cellular immune responses. These factors render the allograft susceptible to invasive viral infection.

Clinical Value of Viral Load Screening and Monitoring

CMV

Cytomegalovirus is the "classic" transplant associated viral infection. It causes fever, malaise, leucopenia, thrombocytopenia, and allograft dysfunction. Even asymptomatic infection with CMV is associated with renal allograft dysfunction, mortality, and graft loss, despite early detection and treatment of asymptomatic infections. Poor outcomes are associated with poor HLA matching and may reflect increased immunogenicity, inflammatory response, or failure to clear CMV. Figure 1 showing CMV virion structure.

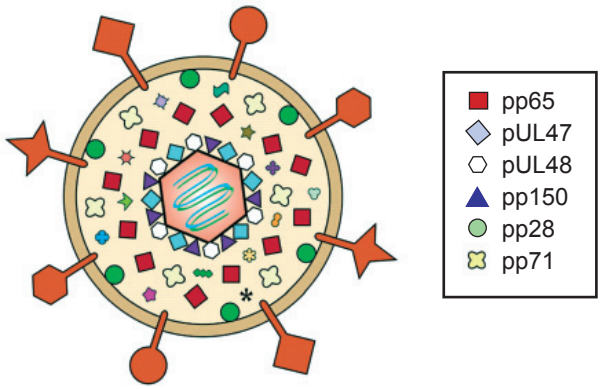


Figure 1. The HCMV virion.
The cartoon represents (not to scale) an average HCMV infectious viral particle. Abundant tegument proteins are listed. The large shapes on the surface of the virion represent various virally encoded membrane glycoproteins.
Source: mmbr.asm.org/content/72/2/249/F1.large.jpg

BKV

The BK virus, named after the initials of the patient in which it was first isolated is the most recently recognized viral pathogen affecting renal transplant recipients. It was originally reported in 1971. Primary infection usually occurs early in the childhood, at a median age of five years, and is characterized by low upper respiratory tract morbidity or is asymptomatic. Following primary infection, BKV remain latent in

different sites, including the Reno urinary tract, as the epidemiologically most relevant latency site, B cells, brain, spleen, and probably other organs. Periodical reactivation may occur in both immunocompetent individuals (in 0 per cent up to 62 per cent) and immunocompromised patients and is evidenced by asymptomatic viruria. In the current era of immunosuppression, BK virus causes nephropathy in up to eight per cent of recipients, and frequently results in allograft loss or permanent dysfunction. It presents as an asymptomatic gradual rise in creatinine with a tubulointerstitial nephritis that mimics rejection on biopsy and produces a treatment dilemma. Viral replication begins early after transplantation and progresses through detectable stages - viruria, then viremia, then nephropathy. Figure 2 showing BK virus genome structure.

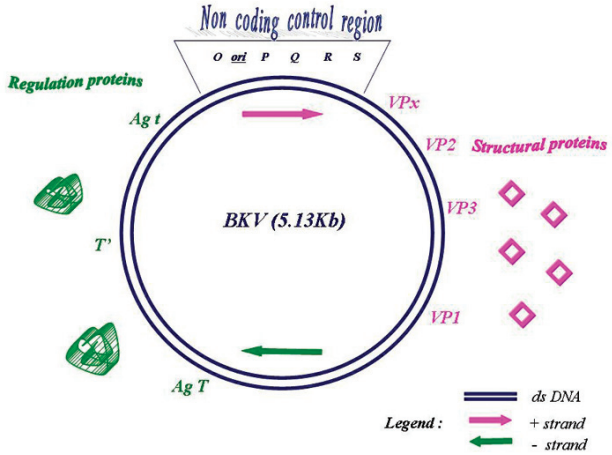


Figure 2. Schematic representation of the gene organization in the BK virus (BKV) genome. The double circle represents the double stranded DNA genomes. The genome is divided into three regions. The early region encodes three regulatory proteins (Agt, AgT, T'). The late region specifies four structural proteins and agnoprotein (VP1, VP2, VP3, VPx). The non-coding control region contains the elements for the control of viral DNA replication (ori) and viral gene expression The arrows indicate the positive and negative strands according to the direction of viral transcription
Source: Mischitelli et al. Virology Journal 2008 5:38

