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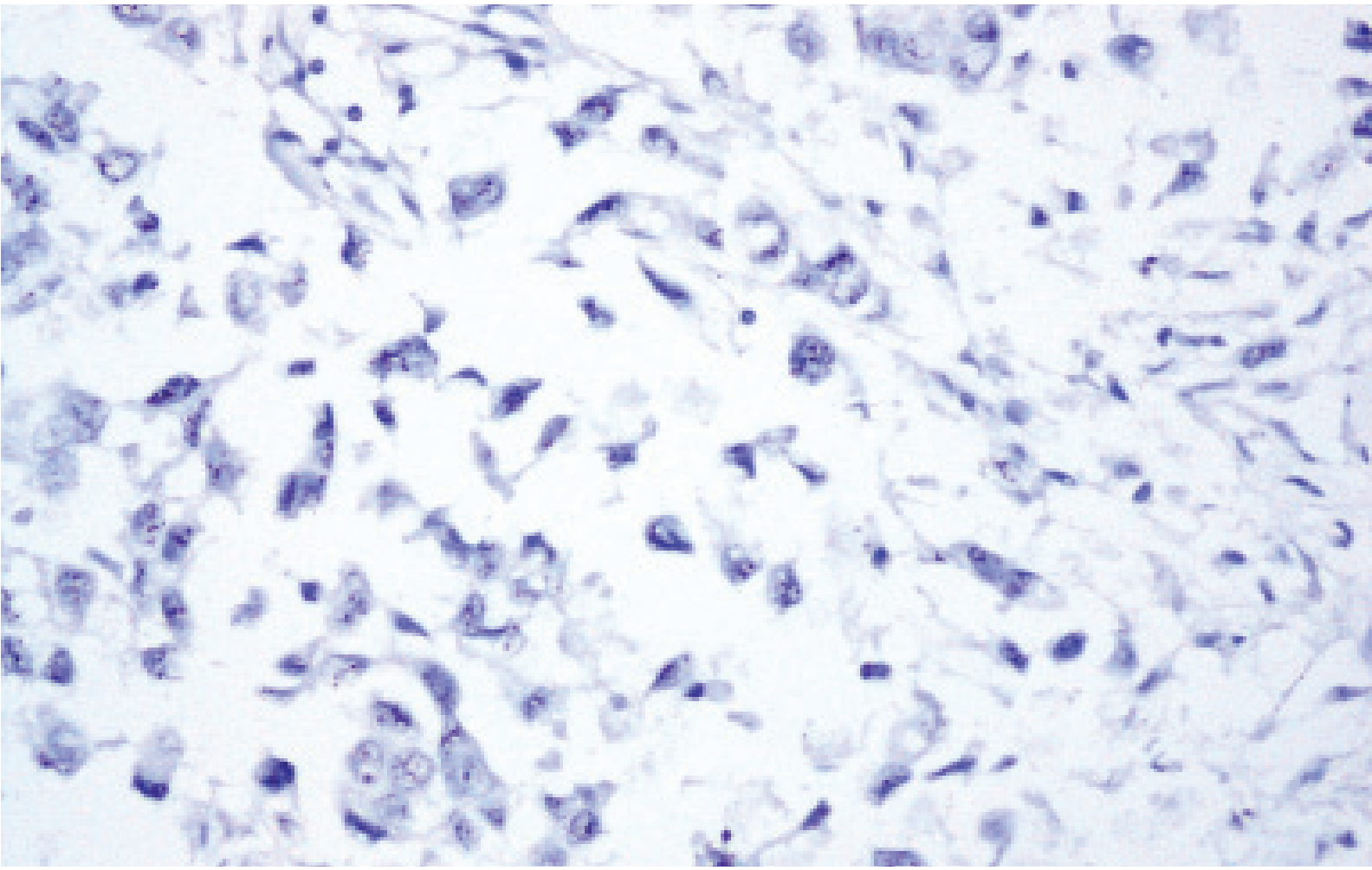

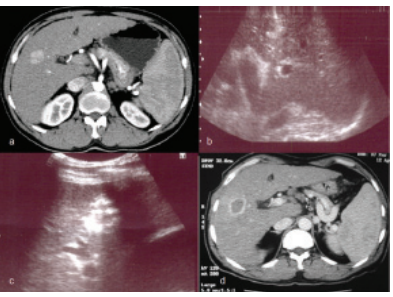

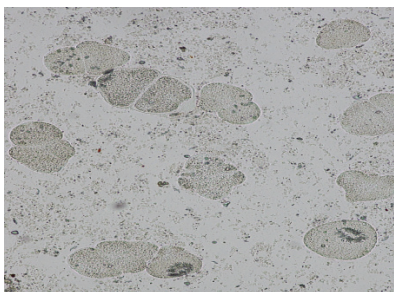

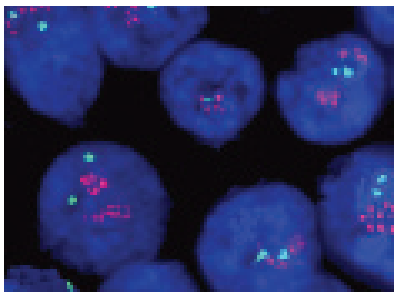

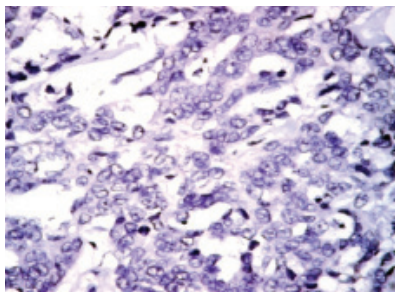

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The Aga Khan University Hospital, Karachi

LABRAD

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Routine and Special Liver Function Tests

Dr Lena Jafri
Chemical Pathology

No single test is able to provide an overall measure of the liver function. Instead, the group of values measured is interpreted collectively to determine the likelihood of liver disease, possible causes and the severity of the disease. Laboratory tests of the liver function can also be used to monitor the progress of the disease and the response to the treatments.

