7-2015

LABRAD : Vol 41, Issue 2 - July 2015

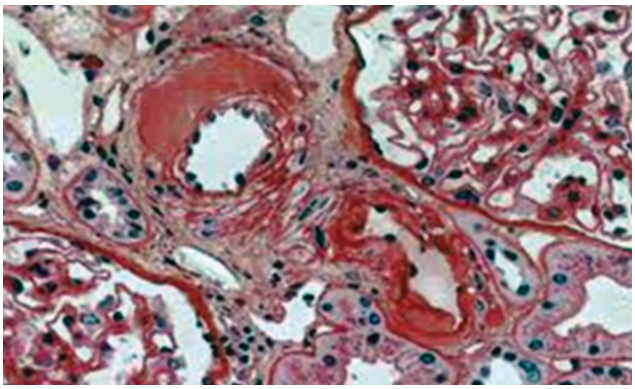
Aga Khan Universiity Hospital, Karachi

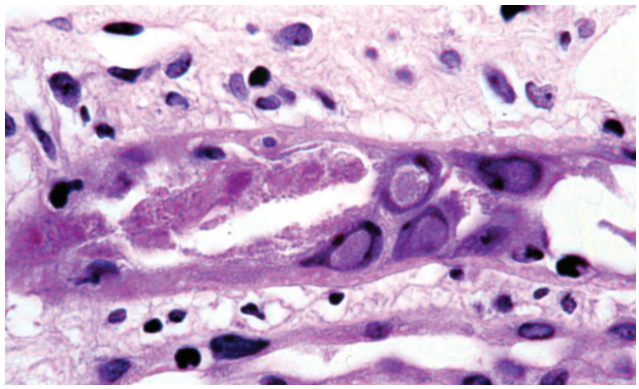
NEWSLETTER OF THE DEPARTMENTS OF PATHOLOGY & LABORATORY MEDICINE AND RADIOLOGY

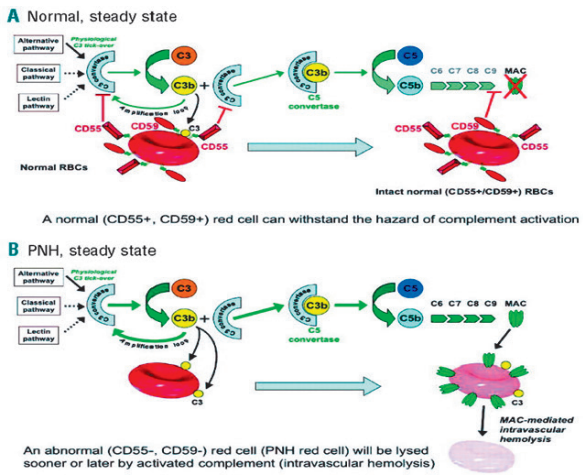
LABRAD

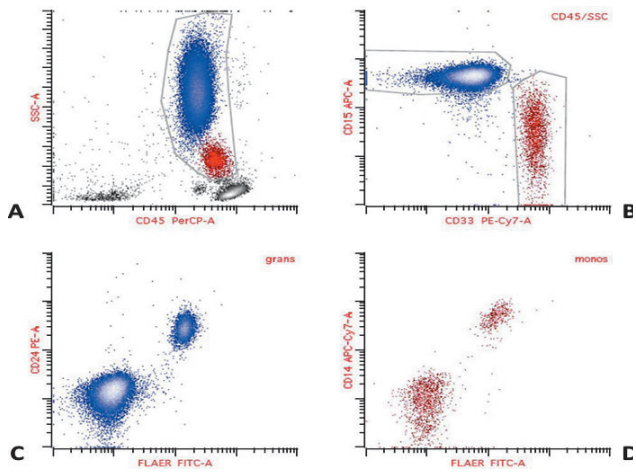
JULY 2015

VOL. 41, ISSUE 2









آغا خان یونیورسٹی ہسپتال، کراچی

The Aga Khan University Hospital, Karachi

LABRAD

A Publication of the Departments of Pathology & Laboratory Medicine and Radiology

July 2015
Volume 41, Issue 2

From the Editor's Desk

The year is flying by and it is time for the latest issue of LABRAD. This edition of LABRAD is non-thematic and includes diverse range of up-to-date topics in the expanding field of laboratory medicine. The LABRAD kicks off with an overview on IgG4 related diseases and its diagnostic challenges. IgG4-related disease is a recently recognized syndrome of unknown etiology most often occurring in middle-aged and older men. The Section of Chemical Pathology has recently started measuring subclass IgG4 in serum which can assist in the diagnosis of IgG4-related disease; however confirmed diagnosis requires a tissue biopsy of the affected organ.

Pathological changes in renal transplant biopsies either due to immunosuppressive drugs or the sequelae of the immunosuppressive state has been discussed later on in the newsletter. One of our hematology residents has elaborated on complement-mediated hemolysis 'Paroxysmal Nocturnal Hemoglobinuria' and how it can be diagnosed by flow cytometry.

This issue also includes an article on the utility of Beta-D-Glucan and Galactomannan testing in diagnosis of invasive fungal infections. Beta-D Glucan and Galactomannan are fungal cell wall antigens which can be measured in serum or bronchoalveolar lavage fluid. These tests have been recently introduced in the Section of Microbiology. Elevated Beta-D-Glucan and Galactomannan levels can help diagnose invasive fungal infections early and can also guide in commencement of therapy as it has both diagnostic as well as prognostic impact.

