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Six Sigma Improvement Cycle

- Define
- Measure
- Analyze
- Improve
- Control

The Aga Khan University Hospital, Karachi
Provocative Testing for Growth Hormone Disorders  

Serum Procalcitonin for Early Sepsis Diagnosis  

Monoclonal Gammopathy of Undetermined Significance (MGUS)  

Understanding Six Sigma Methodology  

Blood Collection for Culture  

Molecular Analysis of Alpha Thalassaemia by Multiple Ligation Probe Assay  

Prognostification of Acute Myeloid Leukemia with FLT 3 Mutation  

Interferon Gamma Release Assay (IGRA) for Detection of Mycobacterium Tuberculosis Infection in the Host  

Meeting Report: The 37th Annual Conference of the Pakistan Association of Pathologists
**From the Editor’s Desk**

The past few issues have been theme based where we have covered reams of new information and technologies on a focused subject. For a change, we have once again adapted a non-thematic approach in this issue since a lot of information can be disseminated by this methodology as well.

This issue contains variety of articles from Pathology, Microbiology and Radiology. Apart from increasing awareness on quality improvements, needle stick injuries, new markers for sepsis, collection technique for blood cultures and prognostic markers for acute myeloid leukemia to name a few, we have also shared information on genetic testing services offered for alpha thalassemia. In addition, the issue also includes information about various other new tests available in different sections of the department.

It is hoped that this issue will further enhance the awareness and education of our physicians in diagnosing common and rare medical conditions.

Dr Natasha Ali
Haematologist

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**Provocative Testing for Growth Hormone Disorders**

**Dr Noreen Sherazi & Dr Shabnum Khawaja**
Chemical Pathology

**Introduction**

The diagnosis of growth hormone deficiency (GHD) or excess in a child or adult with impaired or abnormal growth cannot be made on the basis of auxological criteria alone, since these are common to a variety of growth disorders related to different causes. In our current understanding, any growth disorder that is caused either by impaired growth hormone (GH) secretion or by impaired peripheral action of GH can be classified as GHD and excess growth hormone secretion is labeled as gigantism in a child and acromegaly in an adult.

Normal GH secretion shows an episodic circadian pattern with wide intra- and inter-individual variation, for this reason random serum GH levels are of little value and provocative testing of GH is indicated for biochemical diagnosis of GH disorders. It can be stimulated by physiological or pharmacological ways. Sleep, fasting and exercise are the physiologic stimuli of the GH release. Pharmacological stimuli include L-dopa, clonidine, propranolol, glucagon, arginine, GH-releasing hormone (GHRH) and insulin-induced hypoglycemia. In the diagnosis of GHD, these pharmacological stimuli can be used alone or in a variety of combinations.

The provocative tests done in Section of Chemical Pathology, Department of Pathology and Microbiology are discussed below:

1) For GH Deficiency:
- ‘Growth hormone stimulation test with insulin’ or ‘Insulin hypoglycemia test (IHT)’ or ‘Insulin tolerance test (ITT)’
- Growth hormone stimulation with L-DOPA

2) For GH Excess:
- Growth hormone suppression with glucose by Oral glucose tolerance test (OGTT)

**Growth Hormone Stimulation Test with Insulin**

The patient comes to the laboratory by appointment and overnight fast for 10-14 hours is mandatory for the procedure. Test is started by taking consent, weighing and canulating the patient for emergency I/V access. Required dose of insulin is calculated i.e. 0.1 or 0.15 units/kg body weight. 10 cc syringe with 25 per cent dextrose is kept ready. Baseline sample
of glucose and GH is taken and simultaneously patient’s glycemic status is checked on glucometer. After that multiple sampling of glucose and GH is done at 30, 45, 60, 90 and 120 minutes. The test will be continued further only if blood glucose level drops below 50 mg/dl or < 2.9 mmol or patient is symptomatic (i.e. there is shivering, irritability, drowsiness, sweating, confusion, fatigue, hunger, etc.) If blood glucose level doesn’t drop till 50 mg/dl or no symptoms at all then test is repeated with higher insulin dose i.e. 0.15 units /kg taking 45 minutes glucose level as baseline.

Insulin induces hypoglycemia and as a result GH is released. After stimulation, for a normal individual GH should increase to a peak value of >10 ng/ml when hypoglycemia is achieved. If there is GH deficiency this GH peak would not be observed.

**Growth Hormone Stimulation Test with L-DOPA**

It is pharmacological stimulus of GH to evaluate GH deficiency. No appointment is needed. Patient should fast overnight for the procedure. Consent should be taken and dose of L-dopa tablet for the patient is calculated according to weight i.e. 125 mg if weight is ≤ 15 kg, 250 mg if weight is 16-35 kg and 500 mg if weight is >35 kg body weight. After baseline sampling for serum GH, the tablet of L-dopa should be ingested by the patient with few sips of water. Blood samples are collected at 30, 60, 90 and 120 minutes. Please note that test should be abandoned if patient vomits out ingested tablet (blue vomitus) before 60 minutes sample. After pharmacological stimulation, normally GH peaks in 30 - 120 minutes and its concentration is far above 7.5ng/ml. Deficiency is suspected if levels remain below the desired cutoff.

**Growth Hormone Suppression with OGTT**

When there is clinical suspicion of acromegaly or gigantism GH secretion becomes autonomous it will not suppress with hyperglycemia, where as in normal individuals hyperglycemia causes GH inhibition. However, it should not be done in diabetic patients who have suppressed GH due to hyperglycemia. No prior appointment from the laboratory is required. Patient should fast overnight. After consent, baseline samples for GH and glucose is taken. Anhydrous 75g of glucose in 300 ml of cold water is given to the patient. Multiple sampling is done at 30, 60, 90 and 120 minutes for GH and glucose.

Normally GH suppresses with hyperglycemia or glucose load to < 2ng/ml. In case of acromegaly patient will fail to show suppression and there will be paradoxical rise in GH concentration.

Please note we are also performing insulin growth factor 1 (IGF-1) levels at AKUH Clinical Laboratory for diagnosing various GH disorders.

**Frequently asked questions from Chemical Pathologists regarding GH stimulation with insulin:**

Should the test be conducted if a patient comes with fasting in excess of 14 hours?
Test should not be done because over longer periods of fasting, liver will start to produced glucose endogenously by gluconeogenesis will affect the test results.

Should the test be continued if the fasting blood glucose is high?
Take proper history regarding meal intake and duration of fasting. Start the test if the fasting is below diabetic range.

What will you do if the patient’s blood glucose hasn't dropped down at 30 minutes and patient is also not symptomatic?
It means biochemical hypoglycemia not achieved. Wait till 45 minutes sample and if still high start the test with second bolus of insulin i.e. 0.15units/kg body weight.