Following is a brief description of the tests helpful in diagnosing and managing liver disorders done under the section of Chemical Pathology, Aga Khan University.

Tests Detecting Injury to Hepatocytes

Alanine transaminase (ALT) and Aspartate aminotransferase (AST): ALT is mainly found in the cytosol of the liver cell whereas AST is found in the cytosol and mitochondria. AST is also present in nearly the same levels in the heart, skeletal muscle and the liver. Therefore, mild and moderate liver damage gives high ALT and normal AST value and severe liver damage gives high AST /ALT blood test value. The AST/ALT ratio is approximately 0.8 in normal subjects. In particular, the AST is greater than the ALT in alcoholic hepatitis and the ratio is greater than 2:1. A high transaminase level is characteristic of liver cell death and the highest levels occur early in the disease (Fig 1).

Lactate dehydrogenase (LDH): LDH is localised in the cytoplasm of cells and is thus extruded into the serum when cells are damaged. The total LDH is increased in viral or toxic hepatitis, extrahepatic biliary obstruction, acute necrosis of the liver and liver cirrhoses.

Tests of Cholestases

Gamma Glutamyl Transferase (GGT): GGT is a hepatic microsomal enzyme. Chronic ingestion of alcohol or drugs induces microsomal enzyme production. GGT permits differentiation of liver disease from other conditions in which serum alkaline phosphatase is elevated. Serum GGT levels are normal in Paget's disease, rickets, and osteomalacia, and in children and pregnant women without a liver disorder.

Alkaline Phosphatase: Alkaline phosphatase activity in serum is primarily derived from three sources: Liver, bone, and the intestinal tract. Increased levels of alkaline phosphatase suggest cholestases.

Tests of Detoxification and Excretory Function

Bilirubin: Unconjugated hyperbilirubinaemia occurs in hemolytic disease, Gilbert's syndrome and the Crigler-Najjar group of disorders. Conjugated hyperbilirubinaemia occurs in Dubin Johnson, Rotor

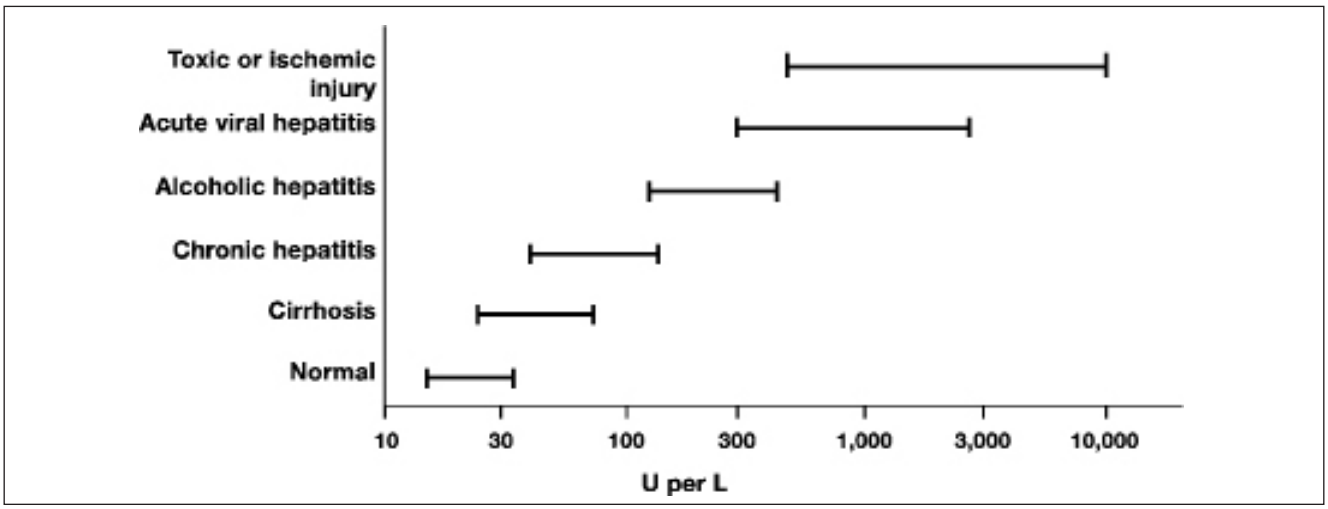

Fig 1. Concentration of Transaminases in Various Liver Disorders.