The pace of change in Laboratory Medicine is dizzying; both in numbers of laboratory tests and in the complexity of the results, as is the emerging need to integrate massive data sets to reach a clinical interpretation. The genomic success stories strongly suggest that personalized medicine will increase the role of clinical laboratories in patient management. These changes have profound implications for the discipline of Pathology and Laboratory Medicine and for how laboratory diagnosticians relate to medicine as a whole. LABRAD gives us an opportunity to connect to the clinicians, our peers and other health care providers. All faculty, technologists and residents are encouraged to keep sending articles or other contributions (interesting images in cytology, test/ procedure updates, and quiz) to us. The Department of Pathology and Laboratory Medicine members contribute extensively to healthcare, education and research arenas. Maintaining our visibility as key players in healthcare is crucial to the profession, I urge you to please send a small write up and photographs of your activities to us so that we could share it with all our readers.

We really hope this issue will provide some cool reading on a hot day!
Happy reading

Dr Lena Jafri
Associate Editor, LABRAD

An Overview of Immunoglobulin G4-Related Disease (IgG4-RD)

Dr Sheharbano Imran
Chemical Pathology

Immunoglobulin G4-related disease (IgG4-RD) is a rare systemic fibro-inflammatory disorder comprised of a collection of disorders that share specific pathologic, serologic, and clinical features. The pathogenesis of IgG4-RD is poorly understood; findings consistent with both an autoimmune disorder and an allergic disorder are present. The common features of IgG4-related disease are lymphoplasmacytic tissue infiltration usually accompanied by fibrosis, obliterative phlebitis, and elevated serum IgG4. However, approximately 30% of patients have normal serum

IgG4 concentrations despite classic histopathological and immunohistochemical findings. The hallmark histopathological features of IgG4-RD are

- A dense lymphoplasmacytic (lymphocyte and plasma cells) infiltrate rich in IgG4-positive plasma cells.
- Fibrosis, arranged at least focally, in a storiform pattern. 'Storiform' is commonly referred to as meaning 'having a cartwheel pattern', but its literal meaning is the appearance of 'a woven mat'
- The venous channels are obliterated by a dense lymphoplasmacytic infiltrate, within both the venous walls and the lumen.

Other histopathological features associated with IgG4-RD are phlebitis without obliteration of the lumen and/or tissue with increased numbers of eosinophil.

IgG4-RD can involve one or multiple sites in the body (salivary gland, orbits, pancreas, Para nasal sinus, lacrimal gland, thyroid, pleura, lymph nodes etc.). With multiorgan involvement, the sites involved can be affected at the same time (synchronously) or at different unrelated periods (metachronously). However, Type 1 autoimmune pancreatitis is the most frequent manifestation of this disease. Following medical conditions that have long been viewed as conditions confined to single organs are part of the spectrum of IgG4-related disease: autoimmune pancreatitis, Mikulicz disease, Kuttner's tumor, Riedel thyroiditis, eosinophilic angiocentric fibrosis, multifocal fibrosclerosis, inflammatory psuedotumor, fibrosing mediastinitis, sclerosing mesenteritis, retroperitoneal fibrosis, periaortitis/ periarteritis, inflammatory aortic aneurysm, cutaneous pseudolymphoma, idiopathic hypertrophic pachymeningitis, idiopathic tubulointerstitial nephritis, , idiopathic hypocomplementemic tubulointerstitial nephritis with extensive tubulointerstitial deposits and idiopathic cervical fibrosis.

Early detection is important to avoid organ damage and potentially serious complications. Diagnosing IgG4- RD is challenging and the awareness of this disease is low. The Clinical Laboratory of Aga Khan University Hospital has initiated the testing serum IgG4 which can assist in the diagnoses of IgG-4 related diseases.

Plasma Neutrophil Gelatinase Associated Lipocalin (NGAL) a Marker of Acute Kidney Injury

Dr Shabnum Khawaja
Chemical Pathology

Acute kidney injury (AKI) is characterized functionally by a rapid decline in the glomerular filtration rate (GFR), and biochemically by the resultant accumulation of nitrogenous wastes such as blood-urea nitrogen and creatinine. It has high incidence in hospitalized patients and poor prognosis in critically ill patients with a high mortality rate. At present, serum creatinine is used to measure the GFR and it is the most commonly used marker of renal function. Diagnosis of AKI still entirely based on an increase in serum creatinine or decrease in urine volume. Due to delay between changes in serum creatinine and changes in GFR inhibits the ability to accurately estimate timing of injury and severity of dysfunction following injury. Unfortunately, serum creatinine is a delayed and unreliable indicator of AKI, for the following reasons:

- Serum creatinine level is influenced by multiple non-renal factors, such as age, gender, muscle mass, muscle metabolism, diet, medications, and hydration status.
- In AKI, the serum creatinine level can take several hours or days to reach a new steady state and thus does not reflect the actual decrease in GFR in the acute setting.

- Because of renal reserve, the serum creatinine level may not rise until more than 50% of the kidney function has been lost.
- An increase in the serum creatinine level represents a delayed indication of a functional change in GFR that lags behind structural changes that occur in the kidney during the early stage of AKI.
- Serum creatinine measurement does not allow differentiation between hermodynamically mediated changes in renal function, such as pre-renal azotemia from intrinsic renal failure or obstructive uropathy.

NGAL for the prediction of AKI

Neutrophil gelatinase-associated lipocalin (NGAL) is a single disulphide-bridged polypeptide chain of 178 amino-acid residues with a molecular mass of 25kDa. It is a small protein expressed in neutrophils and certain epithelia, including the renal tubules. In healthy person it filtered out of plasma by the glomeruli when it passes through the kidneys then reabsorbed back into plasma in the tubules. In kidney inflammation its expression is greatly increased as a human immune response to inflammation and injury to epithelia and accumulation of in blood plasma, with increased concentrations in plasma and urine, could indicate AKI.