Should the test be continued if the patient’s blood glucose hasn’t dropped down at 30 minutes but patient is irritable or drowsy?
Criteria for proceeding the GH stimulation with insulin is either biochemical hypoglycemia i.e. 50 mg/dl glucose or signs of hypoglycemia. In this case we will carry on with the procedure.

What will be the new blood glucose baseline while giving insulin again?
We will wait till 45 minutes sample, if glucose level is still high then restart the test with 45 minutes reading taken as baseline for glucose.

What will you do if on repeating GH stimulation by insulin, blood glucose does not drop?
It could be due to various reasons; firstly if it is happening frequently over the past few weeks then check your insulin vial. If it is closer to expiry, discard and get a new one. Secondly if this is the first case then we should suspect insulin resistance in such patients. Test should be abandoned and a note should be given or communicate with treating physician directly so that he is well aware about the failure of test.
Serum Procalcitonin for Early Sepsis Diagnosis

Dr Sibtain Ahmed
Chemical Pathology

Procalcitonin, a protein that consists of 116 amino acids is a peptide precursor of the hormone calcitonin. Procalcitonin is also produced by the neuroendocrine cells of the lung and intestine. It is released as an acute-phase reactant in response to pro-inflammatory stimuli, especially of bacterial origin. Increased levels of procalcitonin in response to viral infections and noninfectious inflammatory stimuli (autoimmune diseases and chronic inflammatory processes) are much less pronounced, rarely exceeding 0.5 ng/mL. Severe trauma, major burns, multi-organ failure, or major surgery can cause procalcitonin elevations in the absence of sepsis. The optimal cut-off ranges of procalcitonin are variable and are dependent on the clinical setting and the site and extent of the infection. Following are the clinical and diagnostic utilities of procalcitonin.

Procalcitonin Kinetics in Sepsis

Serum procalcitonin has been demonstrated to be an ideal biomarker for differentiating patients with sepsis from those with systemic inflammatory reaction not related to infectious cause. Table 1 shows the comparison of procalcitonin with other biomarkers used to assist sepsis diagnosis.

Differentiates Bacterial from Viral Infection

A commonly encountered situation in the clinical practice is that the signs and symptoms of bacterial and viral infections are widely overlapping, especially in respiratory tract infections. Sometimes the diagnostic uncertainty still remains, even after obtaining a complete clinical history, performing a physical examination and requesting various radiological/ laboratory investigations. It is often difficult to differentiate bacterial from viral causes of sepsis. Serum procalcitonin with more specificity for differentiating bacterial from viral infections considerably improves the differential diagnosis in these cases. Procalcitonin released as an acute-phase reactant is associated with bacterial endotoxin and inflammatory cytokines and it does not increase in viral infections.

Prognostic Marker of Sepsis

Increased levels of procalcitonin are also reported to be associated with higher SOFA (Sequential Organ Failure Assessment score) and APACHE II scores (Acute Physiology and Chronic Health Evaluation). Serial measurements of serum procalcitonin can be of greater value in monitoring response to treatment in septic shock. Decreasing procalcitonin levels in patients with sepsis indicate effective treatment of the underlying infection. Persistently elevated procalcitonin levels indicate a possible treatment failure. When integrated into the management of septic patients, procalcitonin can help clinicians to manage septic patients more efficiently.

Table 1. Compares the Kinetics of C-reactive Peptide, Interleukins, Tumor Necrosis Factor-α with Serum Procalcitonin after a Bacterial Insult

<table>
<thead>
<tr>
<th></th>
<th>Cytokines (interleukins, tumor necrosis factor-α)</th>
<th>C-Reactive Protein</th>
<th>Procalcitonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rise</td>
<td>1-3 hours</td>
<td>12-24 hours</td>
<td>2-4 hours</td>
</tr>
<tr>
<td>Peaks</td>
<td>2 hours</td>
<td>48 hours</td>
<td>8 hours</td>
</tr>
<tr>
<td>Relation to sepsis</td>
<td>Only remain elevated for few hours</td>
<td>Can remain elevated without there being underlying infection</td>
<td>Persists for as long as the inflammatory process continues</td>
</tr>
<tr>
<td>Normalize</td>
<td>8 hours</td>
<td>Late</td>
<td>Early</td>
</tr>
</tbody>
</table>
Utility in Paediatrics

Adding procalcitonin results to clinical assessment improves the accuracy of the early clinical diagnosis of sepsis in neonates. It can also be used in the early diagnosis of bacterial meningitis. It is a useful biomarker in differentiating bacterial from non-bacterial meningitis.

Useful in Neutropenic Patients

Several published studies have shown that the procalcitonin levels accurately predicted blood culture positivity in patients with neutropenia. Procalcitonin measurement also demonstrated the potential to reduce the number of blood cultures drawn in the Emergency Department to better implement resources. Several studies have shown that the use of procalcitonin in targeting rational blood culture utilization allows for more directing allocation of limited health-care resources.

Prediction of Renal Involvement in Paediatric Urinary Tract Infection

Urinary tract infection (UTI) in young children carries the risk of parenchymal damage and sequelae. The location of the infection within the urinary tract influences decisions regarding both therapeutics and follow-up. Because clinical features and laboratory markers of infection at an early age are not specific, it is difficult to make a distinction between lower UTI and acute pyelonephritis. Procalcitonin values have been proved to be more specific for identifying patients who might develop renal damage.

Utility in Organ Transplants Rejection

Procalcitonin possesses both sensitivity and specificity in identifying infection complications among patients undergoing solid organ transplantation. It is useful in differentiating acute graft rejection after solid organ transplantation from bacterial infections.

Antibiotic Stewardship

Müller and colleagues have recommended a procalcitonin guided diagnostic algorithm for patients with suspected sepsis. This is based on data available to date that discourages use of antibiotics at 0.1 - 0.25 ng/mL of procalcitonin levels. Whereas at the levels of >0.5 ng/mL use of antibiotics is encouraged. A rising procalcitonin after starting treatment should prompt a review of antibiotics or source control in patients with systemic infections. In four intervention trials enrolling more than 1200 patients, the success of this algorithm was measured by clinical outcomes, assuming that, if the patient recovered without antibiotics, then there was no serious bacterial illness. Literature shows reduction in the antibiotic prescription rate by 40-50 per cent in patients with lower respiratory tract infection by procalcitonin guided stewardship. Procalcitonin at low concentrations can help to differentiate patients with clinically relevant lower respiratory tract infection who require antibiotic therapy from those with viral infection or minor bacterial infection who do not require antibiotic treatment.

Figure 1. Algorithm for guidance of antibiotic initiation and modification proposed by Müller B et al. Int J Antimicrobial Agents. 2007, 30S:S16-S23
TRALI

Dr Farheen Karim
Haematology

TRALI is a syndrome characterized by the development of new onset acute respiratory distress with hypoxemia (SpO2 <90% on room air) during or up to six hours after completion of a blood transfusion. The incidence of TRALI has been reported to be one in 2000 to one in 7500 transfusions. However, it is felt worldwide that TRALI is an under-recognized and under-reported entity because of lack of awareness.