Table 1. Concentration of Analytes in Prehepatic, Hepatic and Post Hepatic Jaundice

	Pre-hepatic	Hepatic	Post-hepatic
Serum Bilirubin	Increased (unconjugated)	Increased (conjugated)	Increased (biphasic)
Urine Bilirubin	Absent	Increased	Often absent
Urine Bilinogen	Increased	Absent	May be increased or decreased
Stercobilin and colour of faeces	Increased, Dark	Decreased, Pale	Decreased or normal, Pale or normal
Serum Alkaline phosphatase	Normal	Increased	Increased
ALT, AST	Normal	Increased Slightly	Increased
Prothrombin Time	Normal	Prolonged not corrected by IV vitamin K	Prolonged corrected by IV vitamin K

syndrome, and in hepatocellular and cholestatic liver diseases (Table 1).

Ammonia: Ammonia is absorbed from the gut and incorporated into the urea and amino acids by the liver. If there is a severe hepatocellular disease or portal-systemic blood shunting, the blood levels of ammonia rise. An elevated blood ammonia level is a classic laboratory abnormality in patients with hepatic and encephalopathy which is present with altered mental status.

Tests of the Liver's Biosynthetic Capacity

Serum Albumin/Total Protein: The liver is the sole site of albumin synthesis. The protein is not a good indicator of hepatic synthetic function in acute liver disease because of its half-life of 20 days. Albumin levels are decreased in malnutrition, trauma, nephrotic syndrome and in patients with ascites due to large volumes of distribution. The serum total protein value minus the albumin value gives the serum globulin level. Serum globulin can increase dramatically in chronic hepatitis and cirrhosis, and in the presence of hypoalbuminaemia. This emphasises the importance of measuring both albumin and total protein under these conditions.

Specific Etiological Tests of Liver Dysfunction

Alpha-1-Antitrypsin: Deficiency of this enzyme can lead to lung emphysema and liver disease. Decreased plasma levels of this protein may be associated with neonatal hepatitis, neonatal cholestasis, and cirrhosis in infancy, childhood or adulthood.

Alpha-Fetoprotein: Alpha-fetoprotein (AFP) is a glycoprotein that is normally produced during gestation by the foetal liver and yolk sac, the serum concentration of which is often elevated in patients with hepatocellular carcinoma. Elevated serum AFP may also occur in pregnancy, with tumours of gonadal origin, and may be seen in patients with chronic liver disease without hepatocellular carcinoma.

LKM-1 Antibodies (IgG): LKM-1 antibodies are used in the diagnosis of auto immune hepatitis. These antibodies are not seen with type-1 patients of autoimmune hepatitis. LKM-1 antibodies can also be found in patients with chronic hepatitis C.

Caeruloplasmin/Urinary Copper: Wilson's disease results in liver cirrhosis. A characteristic feature is a low serum level of copper binding protein, caeruloplasmin and an increase in hepatic copper. If the caeruloplasmin is normal and Kayser-Fleischer rings are absent, but there is still a suspicion of Wilson's disease, the next test is a 24-hour urine collection test for quantitative copper excretion. Diagnosis is confirmed by a liver biopsy.

Serum Ferritin/ Iron Studies: The presence of iron overload is suggested by elevations in the concentrations of plasma iron and ferritin and by an increase in transferrin saturation. Ferritin, a protein synthesised by the liver is the primary form of iron storage within cells and tissues. Measuring ferritin provides a crude estimate of iron stores in the body though in many cases notable inflammation can elevate serum ferritin.

Other Analytes

Vitamin D: The two major forms of Vitamin D; D2 and D3 are hydroxylated in the liver forming 25-hydroxy D. 25-hydroxy D is the key storage form and is known to provide the overall nutritional status of Vitamin D.

In liver diseases, impaired hydroxylation by the liver can lead to Vitamin D deficiency.

Haptoglobin: Haptoglobin is used to screen for and monitor intravascular hemolytic anaemia. In intravascular hemolysis, haptoglobin levels are reduced whereas in extra vascular hemolysis, the levels are normal. As haptoglobin is an acute-phase protein, any inflammatory process may increase the levels of plasma haptoglobin. Chronic liver disease may result in low haptoglobin levels.

Blood Urea Nitrogen: Since urea is synthesised in the liver, diseases of the liver without renal impairment result in low serum urea nitrogen, although the urea to creatinine ratio may remain normal.

Management of Coagulopathy Associated with Liver Disease

Dr Farrukh Khan
Haematology

Liver disease impacts both the primary and secondary haemostatic pathways through a number of mechanisms. Historically, liver-disease associated coagulopathy has been considered a major contributor to bleeding complications. Both acute liver failure (ALF) and chronic liver disease (CLD) result in reduced synthesis of pro- and anticoagulant factors and are associated with thrombocytopenia and platelet dysfunctions.

Routine correction of liver disease-associated coagulopathy is not required in non-haemorrhagic patients.

Vitamin K deficiency is common in ALF and cholestatic liver disease and replacement of Vitamin K should be considered in these patients. Additional therapy may be required during episodes of variceal bleeding, surgery and prior to invasive procedures. However, the role of plasma and platelet infusion prior to invasive procedures is not well-established.

Paracenteses and central venous access can be undertaken safely even in the presence of coagulopathy and thrombocytopenia, and bleeding post-liver biopsy is not predicted by abnormal laboratory markers.