It is to be one of the earliest and most robustly induced proteins in the kidney after ischemic or nephrotoxic AKI. Its levels rise within two hours of the insult, making it as an early and sensitive biomarker of AKI. Plasma NGAL is a biomarker for the early prediction of AKI following defined clinical injuries such as cardiopulmonary bypass, contrast administration and kidney transplantation. Plasma NGAL can also be used for the early prediction of AKI, even in heterogeneous clinical situations where the timing of kidney injury is unknown, such as in the critical care or emergency settings. NGAL levels can also discriminate between true AKI and prerenal azotemia in unselected patients presenting for emergency care. Reduction in NGAL levels are becoming increasingly used as an efficacy marker in trials for the prevention and/or treatment of AKI. Early plasma NGAL concentrations are predictive of dialysis requirement, mortality and length of hospital stay in a variety of clinical AKI situations. NGAL is emerging as an excellent standalone troponin-like biomarker for the prediction of AKI and its clinical outcomes. It not only aids in diagnosis but levels also correlate with the severity of the AKI and with patients outcomes.

Celiac Disease: Biomarkers for Diagnosis and Monitoring

Dr Hafsa Majid
Chemical Pathology

Celiac Disease (CD) or gluten-sensitive enteropathy is an autoimmune disease caused by ingestion of gluten-containing cereals, in genetically predisposed individuals. It is characterized by inflammation and atrophy of the small intestinal villi, leading to reduced nutrients absorption. Clinical symptoms of CD are fatigue, abdominal pain, diarrhea, effects of malabsorption such as weight loss and growth retardation in children, vomiting, constipation, and bone pains. CD patients may also manifest with symptoms of nutritional deficiencies such as osteoporosis, anemia, neuropathies, carditis, pregnancy problems, or lymphoma. The only effective treatment for CD is adherence to a gluten-free diet.

The prevalence of symptomatic CD is around one percent. The frequency of CD is substantially increased in patients who have a first-degree family member affected with CD. However, most cases remain undiagnosed, only patients with classical variant are investigated and represent only the tip of the celiac iceberg. Recently, it has become increasingly evident that CD exists not only in its classical form, but also more frequently in asymptomatic or silent forms. These patients do not have symptoms, but they exhibit CD specific antibodies and villous atrophy. So a disease which was once believed to be a rare enteropathy of childhood is now considered to be a much more

common multi-organ disease. The exact prevalence of CD in Pakistan is not known, but it is felt to be a common disorder present in all four provinces.

Disease Pathogenesis

The cause of the pathological process leading to CD is prolamins, which are protein components of gluten found in many types of cereal. Gliadin is the most common occurring prolamin. Prolamins are only partly digested in the small intestine. The resulting peptides are taken up into the intestinal wall and deamidated by the enzyme tissue transglutaminase (TTG). The immune system of affected individuals is genetically predisposed to develop immune reactions against both the deamidated peptides and transglutaminase. These reactions cause chronic inflammation and atrophy of the small intestinal villi.

Diagnosis of CD

In recent years, advances in laboratory diagnostic tests for CD have transformed the way the disease is diagnosed and monitored, reducing the number of small bowel biopsies performed significantly. The target antigens are divided into two groups, first group includes autoantibodies [Anti endomysial (EMA) and anti-TTG] antibody tests, and second group comprise antibodies targeting the offending agent, gliadin. Key laboratory investigations comprise the determination of autoantibodies against TTG or endomysium (EmA), antibodies against deamidated gliadin peptides and the CD-associated human leukocyte antigens (HLA) DQ2 and DQ8. Newly published guidelines confirm the pivotal role of serological testing in CD diagnostics.

The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and American College of Gastroenterology guidelines on 'Diagnosis and Management of Celiac Disease' recommend the following:

- In patients with CD, Immunoglobulin-A (IgA) TTG antibody is the preferred single test in individuals over the age of two years.
- In high-probability patients both IgA and IgG-based testing, such as IgG-deamidated gliadin peptides (DGPs) and IgA-TTG should be done and intestinal biopsy should be pursued even if serologic tests are negative.
- If the anti-TTG antibody titer is very high (>10 times upper normal limit), and if this result is reinforced by other positive serology and compatible HLA, it is not necessary to perform a biopsy.
- In subjects suspected of low IgA or selective IgA deficiency, total IgA and IgG-based testing (IgG-DGPs and IgG-TTG) should be performed.
- In patients on a gluten-containing diet all diagnostic serologic testing should be done.
- Combining several tests for CD rather than IgA-TTG alone may increase the sensitivity for CD, especially in high risk group.
- Screening children younger than 2 years of age, the IgA TTG test should be combined with DGP (IgA and IgG).

Diagnostic tests should be carried out in individuals on a gluten-containing diet. A gluten challenge is only performed under exceptional circumstances.

Antibodies against Tissue Transglutaminase

Autoantibodies against TTG are the most important serological markers for CD. They are alternatively known as EmA, depending on the testing method used: EmA are determined using indirect immunofluorescence, while anti-TTG is detected using immunoassay test systems such as ELISA.

EmA and anti-TTG antibodies of immunoglobulin class IgA possess a very high sensitivity and specificity for CD. They virtually never occur in healthy individuals or patients with other intestinal diseases, whereas in untreated CD their prevalence is near 100 percent. Enzyme immunoassays for detection of anti-TTG antibodies are preferred due to their simplicity, cost-effectiveness, and automatability, combined with their high sensitivity and specificity. A multitude of clinical studies have confirmed the efficacy of ELISA method, yielding a sensitivity of 90 percent to 100 percent and a specificity of 95 percent to 100 percent for active CD.

Antibodies against deamidated gliadin peptides

Antibodies against gliadin also occur in patients with CD, and their determination strengthens a diagnosis. Recent scientific knowledge has revealed that only a tenth of the epitopes of the gliadin molecule are diagnostically relevant, and these must be present in deamidated form. These immunoassays assays provide significantly higher sensitivity and

specificity than conventional anti-gliadin assays. Multiple studies have shown that the new test yielded sensitivity (at 95 percent specificity) of 83 percent/94 percent (IgA/IgG).