Diagnosis of TRALI

TRALI is a clinical diagnosis. It should be considered whenever a patient develops hypoxemic respiratory insufficiency during or shortly after transfusion of any blood product. The clinical and radiologic findings in TRALI are as follows:

Clinical Findings

Dyspnea, hypoxemia and bilateral pulmonary edema. Other reported findings include hypotension, tachycardia and fever (1-2°C rise from baseline).

Radiological Findings

Characteristic chest x-ray results show bilateral patchy infiltrates, with alveolar and/or interstitial patterns. The chest x-ray may indicate non-cardiogenic pulmonary edema without cardiac enlargement or other evidence of fluid overload.

Blood Products Implicated in Causing TRALI

All blood products have been associated with TRALI. Products with high plasma content such as fresh frozen plasma, apheresis platelet concentrates and whole blood have been associated with the greatest risk.

Etiology

TRALI may be attributable to the presence of anti-HLA and/or anti-granulocyte antibodies in the plasma of multiparous females or donors who have received previous transfusions.

Pathogenesis

A “Two-Hit theory” has been proposed in the pathogenesis of TRALI:

1. Antibodies to white blood cell antigens (HLA, granulocyte and monocyte) present in the donor’s plasma bind to the white cells of the recipient which results in activation of complement and neutrophils. The aggregation of white cells within the lungs, and subsequent adhesion to the capillary endothelium produce endothelial damage, capillary leakage and the development of pulmonary edema.

2. The clinical condition of the patient (e.g. sepsis, trauma, etc) causes sequestration of primed neutrophils in the activated pulmonary endothelium. This results in endothelial damage, capillary leakage and the subsequent development of pulmonary edema. The two-event theory may explain the development of TRALI when there is no evidence of donor HLA/HNA antibodies.

Treatment

Treatment of TRALI is supportive. Mild forms of TRALI usually respond to supplemental oxygen therapy. Severe forms require mechanical ventilation and ICU support. There is no role for diuretics or corticosteroids. The majority of patients recover within 72 to 96 hours. However, some patients are slower to recover and may remain hypoxic with persistent pulmonary infiltrates up to seven days. Approximately five to ten per cent of cases are fatal in spite of aggressive supportive care.

Importance of Diagnosing and Reporting TRALI

Transfusion-related acute lung injury (TRALI) is a rare but potentially fatal complication of blood product transfusion. The diagnosis and reporting of TRALI will allow better understanding of the incidence, clinical course and associated mortality of this reaction. Furthermore, by identifying cases of TRALI, steps can be taken to prevent further cases of TRALI by investigating donors involved in these cases and deferring them from further donations if they are found to be implicated.
Detection of Partial “D” Antigen or “D Variant”

Huma Warsi
Haematology

D antigen is a protein with many parts, found on the surface of red blood cells. The D antigen is also known as the “Rh factor” and it tells us blood type. People are either D (Rh) negative or D (Rh) positive. The D antigen may show up only weakly. Red blood cells from these people are also Du positive.

Weak D (DU) red cells have historically been defined as having a reduced amount of D antigen, requiring an indirect Antiglobulin test (IAT) for detection. The number of samples classified as weak D however, depends on the characteristics of the typing reagents, which have changed over the years. Weak D expression results primarily from single nucleotide mutations in RHD that encode amino acid changes predicted to be located intracellularly, or in the trans membrane regions of red blood cell rather than on the outer surface of the red cell. The mutations affect insertion of the protein in the membrane, reflected in the reduced number of D antigen sites on the red cells.

Partial D red cells have historically been classified as D positive. These red cells were predicted to be missing portions of D and indeed the majority of partial D phenotypes is due to hybrid genes in which portions of RHD are replaced by the corresponding portions of RHCE. The novel sequences of the hybrid Protein resulting from regions of red joined to RhCE not only can result in the loss of D epitopes but also can generate new antigens.

Clinical Significance During Pregnancy

Mothers who are “partial D” should be considered D (Rh) negative. These mothers could build up antibodies to the part of the “D” antigen they lack. If their baby is D (Rh) positive, the antibodies could destroy the baby’s red blood cells. This leads to hemolytic disease of the fetus and newborn, or “HDFN.” These mothers will need treatment during pregnancy to prevent HDFN.

Source: Blood journal. hematology library Vol 89 no 5 1779-1786
Monoclonal Gammopathy of Undetermined Significance (MGUS)

Dr Muhammad Shariq Shaikh
Haematology

Monoclonal gammopathy of undetermined significance (MGUS) is defined as the presence in the serum of an M-protein <30 g/L, bone marrow clonal plasma cells <10 per cent, no end organ damage (CRAB: hypercalcemia, renal insufficiency, anaemia, bone lesions) and no evidence of B-cell lymphoma or other disease known to produce an M-protein. Paraprotein or M-protein refers to existence in blood of a single homogeneous (monoclonal) immunoglobulin typically secreted by expanded clone of terminally differentiated B cells.

MGUS is an asymptomatic condition and is usually discovered unexpectedly. Typical laboratory and radiographic abnormalities are absent. Its prevalence is approximately three per cent in persons over age 50 which increases to more than five per cent beyond 70 years of age. It is more common in man (M:F 1.5:1) and more than twice as frequent in African Americans as in Caucasians. Common associations include connective tissue disorders, peripheral neuropathies, dermatological, endocrine and liver diseases. It may often be seen as a transient event following solid organ and bone marrow stem cell transplantation.

Approximately 70 per cent are IgG, 15 per cent IgM, 12 per cent IgA and three per cent are biclonal1. MGUS may consist of immunoglobulin light chain only in up to 20 per cent and is only detected by serum free light chain assay. Bone marrow aspirate and trephine does not contribute to the diagnosis as plasma cells are not markedly increased. Immunophenotyping by flow cytometry however, frequently shows two populations of plasma cells, one with a normal immunophenotype (CD38 bright+, CD19+ CD56-) that is polyclonal and a monoclonal population with an aberrant phenotype, most often either CD19-/CD56+ or CD19-/CD56-.

Although the prevalence may differ, abnormal karyotypes when present in MGUS are the same as those found in myeloma. Translocation involving immunoglobulin heavy chain (14q32) gene are detected in nearly 50 per cent of the cases with t(11;14) (q23;q32) being the commonest translocation (15-25 per cent). Deletions of 13q are detected in 40-50 per cent cases however, its relation to progression of MGUS are not clear. Activating K- and NRAS mutations are much less frequent in MGUS (-5 per cent) compared to myeloma (30-40 per cent). Although genetic alterations and gene expression patterns probably can distinguish advanced myeloma from MGUS there are no unequivocal intrinsic differences that distinguish MGUS from myeloma.