Diagnosis

Molecular techniques including PCR are monitoring tests of choice. Qualitative CMV PCR detects only presence/absence of viral DNA and is useful in systemic CMV infection in immunocompromised patients, antenatal diagnosis of congenital CMV infection, CMV retinitis and encephalomyelitis whereas quantitative CMV detects "viral load" which identifies both virologic response (guiding therapy duration) as well as possible presence of antiviral resistance. Molecular Pathology department Aga Khan hospital is performing qualitative and quantitative CMV and quantitative BK virus PCR.

Qualitative CMV PCR

Principle: DNA is extracted from blood followed by PCR amplification of conserve region of the DNA polymerase of CMV by real time PCR. Human apoprotein gene is also amplified simultaneously and serves as internal control. Interpretation of the assay is based on the strength of fluorescence signals.

Quantitative CMV PCR

Principle: DNA extraction from plasma is followed by PCR amplification performed in real time PCR instrument. The PCR primers and probes target genes within the CMV genome and provide viral load in IU/ml. Lower detection limit of the assay is 30 IU/ml. PCR results are shown in Figure 3.

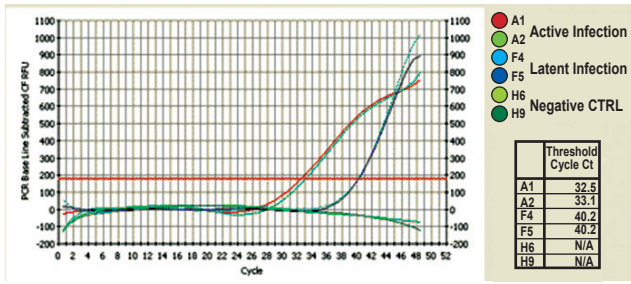


Figure 3. Two positive (active infection and latent infection) clinical samples was tested . All positive samples were amplified and the cycle threshold show the difference between an active infection and a latent infection

Quantitative BKV

Principle: DNA is extracted from serum or urine sample followed by amplification by real time PCR. The PCR primers and probes target genes within the BKV genome and provide viral load in IU/ml. PCR results are shown in Figure 4.

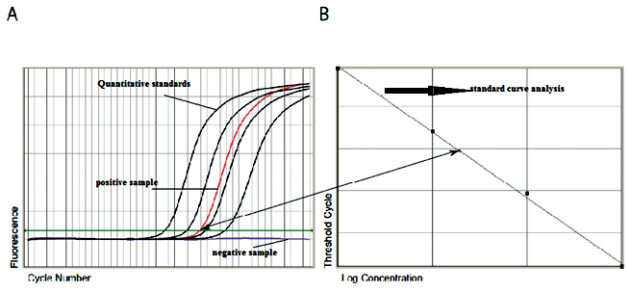


Figure 4. Result interpretation: Quantification Standards (black), a positive (red) and a negative sample (blue) displayed in the Amplification Plot (A) and Standard Curve analysis (B).

Conclusion

Both viruses are opportunists in the setting of transplantation, with potential effects, the clinical

problems currently associated with either virus are fundamentally different. CMV can affect any organ system, with substantial morbidity and mortality, all of which can be essentially controlled by effective antivirals. BKV on the other hand causes severe pathologies in the Reno urinary tract in a limited number of kidney transplant recipients. Access to invasive procedures and biopsy workup is required for definitive diagnosis of CMV and BKV disease. However, for both agents, the most relevant diagnostic study in the clinical setting is early detection and quantification of virus replication in blood. Assays quantifying virus-specific cellular immune responses in real-time are important new avenues to be explored to better predict risk of replication and disease and to optimize clinical management.