Anti-DGP antibodies are a relatively new biomarker for CD diagnosis and management, recently this marker is introduced at clinical laboratories of Aga Khan University. Use of the anti-DGP antibodies in combination with the anti-TTG are shown to significantly increase the serological detection rate for CD. This biomarker is particularly valuable for identifying CD patients with an IgA deficiency, which is frequently associated with CD and in situations where biopsy cannot be performed due to any reason. Determination of antibodies against deamidated gliadin is also suitable for assessing disease activity and for monitoring a gluten-free diet or a gluten-load test.

In conclusion the confirmation of a diagnosis of CD should be based on a combination of findings from the medical history, physical examination, serology, and upper endoscopy with histological analysis of multiple biopsies of the duodenum. While patients with CD should be monitored regularly for residual or new symptoms, adherences to gluten free diet and assessment for complications. Monitoring of adherence to gluten free diet should be based on a combination of serology (IgA-TTG or IgA/IgG-DGP antibodies). In children, special attention to assure normal growth and development is recommended.

References:
A. R. Tapia et al. 'ACG Clinical Guidelines: Diagnosis and Management of Celiac Disease'. The American Journal of Gastroenterology. Vol 108; May 2013.
S. Husby et al. 'European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease'. JPGN. Volume 54; Jan 2012.

Pathophysiology of Paroxysmal Nocturnal Haemoglobinuria and Diagnosis by Flowcytommetry

Dr Nadia Nasir
Hematology

Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare, acquired stem cell disorder caused by a somatic mutation in the X-linked PIG-A gene. The PIG-A protein is involved in the initial stage of synthesis of the glycosylphosphatidyl-inositol

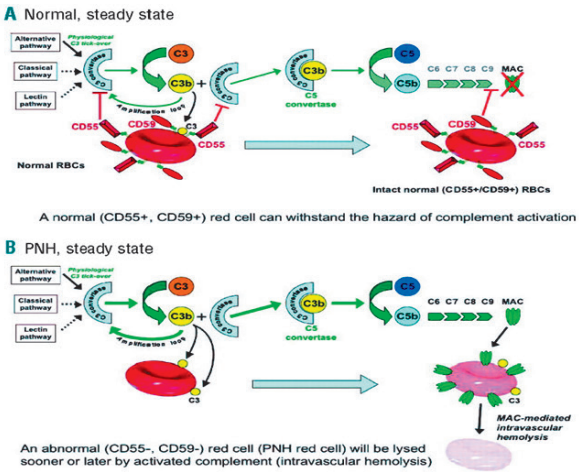


Figure 1: Pathogenesis of PNH adapted from Haematologica April 2010 95: 523-526

(GPI) anchors. There is a partial or absolute defect in the biosynthesis and expression of glycophosphatidylinositol V (GPI) linked structures including CD55 and CD59 on red cells. Absence of these anchors on red cells is largely responsible for intravascular hemolysis associated with clinical PNH.

PNH has three distinctive clinical features that vary greatly from patient to patient and during the course of the disease. First, there is complement mediated and predominantly intravascular hemolysis. These result in clinical features such as, erectile dysfunction, chronic renal failure, pulmonary hypertension, anemia, and,hemoglobinuria.

Second, there is a characteristic thrombotic tendency that occurs in extremities along with hepatic portal (Budd-Chiari Syndrome), splenic, or mesenteric veins.
Third, there is underlying bone marrow failure, which occurs to some degree in all patients, and in

its most extreme form, presents as immune-mediated severe aplastic anemia.

Diagnosis by Flow Cytommerty

1. RBC Antigen Assays

These were the first used to detect PNH because DAF (CD55) and MIRL (CD59) were recognized early on as the proteins whose deficiency was central to the pathophysiology of PNH. However testing of RBCs alone in a routine assay is not adequate for evaluation of these patients, because during acute hemolytic episodes, inadequate red blood cells are available for testing, hence such assays can undersestimate the size of a PNH clone.

2. Leukocyte Analysis

Assessment of PNH populations in leukocytes is widely recognized as the best method for assessing the true size of a PNH clone especially monocytes and granulocytes. Lymphocytes are not a suitable target because of their long life span and variable expression of many GPI-linked proteins. Both monocytes and neutrophils are analysed together to increase sensitivity of the test. Monocytic markers include CD14 and CD33 whearas granulocytic markers include CD66.

3. Analysis by FLAER

One of best reagents available to study GPI-linked antigens on leukocytes is the reagent fluorescent aerolysin or FLAER. This is a fluorochrome-conjugated inactive variant of the bacterially derived protein aerolysin, which binds specifically to the GPI anchor and is absent from GPI anchor–deficient granulocytes and monocytes.

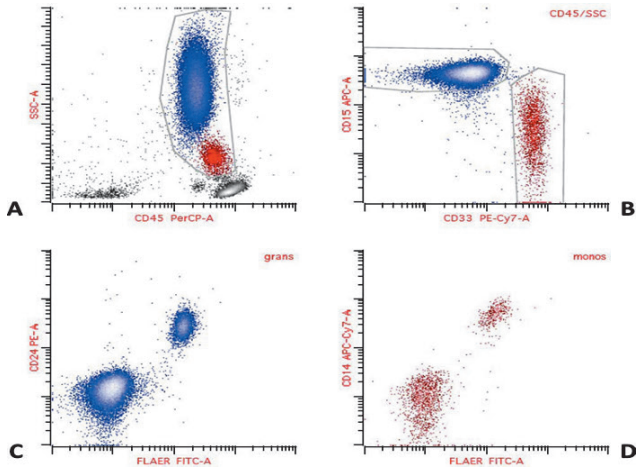


Image adapted from Guidelines for the Diagnosis and Monitoring PNH and Related disorders by flowcytometry; Cytometry Part B (Clinical Cytometry) 78B:211–230 (2010)

A: Initial gate set on a CD45/SSC shows both granulocytes and monocytes

B: Display of CD33 vs. CD15. Granulocytes (CD15 high, CD33) low are clearly separated from monocytes (CD15 low CD33 high) and are easily distinguished.