M-protein levels remain stable with no other evidence of progression in most individuals. However, evolution to an overt plasma cell myeloma, amyloidosis, Waldenstrom’s macroglobulinemia and other lymphoproliferative disorder may occur at a risk of one per cent per year. Factors associated with increased likelihood of progression include higher M-protein level (>4 times with level of 25g/L to that of <5g/L), IgM or IgA MGUS, fraction of plasma cells in bone marrow with abnormal phenotype, DNA aneuploidy and subnormal levels of polyclonal immunoglobulins.

Thus, MGUS should be considered a pre-neoplastic condition and patients should be appropriately counseled and followed by monitoring M-protein levels.
Six Sigma is a statistical measure of quality for process improvement originally developed by Motorola in 1985. It is a metric that quantifies the performance of processes as a rate of Defects Per Million Opportunities (DPM, or DPMO). A Six Sigma-capable process has so little random variation that the standard deviation, when multiplied by six, gives a quantity that meets the customer requirement for that process. It sets a quantitative goal for process performance and in turn quality improvement. It was in March 1998 that Six Sigma was applied to the healthcare industry for the first time. The delivery of healthcare demands the highest level of quality and the most advanced quality management system.

A Six Sigma process is one in which 99.9997% of the products manufactured are statistically expected to be free of defects or errors (3.4 defects per million). Higher sigma values indicate better performance and vice versa. Quality is assessed on the sigma scale with a criterion of three sigma as the minimum allowable sigma for routine performance and a sigma of 6 being the goal for world-class quality.

Six Sigma projects follow two project methodologies with acronyms DMAIC and DMADV. Each methodology is composed of five phases. (Table 2) To improve an existing process the DMAIC methodology is used and to implement a new process or product DMADV is used.

There are several key players involved in launching a Six Sigma project. Prior to starting a project, a multidisciplinary team is formed. Similar to martial arts system formal Six Sigma programs adopt a ranking terminology. For example ‘Black Belts’ and ‘Green Belts’, which denote people with different levels of expertise (and certification). Six Sigma ‘Black Belts’ use a vast array of tools at each stage of Six Sigma implementation to control variation in process quality, and to manage teams and communication. This helps in creating an infrastructure within the organization.

A major difference between Six Sigma and other quality programs is that Six Sigma incorporates a control phase with ongoing checks. This ensured that once improvement is achieved it is not temporary but is maintained over time. Six Sigma

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**Table 1. Sigma Levels and Defects Per Million of Opportunities**

<table>
<thead>
<tr>
<th>Sigma Level</th>
<th>DPMO</th>
<th>Error Free Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six Sigma</td>
<td>3.4</td>
<td>99.9997%</td>
</tr>
<tr>
<td>Five Sigma</td>
<td>233</td>
<td>99.977%</td>
</tr>
<tr>
<td>Four Sigma</td>
<td>6210</td>
<td>99.4%</td>
</tr>
<tr>
<td>Three Sigma</td>
<td>66810</td>
<td>93%</td>
</tr>
<tr>
<td>Two Sigma</td>
<td>308500</td>
<td>69%</td>
</tr>
<tr>
<td>One Sigma</td>
<td>691500</td>
<td>31%</td>
</tr>
</tbody>
</table>

**Table 2. Tools to Dry Sig Sigma Projects**

<table>
<thead>
<tr>
<th>DMAIC</th>
<th>DMADV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Define: process of interest that needs to be improved</td>
<td>Define: goals as per customer demands and satisfaction</td>
</tr>
<tr>
<td>Measure: relevant data, measure performance</td>
<td>Measure: production capabilities and risks</td>
</tr>
<tr>
<td>Analyze: what is affecting our processes</td>
<td>Analyze: to develop alternatives</td>
</tr>
<tr>
<td>Improve/Innovate: redefine the process, remove cause, brainstorm needed</td>
<td>Design: an improved alternative</td>
</tr>
<tr>
<td>Control: documentation of new process and training, monitor the process</td>
<td>Verify: run pilot run to verify design</td>
</tr>
</tbody>
</table>
methodologies are well suited for application to laboratory settings because of the inherent need for statistical precision and quality control in laboratory testing, as well as the highly repetitive nature of laboratory work. It can serve as a quality indicator for pre-analytical, analytical and post analytical processes. Six Sigma values can be calculated for quantitative assays (total allowable error – bias / CV), for internal quality control data and also in proficiency testing. The Clinical Laboratory Improvement Amendments of 1988 and the Joint Commission on Accreditation of Healthcare Organizations requires laboratories to perform external comparison of their performance with others in a process commonly known as benchmarking. The data of Six Sigma studies and projects is evolving and it can become the tool for benchmarking and to compare laboratory error rates with peers.

Example

To better understand the calculations involved in sigma metrics here is an example from laboratory practice. A process improvement initiative using Six Sigma strategies between May to October 2013 was taken at the Section of Chemical Pathology, Department of Pathology and Microbiology, Aga Khan University Hospital (AKUH) to reduce the STAT tests turnaround time in emergency department (ED) laboratory. A team led by pathologist and comprising of two senior technologists conducted the project using ‘Define Measure Analyze Improve Control’ (DMAIC) methodology. Review of total STAT data showed that 0.25 per cent STAT tests were not reported in one hour, which is the cutoff reporting time of Clinical Laboratory of AKUH. In the commencement of the project the problem was defined as average STAT delay of 14 out of six thousand samples per month (DMAIC).

The approach adopted was direct observation and process analysis. Tests that exceeded one hour goal required for completion was considered a delay. The base line delay using Six Sigma calculator was 4.33 on sigma scale (DMAIC). A group member was assigned to daily monitor and records the process flow of STAT delays along with the reason for the delay. Group met fortnightly and brain storming was done, corrective and preventive actions for each delay were taken (DMAIC). Root cause analysis identified delay in processing of specimens, instrument malfunctioning and their late rectification and interruption in data transmission through instrument to central information system. Frequency of delays was more in night shifts. Significant time was taken in sample processing because of inadequate staffing. and in answering phone calls by technologists during busy periods. On spot feedback mechanism after the delays did not exist (DMAIC). On the basis of root cause analysis technologists were educated about the importance of STAT test in patient care. Proper maintenance of instrument by evening staff was ensured to give support to night shift. A new backup instrument with short throughput was arranged. Brief ED laboratory visit by senior technologist was arranged on daily basis to deal with staffing challenges, equipment problems, quality issues, and staff acknowledgments and feedbacks (DMAIC). Process of sample analysis from the time of sample receiving till sign out was revisited and a detailed workflow diagram was created and made available on the soft board for the technologists (DMAIC). To improve informing the results of STAT tests with panic value a voice pager system was also introduced in the ED (DMAIC). On reanalysis of the number of STAT delays it was noted that it reduced from 4.33 to 5.01 on sigma scale. Impact of project was that STAT tests entries are now done on fast track.