The British Committee for Standards in Haematology Blood Transfusion Taskforce (2004) suggested that there is insufficient evidence to support prophylactic transfusion of fresh frozen plasma (FFP) in those with a prolonged prothrombintime (PT) prior to liver biopsy. Furthermore, the response to FFP infusion in patients with liver disease is highly variable, with less than 15 per cent of patients achieving an appropriate correction of PT following infusion of FFP in one study. The use of FFP can result in additional complications; large infusional volumes can lead to volume overload, with subsequent exacerbation of portal hypertension, ascites and intracranial hypertension, in addition to the risk of infection and transfusion related acute lung injury.

In those with disseminated intra vascular coagulation (DIC) and bleeding, FFP administration may be useful. In this setting, in the presence of persistent hypofibrinogenaemia and continued bleeding following FFP, cryoprecipitate or fibrinogen concentrate may be used.

Platelet transfusion should be considered in the presence of bleeding and a platelet count of <10 x 109/l or prior to invasive procedures in those with a platelet count of <50 x 109/l. Increments may be poor following platelet transfusion in those with hypersplenism, active bleeding or coexistent infection. Given the lack of evidence and efficacy in this area it may be more appropriate to adopt an expectant management strategy, whereby FFP is reserved for those who develop bleeding complications. However, this approach is considered too high a risk for some procedures, such as intracranial pressure monitor placement in ALF. Further research is required to determine an optimal management strategy.

Recombinant Factor VIIa (rFVIIa) has been investigated as an alternative agent for the correction of PT in ALF and to reduce blood loss in those with

bleeding varices, prior to liver biopsy, liver resection and during liver transplantation. These studies have demonstrated that although rFVIIa administration leads to normalisation of the PT, there was no associated reduction in bleeding. It is not approved for use in liver disease and is associated with a significant increase in arterial thromboembolic events when used outside its approved indications.

Prothrombin complex concentrates (PCC: containing Vitamin K-dependent coagulation factors) may have an advantage over FFP with smaller volume for delivery of factor replacement and rapid correction of haemostatic parameters. However, caution is required in patients with liver disease due to the risk of DIC and thromboembolic complications. There are no randomised controlled trials assessing the efficacy and reduction in bleeding risk with PCC in patients with liver disease.

Anti-fibrinolytic agents, including tranexamic acid, aprotinin and e-aminocaproic acid, have been used successfully to control bleeding during liver transplantation and reduce transfusion requirements in this setting. However, their routine use has recently been challenged and their role in liver disease outside the transplant setting has not been established yet. The use of desmopressin (1-deamino-8-d-arginine-vasopressin, DDAVP) has been investigated in liver disease but no improvement in clinical outcome was seen in those with variceal bleeding or those undergoing transplantation.

There may be a future role for thrombopoietin mimetics in the treatment of thrombocytopenia associated with CLD. A phase 2 study of Eltrombopag in patients with HCV-associated cirrhosis demonstrated its efficacy in raising platelet counts sufficiently to allow commencement of antiviral therapy.

Further clinical trials are awaited; there may be a further role for these agents in amelioration of thrombocytopenia in patients with CLD prior to procedural interventions.

Significance of Clinical and Laboratory Scoring Systems in Diagnosis of Disseminated Intravascular Coagulation in Chronic Liver Disease

Dr Natasha Ali
Haematology

The liver is the most important organ involved in the regulation of haemostasis. Most of the clotting factors, their inhibitors and a number of proteins responsible for fibrinolysis are produced in the liver, therefore, liver disease impacts both primary and secondary haemostatis pathways through a number of different mechanisms. Abnormalities of the clotting cascade are the predominant features of acute and chronic liver disease. In few patients with hepatic disease, physicians are faced with diagnostic challenge of an underlying bleeding or thrombotic diathesis due to overlap between various clinical syndromes including Disseminated Intravascular Coagulation (DIC).

According to the International Society of Thrombosis and Haemostasis (ISTH), DIC is a syndrome characterised by a systemic intravascular activation of coagulation, with loss of localisation, arising from different causes. It can originate from and cause damage to the microvasculature, which if sufficiently severe can produce organ dysfunction. ISTH also proposed that the working definition of DIC can be divided into two phases:

a) Non-overt DIC: represents subtle haemostatic dysfunction
b) Overt DIC: de-compensated phase of non-overt DIC

Events responsible for DIC in liver disease include liver necrosis, impaired endotoxin clearance, surgery, shock and ascites recirculation. DIC can be recognised as a syndrome suggested by clinical signs and

laboratory tests. The characteristic laboratory findings include: prolonged prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), increased levels of fibrin related markers (Fibrin degradation products, D-Dimer), decreased platelet count, fibrinogen level, plasma coagulation factors and their inhibitors. More specialised and useful tests include: prothrombin activation fragments and thrombin-antithrombin complex (TAT).

In 2001, ISTH proposed two separate scoring systems for overt and non-overt DIC. The overt DIC score consists of a five-step diagnostic algorithm in which a specific score - reflecting the severity of the abnormality found - is given to each of the laboratory tests (Table 1). A score of five or more is considered to be compatible with DIC, whereas a score of less than five may be indicative (but not affirmative) of non-overt DIC. Similarly, using certain clinical and laboratory findings (Table 2), a score of five or greater is compatible with non-overt DIC which could diagnostically define patients with a poor prognosis due to haemostatic dysfunction, independent of developing overt DIC.