C: Display of FLAER and CD24 on the CD15 population showing a clear double negative PNH granulocyte population.

D: Display of FLAER and CD14 on the CD33 positive population showing a clear double negative PNH monocyte population.

Pathological Changes in Renal Transplant Biopsies not Related to Allo-Immune Mechanisms

Dr Mohammad Usman and Dr Saroona Haroon
Histopathology

In renal transplant recipients, a variety of factors lead to renal damage apart from the allo-immune reactions. These morphologic features observed in these conditions can be either directly related to immunosuppressive drugs or the sequelae of the immunosuppressive state. Protocol for obtaining and evaluation of renal biopsy in these conditions is similar to that of a non-transplant biopsy.

Calcineurin Inhibitor (CNI) Drug Toxicity

Calcineurin inhibitors (CNIs) including cyclosporine (CsA) and tacrolimus are used for maintenance immunosuppression after solid organ transplantation. These drugs have the potential to cause both acute and chronic nephrotoxicity. Acute CNI toxicity is one of the important causes of acute graft dysfunction. Acute tubular injury (ATI) is the most common lesion, accompanied by isometric vacuolization of tubular epithelial cell cytoplasm. Both drugs also cause thrombotic microangiopathy (TMA) and microvascular toxicity which manifests as endothelial cell swelling, mucinous intimal thickening, nodular hyalinosis, and focal medial necrosis. Chronic CNI toxicity results in nodular arteriolar hyalinosis (Figure-1), characterized by

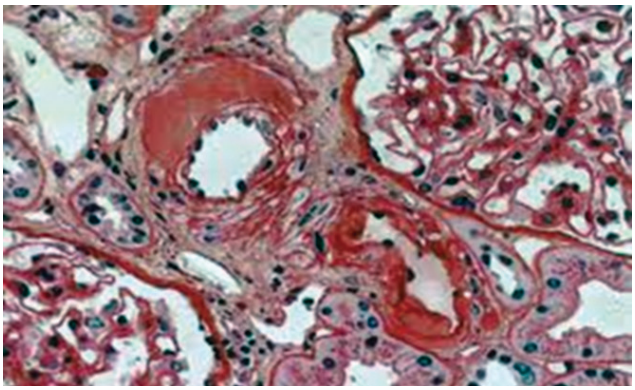


Figure 1: CNI toxicity manifesting as vasculopathic changes including nodular hyalinosis of arteriolar wall and intimal thickening.

hyaline, eosinophilic deposit consisting of fibrin, IgM, C3, and C1q. The ischemic injury caused by vasculopathy leads to interstitial fibrosis.

Infections

Urinary tract infections are common in the early post-transplant period as the recipients are predisposed to bacterial, fungal, protozoal, and viral infections due to immunosuppression.
The infective agents may affect the allograft or the native organs of the recipient. Bacterial infections result in a mixed inflammatory cell infiltrate in the interstitium associated with tubular microabscesses. Cytomegalovirus (CMV) and polyoma (BK) viruses are important causative agents (Figure 2).

Post-Transplant Lymphoproliferative Disorder (PTLD)

It is a rare disorder which is an important differential diagnosis with acute cellular rejection and an early diagnosis of this complication is

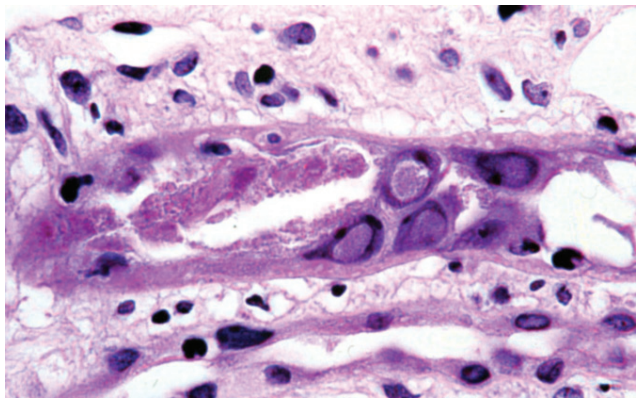


Figure 2: Polyoma (BK) virus infection. Epithelial cells exhibiting intranuclear viral inclusions.

necessary for its successful management. PTLD is characterized by a monomorphic or polymorphic lymphocytic infiltrate containing plasma cells, many of which are atypical. There is typically a diffuse interstitial infiltrate without associated tubulitis or arteritis. Immunophenotyping of lymphocytes helps in the definite diagnosis of its concurrence with rejection.

Acute Tubular Necrosis (ATN)

Acute tubular injury (ATI) or ATN is the main cause of primary nonfunction of the allograft. ATI results from a multitude of causes and including in situ injury in the donor; ischemia during organ harvesting, storage, or transportation of the organ; and ischemic injury incurred perioperatively in the recipient. The morphological picture is similar to that seen in the native kidneys. The histological features of ATN do not correlate well with the allograft function.

Acute Tubulointerstitial Nephritis (ATIN)

Non-immune related ATIN may result from a number of insults such as infection, drug hypersensitivity, viral infection, etc. A predominance of neutrophils in the mixed inflammatory cell infiltrate in the interstitium, especially if associated with tubular microabscesses or leucocyte casts favor the possibility of infection. A predominance of eosinophils raises the possibility of drug hypersensitivity. Viral infections are accompanied by appropriate viral cytopathic effects in addition to the infiltrate.