Meeting Report: Committee on Reference Intervals and Decision Limits (C-RIDL), IFCC Meeting, Symposium and Workshop

Dr Lena Jafri
Chemical Pathology

The Committee on Reference Intervals and Decision Limits (C-RIDL), IFCC Meeting related to 'Global Multicenter Study on Reference Values' was held at the Fukuoka International Convention Center, Japan on 24th and 25th November 2014. It was chaired by Professor Kiyoshi Ichihara, Professor Department of Clinical Laboratory Science, Yamaguchi University Graduate School of Medicine, Chair, C-RIDL, IFCC and the coordinator of the Global Multicenter Study on Reference Values. After a welcome to participants and a short tour-de-table, Prof. Ichihara described background and objectives of the meeting. He updated the group that there were nineteen countries from 5 continents collaborating in this global study; collaborating countries were Turkey, Japan, China, India, Philippines, US, UK, Argentina, Saudi Arabia, Kenya , Nigeria, Russia, Nepal, Bangladesh, Pakistan, Denmark, Egypt and Malaysia. He displayed the current status of each country, the number of analytes proposed, country codes, sample size, test panels and financial support of each region.

It was well attended by, principal investigators and co-investigators of the global C-RIDL study from various countries; India, Saudi Arabia, China, Russia, Nepal, South Africa, Philippines, Pakistan. Bangladesh, Egypt and Malaysia. The meeting continued as each country representative gave a quick update and progress report of their study. Representatives invited from Pakistan were Dr Lena Jafri, from Section of Chemical Pathology, Department of Pathology & Microbiology AKUH and Prof. Dr Brig. Dilshad, from Armed Forces Institute Rawalpindi. The Aga Khan University is one of the collaborators of this study and there is an additional interest in exploring influence of ethnicity on reference intervals within Pakistan. This is a unique opportunity for AKUH since no reference interval is available which has been derived from the local population. AKUH with its countrywide extensive network is well equipped to take on a study of this magnitude.

Prof. Ichihara explained to the members the difference between primary and secondary exclusion criteria. He clarified to the members that to reveal a comprehensive picture of reference intervals, C-RIDL is coordinating this multicenter RI study with recruitment of a sufficient number of individuals to ensure traceability and harmonization of the test results. This was followed by suggestions and tips for recording questionnaire and use of codes for drugs while entering data was advised. The morning meeting was followed by a Symposium "IFCC Global multicenter study on reference values: major findings and their implications" in the afternoon. It was coordinated by Prof. Ichihara and Prof. Rajiv Erasmus from Stellenbosch University, South Africa. This session had several speakers who discussed findings from their respective population. The study collaborators from China, Saudi Arabia, India and South Africa presented their results of their regions using the same IFCC-CRIDL protocol. This was followed by an intense question answer session.

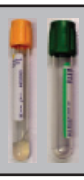
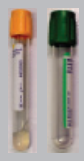
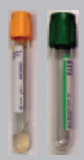
Investigators and co-investigators from various regions of the world at the 'Committee on Reference Intervals and Decision Limits (C-RIDL), IFCC' Meeting

On the 25th Nov C-RIDL meeting continued and all the members gathered at the Fukuoka International Convention Center again. Extensive discussion on statistical tools used like parametric versus non-parametric, bootstrap method for smoothing the reference intervals and prediction of 90% confidence interval of reference intervals limits was done. This meeting was followed by a workshop "Hands-on: Statistical methods for the reference interval study". The new and latest software 'RI-Master' made by Reo Kawano and Prof. Ichihara was introduced to the group. The software was provided to all the participants and a hands-on training session was given. This session enabled the participants to get to know the statistical software, removing curiosity and perhaps some trepidation.

Early morning on the 26th November the group set off for an excursion to Kumamoto where the witnessed volcanic unrest of Mount 'Aso', hot springs and the four hundred year old Kumamoto Castle. This trip provided us an opportunity to interact and share ideas in an informal setting. The event ended with a traditional multi-course Japanese style farewell dinner, Kaiseki (懐石) in a quiet tranquil environment.