Table 1. ISTH Score System for Overt DIC

SCREENING TESTS	FINDINGS	POINTS
PLATELET COUNT	>100,000/µL	0
	50,000-100,000/ µL	1
	<50,000/ µL	2
PROTHROMBIN TIME	(PT upper limit of reference range) >3 secs	0
	(PT upper limit of reference range) =3-5.9 secs	1
	(PT upper limit of reference range) ≥6 secs	2
FIBRINOGEN	>100mg/dl	0
	<100mg/dl	1
FIBRIN MONOMER	No increase	0
	Moderate increase	2
	Severe increase	3

Interpretation: A score of >5 is compatible with overt DIC.

Table 2. ISTH Score for Non-overt DIC

PARAMETER	FINDING	POINTS
Diagnosis	Not associated with DIC	0
	Associated with DIC	2
Platelet count	>100,000/µL	0
	<100,000/µL	1
Prolongation of PT	< 3 seconds	0
	> 3 seconds	1
Soluble fibrin or FDP	Normal	0
	Increased	1
Antithrombin III	Normal	-1
	Low	1
Protein C	Normal	-1
	Low	1

Interpretation: A score of >5 is compatible with non-overt DICt he higher the score the more likely that non-overt DIC is present All the tests required for DIC scoring except fibrin monomer are available at AKU clinical laboratories.

Laboratory Diagnosis of Hydatid Disease

Dr Sana Rajper and Dr Joveria Farooqui
Microbiology

Echinococcosis or hydatid disease is caused by tapeworm Echinococcus most commonly as species *E. granulosus and E. multilocularis*. Humans are the incidental dead-end hosts due to accidental ingestion of eggs. The oncospheres hatch in the intestine, enter the blood stream to migrate to the liver and other viscera where a fluid filled multi-layered cyst with protoscolices develops a hydatid cyst.

Infection of the liver frequently produces no symptoms and remains quiescent for years or produces mass effects. They rarely rupture into the biliary tract or the peritoneal cavity. The right lobe is affected in 60-85 per cent of the cases. Secondary bacterial infection of the cysts results in liver abscesses.

Microscopy: The saline wet preparation demonstrates the daughter cysts or brood capsules with protoscolices in surgically removed tissues. The inner lining of the cyst is a germinal membrane from which numerous daughter embryos develop. These form minute polypoid structures (brood capsules) that line the inner reproductive membrane from which large number of daughter cysts are produced. When embryos break free from the membrane and float in the fluid within the cyst, they are known as hydatid sand (Fig 1). Under the microscope, each grain of sand is in fact a tiny embryonic beginning of a new tapeworm, complete with an inverted scolex and a rostellum armed with hooklets.

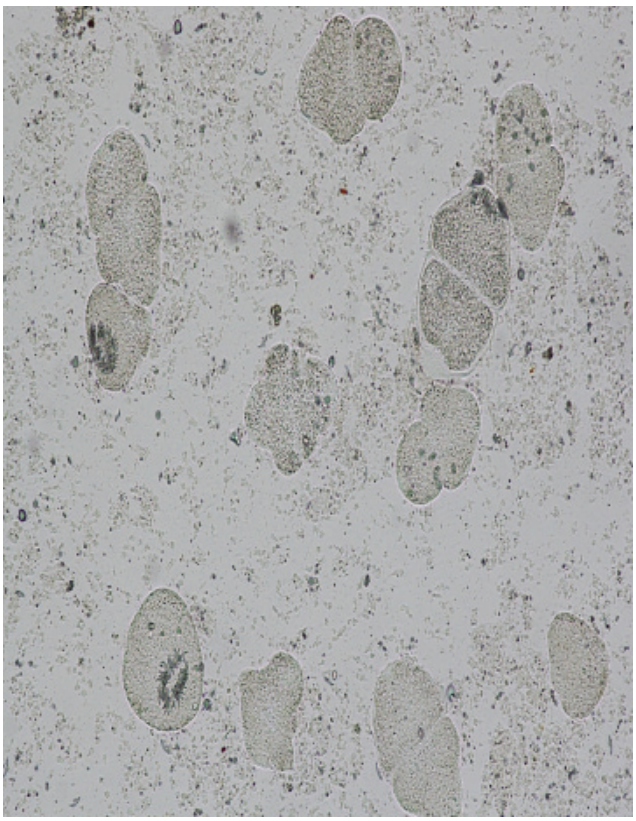

Fig 1. Wet preparation of liver abscess aspirate showing hydatid sands of echinococcus spp. (x 40 magnification).

Serology: Serology is a useful tool for primary diagnoses as well as follow-up after treatment. Most immunodiagnostic techniques involve the detection of Echinococcus specific antibody in the serum of suspected patients using a variety of crude antigens. The problem with methods using crude antigens is the serologic cross-reactivity of Echinococal antibody test. Additional tests using recombinant or purified species-specific antigens may complement the serological diagnoses.

False Positive and False Negative Results: Serologic testing produces both false positive and false negative results.

False positive reactions are more likely to happen in the presence of other helminth infections, cancer, and immune disorders. False negative results occur with varying frequency depending on the site of the lesion and the cyst's integrity and viability. Cysts in the liver more commonly (85-95 per cent) elicit an antibody response than cysts in the lung (65 per cent). Thus, a negative serologic test generally does not rule out echinococcosis. Brain, eye, and splenic cysts often do not produce detectable antibodies, whereas bone cysts are frequently associated with positive serology. Serology is less likely to be positive with cysts at any site if the cysts are intact, calcified or nonviable. Children and pregnant women have negative serology more frequently than other patient populations.