Recurrent and De Novo Renal Diseases

Almost all diseases that occur in the native kidneys can occur de novo or recur in transplant kidneys.

Glomerular diseases account for approximately 10-20 percent of cases of ESRD undergoing transplantation, and overall approximately 20 percent of these patients experience recurrence. The same disease can also occur as de novo disease in the transplanted kidneys. Disease characteristics of the recurrent disease are similar to those of the original disease, but are usually mild in nature. The two most common diseases are membranous glomerulonephritis and focal segmental glomerulosclerosis. A non-glomerular disease that frequently recurs in transplanted kidneys is the primary hyperoxaluria, if kidney transplantation is carried out without concomitant liver transplantation.

Conclusion

Knowledge of the pathologic features either related to allo-immune rejection or other conditions in post-transplant patients is necessary to identify, prevent and minimize the damage by administering proper treatment and management.

Myelodysplastic Syndromes

Dr Aeysha Majeed
Hematology

The Myelodysplastic syndromes (MDS) are a group of clonal disorders of the bone marrow. Their common feature is bone marrow failure as a result of ineffective haematopoiesis rather than reduced haematopoietic activity. MDS is predominantly a disease of the elderly, although it may affect all ages. It can arise de novo or follow previous chemotherapy or radiotherapy for another malignancy.

Classification

French-American-British (FAB) classification
In 1974 and 1975, a group of pathologists from France, the US, and Britain produced the first widely used classification of these diseases. This - French-American British classification was published in 1976, and revised in 1982.

Table 1: (adapted from text book Postgraduate Haematology by Hoffbrand 6th edition)

Name	Description
Refractory anemia (RA	Characterized by less than 5% primitive blood cells (myeloblasts) in the bone marrow and pathological abnormalities primarily seen in red cell precursors
Refractory anemia with ring sideroblasts (RARS	Also characterized by less than 5% myeloblasts in the bone marrow, but distinguished by the presence of 15% or greater red cell precursors in the marrow being abnormal iron-stuffed cells "called "ringed sideroblasts
Refractory anemia with excess blasts (RAEB)	Characterized by 5-20% myeloblasts in the marrow
Refractory anemia with excess blasts in transformation ((RAEB-T	Characterized by 21-30% myeloblasts in the marrow (>30% blasts is defined as acute myeloid (leukemia
Chronic myelomonocytic leukemia (CMML), not to be confused with chronic myelogenous leukemia or CML	Characterized by less than 20% myeloblasts in the bone marrow and greater than $1^{*}10^{9}$/L .monocytes (a type of white blood cell) circulating in the peripheral blood

World Health Organization

Most recently, the WHO has evolved a new classification scheme (2008) which is based more on genetic findings. However, morphology of the cells in the peripheral blood, bone marrow aspirate, and bone marrow biopsy is still the screening test used in order to decide which classification is best and which cytogenetic aberrations may be related.

Table 2: (adapted & modified from text book Postgraduate Haematology by Hoffbrand 6th edition).

Old System	New System
Refractory anemia (RA)	**Refractory cytopenia with unillineage dysplasia** (Refractory anemia, Refractory neutropenia and Refractory thrombocytopenia)
Refractory anemia with ringed sideroblasts (RARS)	**Refractory anemia with ring sideroblasts (RARS)** **Refractory anemia with ring sideroblasts – thrombocytosis (RARS-t)** (provisional entity) which is in essence a myelodysplastic/myeloproliferative disorder and usually has a JAK2 mutation (janus kinase) – New WHO classicication 2008
	Refractory cytopenia with multilineage dysplasia' (RCMD)' includes the subset Refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS). RCMD includes patients with pathological changes not restricted to red cells (i.e., prominent white cell precursor and platelet precursor (megakaryocyte) dysplasia.
Refractory anemia with excess blasts (RAEB)	**Refractory anemias with excess blasts I and II.** RAEB was divided into RAEB-I (5-9% blasts) and RAEB-II (10-19%) blasts, which has a poorer prognosis than RAEB-I. Auer rods may be seen in RAEB-II which may be difficult to distinguish from acute myeloid leukemia.
Refractory anemia with excess blasts in transformation (RAEB-T)	This category was eliminated, such patients are now considered to have acute leukemia **5q - syndrome,** typically seen in older women with normal or high platelet counts and isolated deletions of the long arm of chromosome 5 in bone marrow cells, was added to the classification.
Chronic myelomonocytic leukemia (CMML)	CMML was removed from the myelodysplastic syndromes and put in a new category of myelodysplastic-myeloproliferative overlap syndromes.
	Myelodysplasia unclassifiable (seen in those cases or megakaryocyte dysplasia with fibrosis and others)
	Refractory cytopenia of childhood (dysplasia in childhood) – New in WHO classification 2008

Diagnosis

Morphology

The diagnosis of MDS depends on careful morphological examination of the blood film and bone marrow aspirate and trephine specimens. Common abnormalitiesinclude:

- Peripheral blood. Red cells – anisopoikilocytosis, macrocytosis. Neutrophils – hypogranulation (decrease granules), pseudo- Pelger forms(bi-lobed neutrophils). Platelets – giant forms (large sized).

- Bone marrow. Erythroid cells – multinuclearity, nuclear budding, ring sideroblasts.
  Myeloid cells show hypogranularity, increased blast cells. Megakaryocytes – giant forms or micromegakaryocytes.

Where there are changes in all three lines the term 'trilineage dysplasia' is used. The bone marrow trephine biopsy usually confirms marrow hypercellularity, although fibrosis and even hypocellularity may occur.

Genetics

Around 50 percent of cases of MDS show cytogenetic abnormalities. Common changes include monosomy 7 or 7q−, trisomy 8and monosomy 5 or 5q−. The incidence of chromosome abnormalities increases with the severity of the disease and risk of leukaemic transformation.