Applying Six Sigma methodologies in healthcare helps in improving issues of non-compliance and enhancing effectiveness in laboratory staff. Importance of Six Sigma in laboratory is gradually increasing and is a tool by which clinical laboratories can improve their processes and achieve their quality goals.
Blood Collection for Culture

Dr Naima Fasih
Microbiology

Purpose(s)

To ensure provision of safe, timely and convenient process for the patients undergoing of blood specimen collection.

Responsibility

All assigned consultants, registered nurses, assigned nursing staff, laboratory personnel and unit receptionist.

Equipment

Culture bottles (Two for adults and one for peads)
Skin disinfectant
Option 1: Sterile alcohol swab and two per cent chlorhexidine gluconate in 70 per cent isopropyl alcohol impregnated swab
Option 2: Sterile alcohol swab and 10 per cent povidine iodine
Option 3: Sterile alcohol swab (only for <2 months children of age)
Clean Gloves
Syringe 10 ml (02)
Patient Identification sticker
Alcohol wipes to disinfect bottle septum
Tourniquet

Procedure

Preparatory Phase

Explain procedure to patient/family.
Verify the patient’s identification by checking the patient name and identification number.
Wash or sanitize hands and wear latex gloves.
Follow Standard Precautions for all patients.
Assemble necessary equipment before preparation of the patient’s skin.

Check the expiry of the culture bottle.
Adult: Two bottles (One aerobic + One anaerobic)
Children: One bottle (paediatric)
Remove the cap from culture bottles. Clean septum with (sterile) alcohol wipe (do not use iodine as it damages the septum). Allow it to dry.
(see Figure 1)

Step 1: Vigorously cleanse the skin over the venipuncture site with 70 per cent isopropyl or ethyl alcohol to remove surface dirt and oils. Allow to dry.
(see Figure 2)

Step 2: Disinfect a five centimeter area in concentric circles (see Figure 3), moving outward from center of the site using two per cent chlorhexidine gluconate in 70 per cent isopropyl alcohol (impregnated swab) or 10 per cent povidine iodine.

Step 3: Leave the disinfectant to act for 60 seconds before venipuncture.

Step 4: Clean patient’s skin again with alcohol swab.
DO NOT touch the venipuncture site after preparation and prior to phlebotomy.

**Appropriate amount of Blood for Culture Draw**

Adult: 10 ml blood per culture bottle is recommended.

Children: 1ml/year of age

**Order of Inoculating Blood Culture Bottles**

Inoculate the aerobic bottle first followed by the anaerobic bottle. Do not change needle during transferring of blood from one bottle to other. If an inadequate amount of blood was obtained (less than 5 cc), and repeat phlebotomy cannot be performed; all blood should be preferentially inoculated in the aerobic culture bottle.

**Transport to Laboratory**

As soon as possible.
In case of delay bottle should be kept at room temperature for 4 hours.
Inoculated blood culture bottle will never freeze or refrigerate.

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**Inadequacies of Salmonella Serology for Diagnosing Enteric Fever**

Dr Joveria Farooqi
Microbiology

Enteric fever is a systemic infection with typhoidal Salmonella species, including Salmonella Typhi, S. Paratyphi A, B and C, which are transmitted through feco-oral route. The burden of typhoid and paratyphoid fever is highest in South Asia compared to the rest of the world especially in adolescents and children. Annual incidence of typhoid fever is around 250 to 500 per 100,000 child-years in low socioeconomic communities of Karachi. The diagnosis is suspected on fever of more than three days duration, sore throat and abdominal pain. These are all nonspecific symptoms and could occur in other endemic infections, like malaria and dengue.

Traditionally the definitive diagnosis of enteric fever is by isolation of Salmonella Typhi or S. Paratyphi A, B or C from blood, stool and bone marrow cultures. The sensitivity of blood culture ranges from 50 per cent to 80 per cent with a specificity of 100 per cent. Sensitivity of blood cultures in diagnosing typhoid fever in many parts of the developing world is further limited because of prior antibiotics.

Although bone marrow cultures may increase the likelihood of bacteriologic confirmation of typhoid, these are difficult to obtain and relatively invasive. The greatest advantage of culture-based diagnosis is availability of susceptibility profile of the isolate. This can direct physician to prescribe appropriate antibiotic therapy ensuring cure and eradication, thus reducing transmission.

Detection of raised titers of agglutinating serum antibodies against the lipopolysaccharide (LPS) (O) or flagellum (H) antigens of serotype Typhi (the Widal test) has been in use for a long time. The Widal test is easy, inexpensive, and relatively noninvasive. It can be of diagnostic value when blood cultures are not available or practical. The results must be interpreted cautiously because of the low sensitivity of the test. The serologic diagnosis of typhoid is also fraught with problems because results of a single Widal test may be positive in only 50 per cent of cases in endemic areas, and serial tests may be required in cases presenting in the first week of illness.
Detection of specific antibodies to typhoidal Salmonellae (Typhi dot) is also used as supportive tests for typhoid diagnosis. Rapid salmonella serological tests, which detect IgM and IgG antibodies to a Salmonella typhi-specific outer membrane protein, were considered sensitive and specific for the diagnosis of enteric fever when they were first introduced. Serological diagnosis has its own limitations. Initial studies showing high sensitivities were hopeful because of the chance of detecting typhoid earlier. It soon became evident that high sensitivity meant large number of false positive results; implying 10-15 days of unnecessary antibiotic therapy administered to patients without enteric fever. Antibodies against other gram-negative bacteria, like E. coli from urinary infection and non-typhoidal Salmonella species causing diarrhoea; viruses, e.g. dengue; and even malaria can cross-react with the Salmonella-OMP in the kit, thus giving rise to false positive results.

False negative results are also a problem when the test is performed in the first week of infection. Sensitivity (40-77 per cent) and specificity (80-95 per cent) of serological tests for enteric fever is inadequate for clinical diagnosis considering its high prevalence in Pakistan. Therefore, experts recommend that these serological tests should not be used for diagnosis of enteric fever, except as evidence of past infection.

Detection of Salmonella DNA in blood and urine through nested or multiplex PCR have yielded hopeful results, but it is still not established as a diagnostic method for enteric fever. There is a need for more efficient rapid diagnostic test for typhoid especially during the acute stage of the disease. Until then, culture remains the method of choice and it becomes even more important to curtail the use of antibiotics before blood cultures are sent.

In view of this evidence recent recommendations based on high quality data from studies conducted in endemic areas strongly discourage the use of such tests for enteric fever. Thus, in keeping with international evidence-based recommendations, the Aga Khan University Clinical Laboratories will shortly discontinue rapid typhoid serological test.

For diagnosis of typhoid fever, blood, stool and bone marrow cultures are gold standard, and are advised in suspected cases.