Serologic Methods: A number of techniques are currently employed, these include indirect hemagglutination (IHA), indirect immunofluorescence, latex agglutination, double diffusion immunoelectrophoresis, counter-current immunoelectrophoresis (CIEP), radioimmunoassay

(RIA), enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunoelectrotransfer blots (EITB), enzyme-linked immunoelectrodiffusion assay (ELIEDA), time-resolved fluoroimmunoassay (TR-FLA) and immunoblot.

Screening Test: The methods most frequently employed for initial screening tests are IHA and ELISA. Currently, our laboratory is using the IHA method for detection of Echinococcal antibody.

Indirect Hemagglutination Test (IHA) : The indirect hemagglutination test aids in the qualitative detection and quantitative determination of specific antibodies to *Echinococcus granulosus* in human serum. It is based on the principle of indirect erythrocyte agglutination (Fig 2).

Serum dilutions of 1:32 to 1:64 and higher provide diagnostically useful titers. Mid-range serum titers lie between 1:512 and 1:2048. Low serum titers (1:32 to 1:128) should only be accepted as positive when evaluated in conjunction with a second serological. method (for example, CFT, IFT or ELISA).

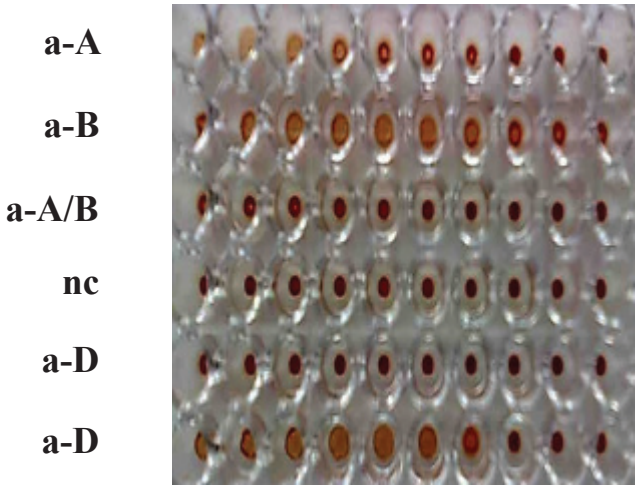

Fig 2. Indirect Heamagglutination Test for the diagnosis of both Echinococcal Liver Cyst and Amoebic Liver Abscess.

Laboratory Diagnosis of Amoebiasis

Dr Sana Rajper and Dr Joveria Farooqui
Microbiology

Amoebiasis is caused by the protozoan, *Entamoeba histolytica*. The parasite exists in two forms: a cyst stage, which is the infective form, and a trophozoite stage, which is the form that causes invasive disease. Majority of infections occur in developing countries due to poor socio-economic conditions and low sanitation levels.

Amoebic liver abscess is the most common extra-intestinal manifestation of amebiasis. Amoebae establish hepatic infection by ascending the portal venous system. Patients are in the acute stage after one to two weeks of fever and right upper quadrant pain. Concurrent diarrhoea may also occur. Occasionally, patients have a more chronic presentation with months of fever, weight loss, abdominal pain, hepatomegaly and anaemia. Hepatic vein and inferior vena cava thrombosis secondary to amoebic liver abscess may also be present.

Laboratory Diagnoses

Microscopy: Amoebic liver abscesses contain acellular, proteinaceous debris, an anchovy paste, and a chocolate coloured fluid, consisting predominantly of necrotic hepatocytes. If an unstained saline amount of the aspirate is observed under the microscope within 2 hours, trophozoites may be seen in fewer than 20 per cent of cases and are often present only in the peripheral parts of the abscess, invading and destroying adjacent tissue.

Serologic Testing: Serum antibodies will be detectable in 92-97 per cent of cases presenting with amoebic liver abscess. Eventually 99 per cent of the patients will have positive antibody tests, but serologic testing may be negative in the first seven days. Up to 25 per cent of uninfected individuals in endemic areas have antiamoebic antibodies due to previous, often undiagnosed infection with *E. histolytica*. Consequently, positive serology in these populations is not nearly as helpful, although negative serology still assists in excluding this infection. Currently, we are performing IHA test for diagnosing amoebiasis. Other methodologies are agar gel diffusion and counter-immunopheresis which are less sensitive.

Indirect Heamagglutination Test (IHA): It aids in the qualitative detection and quantitative determination of specific antibodies to *Entamoeba histolytica* in human serum. It is based on the principle of indirect erythrocyte agglutination (refer to Fig 2 on page 9).

Serum dilutions of 1:32 and higher provide titers useful for diagnosis that indicate the presence of an invasive amoebiasis. Mid range serum titers lie between 1:256 and 1:2048. Low positive serum titers ranging from 1:32 to 1:54 should only be accepted as positive when evaluated in conjunction with a second serological method like immunoflourescence. Titers below 1:32 are generally considered to exclude an invasive amoebiasis.

For this reason, the method is especially well suited for the differential diagnosis of invasive amoebiasis in cases of abdominal pain that has not been clearly diagnosed.