Management

The goals of therapy are to control symptoms, improve quality of life, improve overall survival, and decrease progression to acute myelogenous leukemia (AML). The International Prognostic Scoring System (IPSS) – based on the number of blood cytopenias, percentage of bone marrow blasts and karyotype – is simple prognostic tool which can be used to direct treatment.Supportive care with blood product support and hematopoeitic growth factors (e.g. erythropoietin) is the mainstay of therapy.

Fluorescent Optical Method for Platelet Estimation: An Advanced Clinical Parameter in Laboratory Hematology

Noor Rahman Khan and Muhammad Shariq Shaikh
Hematology

Platelets, also called "thrombocytes", are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clogging blood vessel injuries. Platelets are cytoplasmic fragments of megakaryocytes of the bone marrow and hence lack nucleus. Approximately 70 to 80 percent of platelets circulate in the blood, 20 to 30 percent are stored in the spleen. A normal platelet count ranges from 150,000 to 450,000 platelets per microliter of blood.

Complete blood count (CBC) is a basic test ordered routinely by physicians as a part of initial diagnostic work-up on their patients. Platelet count estimation is an important integral part of a CBC report and its reliability is highly desired in the diagnostic and treatment process of several clinical conditions. For instance, it is very important to know the accurate platelet count of a patient with thrombocytopenia to decide appropriate treatment strategy particularly when platelet transfusion is also being considered.

Various methods for platelet estimation can be utilized like microscopy using a haemocytometer, immunophenotyping utilizing monoclonal antibodies and a flow cytometer, impedance method and the fluorescent optical method. The majority of modern automated hematology analyzers in use incorporate the impedance method for platelet estimation.

In impedance measurement (resistance measuring principle), cells are passed one after the other through a capillary opening. The passing cell produces an electrical resistance and thus an electronic signal which is proportionate to its volume. Hence, the cells are identified based on their size. In a normal specimen, erythrocytes which also are non-nucleated cells can be clearly distinguished from platelets based on difference in their size. However in pathological conditions, where platelets are larger than 30 fL (e.g. large and giant platelets in Bernard-Soulier Syndrome) or when erythrocytes are smaller than 25 fL (e.g. red cell fragmentation syndromes or microcytosis) a clear separation may not be achieved. In such cases, platelet count must be verified by an alternate method.

Fluorescent optical method of platelet estimation is an advanced technique in which the platelet RNA is first stained with a patented fluorescence dye specifically developed for diode lasers. Subsequently, the platelets are recorded flow-cytometrically by means of semi-conductor laser technology. As erythrocytes and platelets differ in their RNA content, this nucleic acid staining enables the analyzer system to properly differentiate platelets from other cells.

Several models of Sysmex X-class hematology analyzers incorporate this sophisticated optical method of platelet estimation. At AKUH clinical laboratories, the state-of-the-art Sysmex XE-5000 automated hematology analyzer (Sysmex Corporation, KOBE, JAPAN) can perform platelet counts by both impedance and optical methods. In case of interference in the platelet impedance count, the instrument automatically alerts the user to switch to optical method. An automatic algorithm using pre-defined criteria can also be activated for this purpose. Thus fulfilling the requirements of good laboratory practice, an accurate and precise platelet count result is reported on every patient specimen.

Introduction of Beta-D-Glucan and Galactomannan Testing for Diagnosis of Invasive Fungal Infections

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Fungi are increasingly reported as major pathogens in critically ill and immunocompromised patients. Invasive fungal diseases are important causes of morbidity and mortality today. Several factors are considered to contribute in the increasing trend of invasive fungal infections, including hematological malignancies, stem cell transplantation, use of chemotherapeutic drugs and immunosuppressive agents, broad-spectrum antibiotics, prosthetic devices and grafts, and more aggressive surgery. Patients with burns, neutropenia and HIV infection are also predisposed to fungal infections. Yeasts most frequently seen in clinical practice are Candida spp and Cryptococcus spp. The most frequent filamentous fungi (molds) isolated are Aspergillus spp., but Fusarium spp., Scedosporium spp. and Mucoraceous molds are increasingly seen.

The symptoms of invasive fungal infections (IFI) are nonspecific which can impede timely diagnosis. Definitive diagnosis of invasive fungal infections requires cultures of tissue, blood, or other fluids. Routine diagnostic tests are time consuming, so empirical treatment can be commenced, based on clinical suspicion, which can also make a specific fungal diagnosis difficult. Fungal infections of the airway may be evident on chest radiography or computed tomography. Bronchoscopy is also used to better visualize the infected area and to obtain tissue samples.

Aspergillosis is common in patients with hematological malignancies. Invasive aspergillosis manifests primarily as pneumonia or sinus infections, or asymptomatic lesions in the lung parenchyma. It is rapidly progressive and can disseminate hematogenously. Definitive diagnosis of Invasive Aspergillosis (IA) can be made on histopathology and culture on biopsy tissue from suspected site. Obtaining such specimens can be a hurdle as it requires invasive procedure, which is not always possible and can delay diagnosis in this regard.

Current guidelines regarding diagnosis of IFI recommends use of fungal cell wall antigens like Beta-D Glucan and galactomannan in serum or Bronchoalveolar lavage (BAL) fluid. Beta-D glucan is a found in most fungi, both yeasts and molds, while galactomannan is specific to Aspergillus. High levels can be detected in serum or BAL fluid, which can help diagnose IFI early and can also guide in commencement of therapy as it has both diagnostic as well as prognostic impact. Use of these antigens in diagnosis and monitoring of IFIs has led to decrease in unnecessary use of antifungals.