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**CURRENT DIAGNOSIS**

- In the absence of localizing signs, the early stage of the disease may be difficult to differentiate from other endemic diseases such as malaria or dengue fever.
- The presentation and diagnosis of typhoid fever may be tempered by coexisting morbidities and early administration of antibiotics.
- The presentation of typhoid may be more dramatic in children younger than 5 years of age, with comparatively higher rates of complications and hospitalization.
- The sensitivity of blood cultures in diagnosing typhoid fever may be limited in many developing countries because of antibiotic prescribing.
- Multidrug-resistant (MDR) typhoid is a more severe clinical illness with higher rates of toxicity and complications. In particular, recent cases of quinolone-resistant typhoid may be more severe.

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**Routine Screening for Macroprolactin in Hyperprolactemtic Sera**

Dr Noreen Sherazi
Chemical Pathology

It is well established that prolactin occurs mainly in three molecular forms: a monomeric prolactin with molecular weight ~23 kDa; a larger form named “big prolactin” (~50 kDa) and a “big, big prolactin” with a molecular weight over 100 kDa which is also called macroprolactin (MaPRL). In the majority of cases, MaPRL consists of monomeric prolactin connected with immunoglobulin; most
Introduction

The Alpha thalassaemia is an inherited disorder of α globin gene, which encompasses all those conditions in which deficit production of α globin chain of hemoglobin (Hb) occurs. Due to underproduction of α globin chain, γ and β globin chain synthesis is excessively increased to compensate during fetal and adult life.

Individuals having mutation in one allele of α globin gene on chromosome 16 are said to have α thalassaemia trait, they may be associated with mild anemia or with normal hemoglobin. Compound heterozygotes and some homozygotes for α-thalassaemia have moderately severe anemia characterized by presence of HbH disease. In neonatal period some individual who make very little or no alpha globin chains have a very severe form of anemia which, if untreated, causes death called the Hb Bart’s hydrops foetalis syndrome.

Genetics of Alpha Globin

Alpha globin gene is located on chromosome 16 (16p 13.3) whereas beta globin gene resides on chromosome 11 (11p 15.4). There are two functional α genes present on each chromosome 16. The size of the cluster gene is 70 kb which consist of three pseudo gene ψ, έ, α2, and α1 and four functional genes, έ, α2, α1 and φ. α gene carry three exon and two introns. While, 10 variable number of tandem
repeats are located around α globin gene locus. Two α genes lie adjacent to each other. As each diploid cell contains two sets of each chromosome, hence there are four alpha globin alleles are present in a normal individual Figure 1.

Epidemiology

The prevalence of α thalassaemia is also like others common globin gene disorder (β thalassaemia and sickle cell trait). It demonstrates high frequency throughout all the tropical and subtropical region of the world. In some areas, the carrier frequency of α- thalassaemia may be as high as 80-90 per cent of the population. Of all globin disorders, α- thalassaemia is the most widely distributed and therefore many individuals in these areas have interacting combinations of these variants (e.g. both α and β thalassaemia). Due to differences in the interactions between various molecular defects underlying α- thalassaemia, HbH disease is predominantly seen in South East Asia, the Middle East and the Mediterranean. Similarly the Hb Bart’s Hydrops foetalis syndrome is predominantly seen in South East Asia.

Molecular Analysis

Due to advent of latest molecular tools, it has becomes possible to accurately and rapidly diagnose α- thalassaemia, while identify the precise defects underlying these disorders. Ultimately, most α globin chain rearrangements have been characterized by MLPA and DNA sequence analysis. For suspected but currently unknown rearrangements, Southern blotting or MLPA analysis may be used. Southern blot was the classical method to detect deletions causing α-thalassaemia before new techniques were introduced. Currently, MLPA is the method of choice. It is based on ligation of multiple probe-pairs hybridized across a large region of interest, followed by semi-quantitative amplification using universal tag PCR primers and subsequently fragments analysis.

Method

MLPA is performed on DNA samples for analysis alpha thalassamia. It can detect both deletion and duplication of alpha globin gene. The protocol consists of five steps spread on two days. MLPA test for alpha thalassemia is offered by the Molecular Pathology Section.

The steps are follows:

1. Hybridization reaction overnight incubation - Day one
2. Ligation of probes – Day two
3. PCR amplification Day – two
4. Capillary Electrophoresis
5. Interpretation of data

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Description</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>αα/αα</td>
<td>Normal –All four α alleles are present</td>
<td>Normal hematological profile</td>
</tr>
<tr>
<td>α/-αaa</td>
<td>Only one α allele absent Genetically silent carrier</td>
<td>Clinically asymptomatic usually, diagnosed during antenatal screening</td>
</tr>
<tr>
<td>α/-α or --/aa</td>
<td>Two α alleles are absent Heterozygous or Homozygous trait</td>
<td>Mild hypochromic, microcytic anemia (iron deficiency)</td>
</tr>
<tr>
<td>--/-α</td>
<td>Three α alleles are absent HbH disease</td>
<td>Moderate anemia, jaundice and Hepato- splenomegaly (folate deficiency)</td>
</tr>
<tr>
<td>--/--</td>
<td>Totally absent α chain Hydrops fetalis</td>
<td>Intrauterine death, skeletal deformities, cardiovascular problems, growth retarded</td>
</tr>
</tbody>
</table>
Table 2. Alpha-Thalassaemia Mutations in Different Ethnic Groups

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Type of Thalassaemia</th>
<th>Mutation(s)</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>α+</td>
<td>- α3.7</td>
<td>Common</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- α4.2</td>
<td>Less common</td>
</tr>
<tr>
<td></td>
<td>α+ - α0</td>
<td>α Koya Dora α</td>
<td>Relatively rare</td>
</tr>
<tr>
<td></td>
<td>α+ - α0</td>
<td>α IVS 1-117 α</td>
<td>Relatively rare</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α PA3(AATA- -)</td>
<td>Also found in Hindustani from Surinam</td>
</tr>
<tr>
<td>Middle East</td>
<td>α0</td>
<td>- - MED 1</td>
<td>Common in Iran, Palestinians, Arab population</td>
</tr>
<tr>
<td></td>
<td>α+</td>
<td>- α3.7</td>
<td>Common in Iran, Palestinians, Arab population</td>
</tr>
<tr>
<td></td>
<td>α+ - α0</td>
<td>α PA1(AATAAG)</td>
<td>Relatively common in Arab population</td>
</tr>
<tr>
<td>South-East Asia</td>
<td>α0</td>
<td>- - SEA</td>
<td>Most common deletion among Asians, world wide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- - FIL</td>
<td>Mainly in Philippinians, Common among Thai</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- -THAI</td>
<td>Relatively common</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- α3.7</td>
<td>Relatively rare</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- α4.2</td>
<td>Highly unstable α-chain, found in Thai, Laotian</td>
</tr>
<tr>
<td></td>
<td>α+</td>
<td>α Constant Spring</td>
<td>One of the most common non-deletion variants in Chinese</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α Suan Dok</td>
<td>Highly unstable α-chain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α Quong Sze</td>
<td>Highly unstable α-chain</td>
</tr>
<tr>
<td></td>
<td>α+</td>
<td>α Pakse</td>
<td>Highly unstable α-chain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α init A-G</td>
<td>Common in Vietnam</td>
</tr>
<tr>
<td></td>
<td>α+</td>
<td>α init -TG</td>
<td>Common in South-East Asia</td>
</tr>
</tbody>
</table>

Source: Adapted from Barbara J. Bain, Haemoglobinopathy Diagnosis 2nd edition 2006 [Bain, 2006126/id]
Prognostification of Acute Myeloid Leukemia with FLT 3 Mutation

Madiha Salahuddin & Dr Zeeshan Ansar Ahmed
Molecular Pathology

Introduction

Acute myeloid leukemia (AML) is a severe progressive malignancy that generally has short survival because of heterogenous origin, less responsive to treatment and tendency to early relapse. Abnormal activity of receptor tyrosine kinase has long been the main pathogenic finding in leukomogenesis, such as mutant forms of KIT, ABL, and platelet-derived growth factor receptor (PDGF-R) are among the constitutively activated tyrosine kinases that have been identified.