Details Of Therapeutic Drug Monitoring

Hafsa Majid, Chemical Pathology

S. No	Drug	Time of Sample Collection	Therapeutic Ranges	Reporting
1.	Valproic Acid 	Trough (predose)	50 - 100 µg/ml	Same day
2.	Tegretol 	Trough (predose)	4-12 µg/ml	Same day
3.	Phenytoin 	Trough (predose)	10-20ug/ml	Performed from Monday to Friday and reported same day
4.	Phenobarbital 	Trough (predose)	10-40ug/ml	Performed from Monday to Friday and reported same day
5.	Gentamicin 	Peak testing (after 2-4 hours post dose) Trough (predose)	**Peak:** Less severe infection 5-8 ug/ml Severe infection 8-10 ug/ml **Trough:** Less severe infection<1.0ug/ml Moderate infection<2.0ug/ml Severe infection<4.0ug/ml **Toxic:** Trough > 2.0 ug/ml Peak > 10.0 ug/ml	Same day
6.	Vancomycin 	Trough Vancomycin concentrations are the most accurate and practical method to guide Vancomycin dosing. Serum trough concentrations should be obtained prior to the fourth or fifth dose.	Therapeutic concentration >10 ug/ml Trough 15-20 ug/ml	Same day
7.	Amikacin 	Peak testing (after 2-4 hours post dose) Trough (predose)	Trough 5-10 ug/ml Peak 20-25 ug/ml **Toxic:** Trough > 10 ug/ml Peak > 35 ug/ml	Same day

S. No	Drug	Time of Sample Collection	Therapeutic Ranges	Reporting
8.	Cyclosporin 	Trough (predose)	**Post renal transplants:** Upto 2 months 200-300 ng/ml 3 months 200 ng/ml 4-12 months 80-180 ng/ml **Post liver transplants:** ≤ 1 month 350-450 ng/ml 2-6 months 250-350 ng/ml >6 months 170-240 ng/ml **Post cardiac transplants:** <6 weeks 300-420 ng/ml 6-12 weeks 180-300 ng/ml >12 weeks 120-180 ng/ml	Performed from Monday to Saturday and reported same day Results of inpatients undergoing transplant are reported within 4 hours after receiving specimen.
9	Cyclosporin C2 Monitoring 	Peak testing (after 2-4 hours post dose)	**Post renal transplant:** Upto 1 month 1700 ng/ml 2 months 1500 ng/ml 3-4 months 1200 ng/ml 5-6 months 1000 ng/ml 7-12 months 800 ng/ml	Performed from Monday to Saturday and reported same day Results of inpatients undergoing transplant are reported within 4 hours after receiving specimen.
10.	Tacrolimus 	Trough (predose)	5-20 ng/ml	Performed on Wednesday and reported next day Results of inpatients undergoing transplant are reported within 4 hours after receiving specimen.
11.	Methotrexate 	Trough (predose) Collect sample after 72 hours of therapy	Non-toxic concentration <0.1 umol/l	Same day
12.	Lithium 	Trough (Predose or after 12 hours post dose)	Therapeutic Range: 0.6 - 1.2mmol/l Toxic: >2.0mmol/l	Same day
13.	Theophylline 	Peak testing (after 2-4 hours post dose) Trough (predose)	Therapeutic Range 10-20ug/ml	Same day
14.	Digoxin 	Peak tissue concentration (after 8 hours post dose) Trough (predose)	Therapeutic Range 0.9-2.0ng/ml Toxic >2.0ng/ml	Same day
Special instruction: Mention drug dosing frequency, route and sample is trough or peak. Mention STAT on the request slip if results are needed urgently.				