Galactomannan levels, however, can be falsely normal, especially if the patient is already on antifungal treatment. Falsely normal levels are also witnessed in chronic granulomatous diseases and Job's syndrome. Falsely positive results are seen in patients with altered intestinal barrier (galactomannan is also found in certain foods e.g. cereals), in patients on treatment with piperacillin-tazobactam or amoxicillin-clavulanate and in patients infected with Penicillium, Histoplasma, Alternaria, Paecilomyces or Geotrichum. Beta-D Glucan levels usually do not rise in infections due to mucoraceous molds (Rhizopus, Mucor) and Cryptococcus neoformans. Falsely high results are seen when patient is on beta lactam antibiotics or hemodialysis or if specimen is contaminated with talc.

Regarding galactomannan, a single positive index of >0.7 or two consecutive samples of >0.5 in serum is considered to be positive and < 0.5 is negative. Serum value of >1.0 is considered a sign of therapeutic failure. In BAL sample, a single positive index of >1.0 is positive. In CSF sample a single positive index of >0.5 is positive. For Beta-D Glucan, data for neonates and infants less than six months are lacking. It is considered positive if levels are >80 pg/ml, Indeterminate if 60-80 pg/ml (repeat test recommended) and negative if levels are <60 pg/ml.

These tests have been introduced in Clinical Microbiology Laboratory at Aga Khan University Hospital. These investigations can be done in patients with high clinical suspicion of IFI like aspergillosis, candidemia, etc. The results must always be correlated to the clinical history, radiological findings and culture results before making the final diagnosis.

Basic Concepts in Hematopoietic Stem Cell Transplantation

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Hematopoietic stem cell transplantation (HSCT) is the therapeutic modality of transplanting blood containing hematopoietic stem cells for treating refractory blood diseases like leukemia and aplastic anemia, some solid tumors and also refractory autoimmune diseases.

Hematopoietic stem cells are cells that in due course become RBCs, WBCs and platelets. These have regenerative capacity and are located in bone marrow but can be mobilized to peripheral blood due to G-CSF, severe infections and treatment with antineoplastic drugs. Cord blood also contains these cells. Accordingly HSCT can be classified as per type of blood that contains these stem cells into: Bone marrow transplant (BMT), Peripheral blood stem cell transplant (PBSCT) and Cord blood transplant (CBT).

It can also be classified a/c to source of stem cells into:

1. Autologous: When transplanted blood is of patient him/herself.
2. Allogeneic: When the blood is obtained from another person.

    a. Syngeneic: if donor is identical twin.
    b. Allogeneic related: If donor is blood relative other than an identical twin
    c. Allogeneic unrelated: When donor is an unrelated person.

Common Indications:

Autologous	Allogeneic
Multiple myeloma	AML, ALL , MDS
Non-Hodgkin lymphoma	CML and other MPDs
Hodgkin disease	Chronic lymphocytic leukemia
Acute myeloid leukemia	Multiple myeloma
Neuroblastoma	Non-Hodgkin lymphoma and Hodgkin disease
Germ cell tumors	Thalassemia major and Sickle cell anemia
Autoimmune disorders - SLE, systemic sclerosis	Inherited bone marrow failure syndromes
Amyloidosis	Aplastic anemia, PNH and pure red cell aplasia
	Severe combined immunodeficiency (SCID)
	Inborn errors of metabolism - Eg, mucopolysaccharidosis and Gaucher disease

HSCT Procedure

Selection of donor (for allogeneic HSCT

Stem cells are normally obtained from an HLA matched donor (Usually sibling). When no donor is available among blood relatives, a search is made for an unrelated matched donor. Allogeneic HSCT is also possible even if there is not a perfect HLA match between donor and patient (mismatch HSCT), but the chance of severe GVHD and graft failure is high. Once the donor is identified, he is screened for general health and well being.

Pretransplant Conditioning

The patient is given chemotherapy and radiotherapy to kill all cancer cells and to eliminate or weaken normal blood cells, to create favorable environment for transplanted stem cells to get grafted. This makes the patient immunocompromised so there is need of isolation in bio clean room and provision of antifungal, antiviral and antibacterial drugs. Conditioning starts approx 7-10 days before transplantation of stem cells.

Harvesting Hematopoietic Stem Cells

From bone marrow: It is done under general anesthesia, through a bone marrow aspirate needle by repeatedly inserting it into the iliac bone to acquire the bone marrow in amount 15ml/kg of patient's body weight. A dose of 1 X 108 and 2 X 108marrow mononuclear cells per kilogram are required to establish engraftment in autologous and allogeneic marrow transplants, respectively.

From peripheral blood: Stem cells are collected once they peak in peripheral blood after the administration of G-CSF. This is done using blood cell separator (Aphresis) which efficiently separates a fraction of stem cells through centrifugal separation. For a typical donor, approximately 24 L of whole blood can be processed over two days to collect approximately 500 million CD34+ cells, a cell progenitor population enriched for hematopoietic stem cells.

In Autologous HSCT, harvesting is done before conditioning. In allogeneic HSCT, donor has to be hospitalized for few days for pre-harvest health check, harvesting and health check after collection.

Transplantation of Stem Cells

The collected stem cells are then transfused to patient through intravenous route. This is just like routine blood transfusion.

Engraftment and Hematopoietic Recovery

Engraftment is the hematopoietic reconstitution i.e. the start of blood cell formation by infused stem cells in the recipient's bone marrow. Normally the day on which absolute neutrophil count reaches at least 500/uL for three consecutive days for the first time is taken as the date of engraftment. It usually takes several weeks before the number of blood cells starts to return to normal. In some people, it may take longer.

Transfusion of blood products is required throughout the process from conditioning to time of hematopoietic recovery.

After the Transplant

Usually the patient is discharged from the hospital approximately one - two months after transplantation. He/she remains on immunosuppressive and anti microbial drugs for a certain period to avoid GVHD and infections. The speed of recovery and return to productive life varies, depending on patient's age, condition, disease involved and post-transplant complications.

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