FMS-like tyrosine kinase-3 (FLT3) is a member of the PDGF-R subfamily of receptor tyrosine kinases, which was originally identified by its expression in hematopoietic stem/progenitor cells, and its importance in normal lymphohematopoietic stem cell function is now well established.

Structure and physiological role in haemopoiesis

FLT3 gene located on chromosome 13 (13q12); it is comprised of 24 exons extending over more than 100 kilobases. FLT3 shares the structural features of the other members of its subfamily, namely five immunoglobulin-like domains in extracellular region, a single transmembrane sequence, and, intracellularly, a short juxtamembrane portion followed by the interrupted kinase domain. FLT3 is expressed in virtually expressed in every tissue thus far examined. In human hematopoietic cells, FLT3 expression is restricted to the CD34-positive fraction of bone marrow and a smaller fraction of CD34-negative cells destined to become dendritic cells. Its activation send message to biochemical pathways that promote cell growth and inhibit apoptosis during normal haemopoiesis.

Figure 1. A model explaining the loss of some FLT3/ITD mutations at relapse
Biological Consequences of Abnormal FLT3 Activation

Molecularly, mutation-activated FLT3 induces transformation in immature clonal neoplastic (Blast) cells Figure 1. These cell completely blocks G-CSF-induced differentiation and suppresses the expression of myeloid transcription factors the profound biochemical consequences of its constitutive activation. FLT3 mutations have also been seen in myelodysplastic syndrome (MDS) in about 3-5 per cent of newly diagnosed patients. In MDS patients without FLT3 mutations, they sometimes appear when these patients progress to AML.

Significance of FLT3 in Leukemia

Expression of FLT3 has been identified in several types of leukemia and lymphoma. In addition, FLT3 expression was demonstrated in most pre-B cell, myeloid, and monocytic leukemia. Recently, it was discovered that FLT3 is universally expressed in primary AML blasts. Moreover, FLT3 mutations at diagnosis have been found to be occasionally acquired at the time of relapse. In other types, a lesser portion of chronic myelogenous leukemia (CML) blast crisis, as well as chronic lymphocytic leukemia (CLL) and even T-cell ALL ALL cells have been shown to express FLT3. A number of FLT3 activating mutations have been found in leukemia patients; the first of these to be identified was the FLT3/ITD mutation. Subsequent to this an activating point mutations at aspartate 835 (or isoleucine 836) in the activation loop of the FLT3 kinase domain was discovered. Both types of mutations are primarily found in AML. Patients with this abnormality were found to have an increased incidence of leukocytosis and a decreased overall survival (OS) when compared with patients without FLT3/ITD mutations. Numerous groups around the world have now confirmed and extended these findings in both adult and pediatric AML.

Method for FLT3 Detection

DNA is extracted by Qiagen Blood DNA isolation kit, from fresh bone marrow or peripheral blood. Extracted DNA is then subjected to amplification using ITD primers and also with D835 primers in separate PCR reactions. Amplified products for D835 mutation are digested with restriction enzyme EcoRV. Gel electrophoresis is performed to analyze amplification and EcoRV digested products Figure 2. This test is being offered by the Molecular Pathology section, Department of Pathology and Microbiology, Clinical laboratories, AKUH.
Interferon Gamma Release Assay (IGRA) for Detection of *Mycobacterium tuberculosis* Infection in the Host

Maheen Hassan, Zahida Amin & Madiha Salahuddin
Molecular Pathology

**Background**

Tuberculosis (TB) is the leading cause of death and a major cause of morbidity worldwide. Pakistan ranks sixth globally, among the 22 high TB burden countries, according to the World Health Organization (WHO). It is estimated that one third of the world’s population is latently infected with *Mycobacterium tuberculosis* (*Mtb*). However, only a small proportion of these progress to active disease. Traditionally, exposure to Mtb has been assessed with the use of a tuberculin skin test (TST) or Mantoux test. In a TST reaction, purified Mycobacterial peptide antigens are injected intra-dermally into the skin and a delayed type hypersensitivity response (DTH) to the antigens is measured after 48 h by measuring induration at the site of peptide injection. While this gives an indication of Mtb exposure in the host, it does not discriminate between *Mtb* and other mycobacteria of the *Mtb* complex such as *M. bovis* BCG. Therefore, the TST reaction is affected by BCG vaccination status of the host.

**Principle of the Assay**

Test based on the principle that T-cells from a whole blood sample, when exposed and incubated with a specific *Mtb* antigen, will produce interferon-gamma (IFN-γ) in an individual who has been infected with *Mtb* (Figure 1). An IFN-γ release assay, commonly known as IGRA is one in which IFN-γ released by T cells in response to exposure to *Mtb* antigens is measured. The Quantiferon TB Gold In-Tube (QFT) is a whole blood based IGRA that measures the cell-mediated immune response (IFN-γ) response to three specific proteins, ESAT-6, CFP-10 and TB7.7 which are found in *Mtb* but not in other species of the *Mtb* complex, such as *M. bovis* BCG. The QFT assay has two stages; firstly, it involves incubation of whole blood with *Mtb*-specific antigen, control antigen (mitogen) and nil control. The TB antigen tube assesses the IFN-γ response to highly specific TB antigen, the negative control tube adjusts for background noise and the positive control tube (Mitogen) indicates the effectiveness of T-cell mediated responses in the patient. Secondly, plasma is harvested from tubes and IFN-γ level is determined by an enzyme linked immunoabsorbent assay (ELISA).

**Figure 1. Principle of Mtb Interferon gamma release assay**

**Interpretation of the QFT Assay**

The QFT is an indirect test for *Mtb* infection: T-cells reactive to *Mtb* specific antigens are only present in those infected with *Mtb*. Results are interpreted (as shown in Table 1). A test for *Mtb* is considered positive if the amount of IFN-γ in response to TB antigen (minus Nil) is >=0.35 International Units (IU)/ml.