Improvement of Bacteriological Yield of Liver Abscess by Use of Anaerobic Chambers

Dr Sadia Shakoor
Microbiology

Pyogenic liver abscesses are often caused by anaerobic bacteria. As many as 25 per cent of the bacteriologic yield from pyogenic liver abscesses comprise of anaerobes. Multiple abscesses are difficult to drain and therapy is primarily medical. Antibiotic therapy of complicated abscesses requires growing causative pathogens and subsequent sensitivity testing.

Growth of anaerobes in the microbiology laboratory is often challenged by prolonged laboratory transport times resulting in death of anaerobes, prevalence of fastidious anaerobes in clinical specimens, and differing growth requirements of different anaerobes unmet by various anaerobic culture systems. The latter issue has been overcome by the use of automated anaerobic chambers. Such chambers allow for hassle-free culture of anaerobes without the added cost of multiple gas-generating kits and anaerobic jars.

Additional provision for workspace in the chambers allows for processing, sensitivity testing and identification of anaerobes within the anaerobic environment of the chamber without having to remove bacterial Petri plates.

Incorporating such automated anaerobic chamber systems in microbiology laboratories increases the yield of anaerobic isolates and allows for appropriate sensitivity testing and identification of anaerobes in clinically relevant specimens (Fig 1).

Fig 1. Anaerobic Chamber in use at Aga Khan University Clinical Microbiology Laboratory.

Radiofrequency Ablation of Liver Tumours

Dr Rana Shoaib and Dr Muhammad Azeemuddin
Radiology

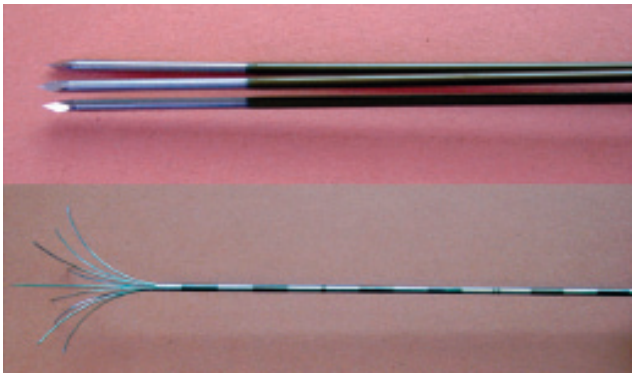

Fig 1. Needles used for RFA.

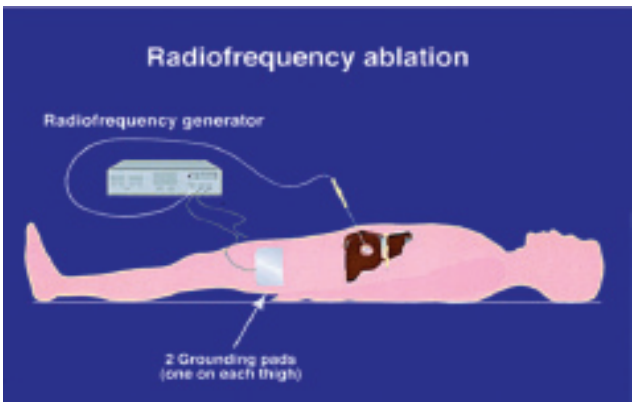

Fig 2. A schematic diagram showing an RF needle electrode deployed in a liver tumour.

Radiofrequency ablation, commonly referred to as RFA, is a minimally invasive treatment for liver cancer. It is an image-guided procedure which produces coagulative necrosis of tumor through local tissue heating (Fig 1 and Fig 2).

In this technique various imaging modalities such as ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) are used to help guide a needle electrode into a lesion. High-frequency electrical currents are then passed through the electrode, creating heat that destroys the tumour cells.

Indications

a) Hepatocellular carcinoma.
b) Liver metastasis, most commonly colorectal, especially if the patient is not an operative candidate.

Inclusion Criteria for Hepatocellular Carcinoma (HCC)

a) Single tumour ≤ 5cm. (lesions > 5cms can be considered for RFA alone or in combination with TACE. However, there is a weak level III evidence up till now and can be considered as pilot in our institution).
b) Multicentric < 3 lesions, each ≤ 3 cm.
c) Child-Pugh class A or B.
d) Tumours accessible by percutaneous route.

Inclusion Criteria for Metastatic Disease of Colorectal Cancer

a) Oligometastases to the liver ≤ 5cm. (those patients with metastatic disease may qualify if extrahepatic disease is deemed curable).
b) When multiple ≤ 3 lesions, each ≤ 3 cm.

Exclusion Criteria for HCC and Metastatic Disease

a) Single mass (primary or metastatic) > 5 cm (in longest axis).
b) When > 1 lesions any one lesion > 3 cm (in longest axis).
c) When > 3 lesions (NOTE: Should not be considered as absolute contraindication if successful treatment of all metastatic deposits can be accomplished).
d) Tumour abutting a major hepatic biliary duct or < 1 cm from the main biliary duct.
e) When ≤ 2 cm from large hepatic vein or portal veins.
f) Tumour adjacent to hepatic hilum and gallbladder.
g) Exophytic location of the tumour (due to the risk of tumour seeding).
h) Along the liver surface or < 1 cm deep to the liver capsule (hydrodissection can still be considered) [Relative Contraindication].
i) Lesions adjacent to GIT, kidney, adrenal (hydrodissection can still be considered). [Relative Contraindication].
j) Extrahepatic spread (unless curable) [Relative Contraindication].

k) Intrahepatic bile duct dilation [Relative Contraindication]
l) Evidence of vascular invasion,
m) Refractory coagulopathy
n) Ascites [Relative Contraindication]

Anaesthesia

This procedure is generally performed using local anaesthesia and mild sedation. However, deep sedation may be used if necessary.