THE AGA KHAN UNIVERSITY HOSPITAL, CLINICAL LABORATORIES - KARACHI

Health Screening Package

Package Code : HSP

Tests	Price
CBC	700
Urine D/R	380
Stool D/R	390
Fasting Gulcose	370
Fasting Cholestrol	600
Serum Creatinine	660
Liver Function Test	1,450
Total	**4,550**
Discount	**910**
Package Price after discount (rounded to 10)	**3,640**

Extended Health Screening Package

Package Code: EHSP

Tests	Price
CBC	700
Urine D/R	380
Stool D/R	390
Fasting Gulcose	370
Serum Creatinine	660
Liver Function Test	1,450
Lipid Profile	2,250
HepBs Ag	1,300
HepC AB	2,400
Total	**9,900**
Discount	**1,980**
Package Price after discount (rounded to 10)	**7,920**

Macrocytic Package

Package Code: MACP

Tests	Price
Vitamin B12	2,000
RBC Folate	1,990
Total	**3,990**
Discount	**790**
Package Price after discount (rounded to 10)	**3,200**

Bone Health Screening Package

Package Code: BHSP

Tests	Price
Calcium	600
Albumin	400
Corrected Total Calcium (calculated value)	60
Phosphorus	620
Magnesium	1,040
Creatinine	660
PTH	2,650
ALP	500
25 OH VIT D	3,500
Total	**10,030**
Discount	**2,000**
Package Price after discount (rounded to 10)	**8,030**

Renal Function Test Package

Package Code : RFTP

Tests	Price
Serum Sodium	410
Serum Chloride	410
Serum Potassium	410
Serum Bicarbonate	410
Blood Urea Nitrogen	600
Serum Creatinine	660
Urine D/R	380
Anion Gap (calculated value)	60
Total	**3,340**
Discount	**660**
Package Price after discount (rounded to 10)	**2,680**

Extended Renal Function Tests Package

Package Code : ERFTP

Tests	Price
Serum Sodium	410
Serum Chloride	410
Micro Albumin	1,050
Serum Potassium	410
Serum Bicarbonate	410
Blood Urea Nitrogen	600
Creatinine Clearance (ml/min)	1,600
Urine D/R	380
Anion Gap (calculated value)	60
Serum Calcium	600
Serum Phosphorous	620
Serum Parathroid Hormone	2,650
Serum Uric Acid	650
Albumin / Creatinine Ratio Per 24 hours	50
Total	**9,900**
Discount	**1,980**
Package Price after discount (rounded to 10)	**7,920**

Osteoporosis Package

Package Code : OSTPP

Tests	Price
Calcium	600
Albumin	400
Corrected Total Calcium (calculated value)	60
Phosphorus	620
25 OH VIT D	3,500
PTH	2,650
ALP	500
NTX	6,100
Total	**14,430**
Discount	**2,880**
Package Price after discount (rounded to 10)	**11,550**

Hepatitis Screening Package

Package Code : HEPSP

Tests	Price
SGPT (ALT)	380
Hepatits B Surface Antigen	1,300
Hepatits B Surface Antibody	1,600
Anti HCV	2,400
Total	**5,680**
Discount	**1,130**
Package Price after discount (rounded to 10)	**4,550**

Microcytic Package

Package Code : ANEMP

Tests	Price
Serum Iron	840
TIBC	780
Serum Ferritin	1,750
Total	**3,370**
Discount	**670**
Package Price after discount (rounded to 10)	**2,700**

Cardiac Risk Package

Package Code : CARP

Tests	Price
Fasting Gulcose	370
HsCRP	1,600
Lipid Profile	2,250
Homocysteine	2,400
Total	**6,620**
Discount	**1,320**
Package Price after discount (rounded to 10)	**5,300**

Antenatal Package

Package Code :ANTP

Tests	Price
CBC	700
Rscreen	1,200
Urine D/R	380
HBSAG	1,300
HCV	2,400
RUBIGG	1,750
RUBIGM	2,150
Anti-TPO	900
Total	**10,780**
Discount	**1,610**
Package Price after discount (rounded to 10)	**9,170**

Hemolytic Panel

Package Code : HEMANE

Tests	Price
Reticulocyte Count	500
LDH	600
Direct Coombs Test	660
Total	**1,760**
Discount	**350**
Package Price after discount (rounded to 10)	**1,410**

hospitals.aku.edu/Karachi/clinical-laboratories