**Utility of the Assay**

The QFT - IGRA is intended for use as a diagnostic aid for *Mtb* infection, whether active tuberculosis disease or latent TB infection (LTBI), and is intended for use in conjunction with risk
assessment, radiography and other medical and diagnostic findings. It is useful for detection of \textit{Mtb} in patients where differential diagnosis of this infection may lead to a change in treatment and management of the case. This is a qualitative test. The IFN-$\gamma$ response to TB antigen should not be used to monitor disease progression. A QFT-G Positive result cannot distinguish between latent and active TB.

The advantage of this test is that specific antigens to \textit{Mtb} are used and one patient visit is required. In contrast to TST, it is unaffected by BCG vaccine. Individuals who are on anti-inflammatory drug treatment such as tumor necrosis alpha (TNF-$\alpha$) inhibitors for rheumatoid arthritis may be at risk of developing TB if they already have latent TB. In such cases, QFT may be useful assessing \textit{Mtb} infection in rheumatic disease patients receiving immunosuppressive therapy.

The disadvantage is that QFT is an expensive test and requires laboratory expertise. A factor which limits the efficacy of the QFT test is the cell mediated immune response in the host. Therefore, if immunity is compromised in the patient and T cell responses are diminished such as in patients with human immunodeficiency virus (HIV) infection, or those with oncological disorders, the QFT may give inaccurate results. However, QFT is more sensitive and specific than the TST for detecting tuberculosis infection in HIV +ve individuals. In children, the utility of QFT is still unclear as in young children cell mediated immune responses are not fully developed and this may result in false negative results.

### Table 1. Interpretation of IGRA – QFT Assay Results

<table>
<thead>
<tr>
<th>Interpretation</th>
<th>Nil*</th>
<th>TB Responset</th>
<th>Mitogen Responset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≤8.0</td>
<td>≥0.35 IU/ml and &gt;25% of Nil</td>
<td>Any</td>
</tr>
<tr>
<td>Negative**</td>
<td>≤8.0</td>
<td>&lt;0.35 IU/ml or &lt;25% of Nil</td>
<td>≥0.5</td>
</tr>
<tr>
<td>Indeterminate++</td>
<td>≤8.0</td>
<td>&lt;0.35 IU/ml or &lt;25% of Nil</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Positive</td>
<td>&gt;8.0</td>
<td>Any</td>
<td>Any</td>
</tr>
</tbody>
</table>

* The interferon gamma (IFN-$\gamma$) concentration in plasma from blood incubated without antigen.
+ The IFN-$\gamma$ concentration in plasma from blood stimulated with a single cocktail of peptides representing early secretory antigenic target-6 (EAST-6), culture filtrate protein-10 (CFP-10), and part of TB 7.7 minus Nil.
$ The IFN-$\gamma$ concentration in plasma from blood stimulated with mitogen minus Nil.
** Interpretation indicating that \textit{Mycobacterium tuberculosis} infection is likely.
++ Interpretation indicating an uncertain likelihood of \textit{M. tuberculosis} infection.
An Analysis of Needle Stick Injury in the Department of Radiology

Amin Rajani and Raza Sayani
Department of Radiology

Needle stick injury is a global issue and a major threat to health care workers. Occupational exposures to percutaneous injuries are a substantial source of infections with blood borne pathogens such as hepatitis B virus, (HBV), hepatitis C virus, (HCV) or Human immunodeficiency virus, (HIV) among health-care workers. NSI’s are generally under reported and data regarding them are usually sparse, especially in developing countries like Pakistan. Obtaining accurate figures for the incidence of needle stick injuries is very difficult. Its frequency in Radiology Department is generally considered low as compared to surgery; however the Radiology Department staff and doctors are no exemption. The frequency is increasing with the rise in interventional procedures in the department. We carried out a cross sectional survey in our department to identify the frequency by recording all self-reported needle stick injury data of health care workers of the department.

In the last decade (year 2000-2010), 55 health workers reported NSI. Radiographers and radiology residents received maximum injuries Figure 1. The main cause identified was cannulation Figure 2. However, many injuries occurred during disposing of the used needles. In majority of cases the patients were not infected with any known blood borne infection.

Over the years at institutions level as well as departmental level, we have regularly emphasized the importance of infection control and NSI particularly. Additionally, faculty as well as residents who are inducted, go through sessions and training for intervention procedures where they are taught measures to be taken when such an event occurs. Similar sessions are conducted for Technologists and Nursing staff as well.

Timely surveillance is performed if a needle stick injury does occur. However, the best measure is prevention which can only be done by repeated re-enforcements through various techniques e.g. flyers, videos and lectures.
Meeting Report: The 37th Annual Conference of the Pakistan Association of Pathologists and The 2nd Joint Conference of the Societies of Pathology

Sibtain Ahmed
Chemical Pathology

The 37th Annual Conference of the Pakistan Association of Pathologists / 2nd Joint Conference of the Societies of Pathology was held in the ‘Mughal City of Gardens’ Lahore from December 20th to 22nd, 2013. The theme of the conference was “Young Pathologists – Our Future.” In order to maximize participation of young pathologists, the organizing committee had arranged scholarships for 100 young pathologists including residents and technologists from across the country to attend the conference. Scholarship consisted of travel expenses, accommodation and registration fee.

The first day began with inauguration ceremony on 20th December. This was followed by the “Razi lecture”, by Dr. Mohammad Akhtar from King Faisal Specialist Hospital Riyadh Kingdom of Saudi Arabia on ‘Breast Cancer’ and impact of new diagnostics including molecular modalities in breast cancer diagnosis were highlighted. It was followed by scientific sessions in which young scientists were given a chance to present researches and studies they have conducted in their respective disciplines. A plenary session was also organized in which senior Pathologists delivered talks on various aspects of Pathology and Laboratory Medicine. In the evening organizers had planned a get together at the famous food street. A blend of rich cultural heritage and delicious food was a refreshing experience towards the end of a busy day.

Second day December 21, 2013 started with ‘meet the experts’ session, a great learning opportunity for maturing pathologists to acquire knowledge from senior professionals in their respective fields. It was followed by free paper sessions in the different disciplines of pathology. A general body meeting was also held during the day. Several selected abstracts were also displayed as posters which portrayed research advances in the field of pathology. After the sunset the exhausting day was masked by a shopping trip and a wonderful dinner in the surroundings of the famous Gaddafi Stadium.

On the third day of the conference two scientific sessions were held. The conference closing ceremony was held in afternoon and best oral and poster presentations were announced.

Workshops of Histopathology, Hematology, Microbiology, Chemical Pathology and Immunology were also arranged and were facilitated by renowned experts in the different disciplines of Pathology respectively. The conference not only excelled in presenting exciting developments in the field of pathology and laboratory medicine, it provided an excellent platform in bringing together the future pathologist and the giants from the different disciplines of pathology thus allowing opportunity for a healthy debate.