Complications

RFA is a relatively low-risk procedure with a low rate of morbidity and mortality. However, few complications are associated with RFA. These include postablation syndrome which presents with low-grade fever, delayed pain, malaise, myalgia, nausea, and vomiting.

Other rare complications include shoulder pain, Cholecystitis, bile duct injury, Hemorrhage, Pneumothorax, Hemothorax or Pleural effusion, Hemobilia, and Infection.

RFA is considered superior to ethanol injection in the treatment of small HCC. RFA results in a higher rate of complete necrosis and requires fewer treatment sessions than percutaneous ethanol injection (Fig 3 and Fig 4).

Radiofrequency ablation (RFA) at AKUH

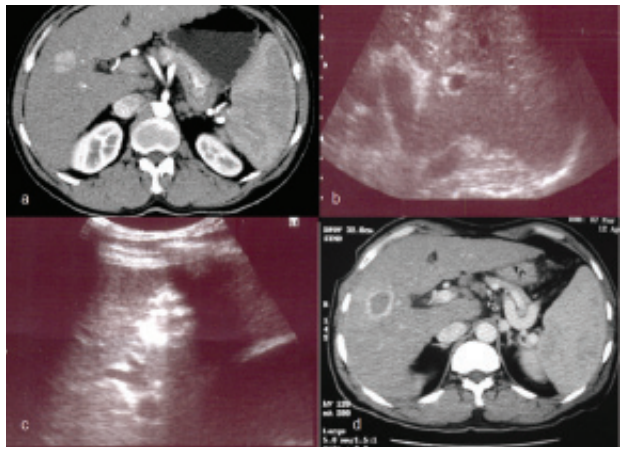

Fig 3. First case of RFA at AKUH: 41-year-old male with HCV, early child's B cirrhosis, A small hepatoma in liver (a) He was successfully subjected to RFA treatment (April, 2010) in Radiology without any major complication (b and c) Post RFA CT scan (d) Typical ring enhancement (inflammatory) and lesion is entirely avascular.

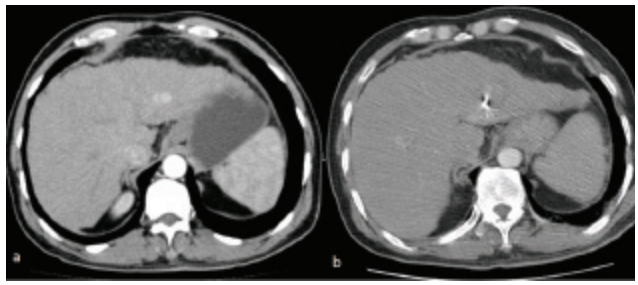

Fig 4. Another male patient with lesion in left lobe of liver (a) RFA performed under CT guidance (b) Needle can be seen.

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HER2 in Breast Cancer: FAQs

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The arrival of Herceptin for the treatment of HER2 amplified breast cancer patients has promoted considerable interest in the biology of HER2 receptor. Recent studies have established HER2 as an independent prognostic and predictive factor and a potential therapeutic target. Following is an overview that addresses some basic and frequently asked questions about HER2 gene amplification, and its role in breast cancer management.

What is HER2?

HER2, discovered in 1980, is a cell surface receptor that belongs to the epidermal growth factor receptor (EGFR) family. According to HUGO nomenclature committee, its official name is ERBB2. It is located on chromosome 17q12. Other members of the EGFR family include HER1, HER3 and HER4. Tyrosine kinase activity that initiates HER2 dependent signaling pathways resides in its cytoplasmic domain (Fig 1). Activated HER2 receptor phosphorylates a variety of substrates, which leads to initiation of intracellular signalling mechanisms involved in tumorigenesis.

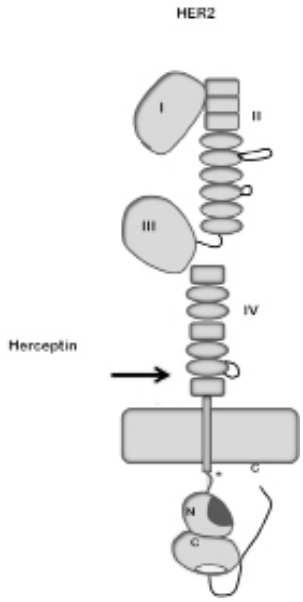

*Fig 1. Diagram of HER2 receptor. Domains I-IV constitutes ECD. A single transmembrane domain spans the cytoplasmic membrane. * indicates the site of PKC-mediated threonine phosphorylation. N and C represent amino and carboxyl terminal lobes of kinase domain. Arrow points to the site targeted by Herceptin (redrawn from Landgarf, BCR 2007, 9:202).*

Why HER2 is important in breast cancer?

According to a recent survey, the annual incidence of breast cancer worldwide in females contributes approximately 30 per cent to the total number of cancer cases reported each year. Whereas, in 25-30 per cent breast cancer patients' gene amplification of HER2 confers rapid tumour growth, lymph node metastases, high risk of post-surgery recurrence and poor survival. Moreover, scientific literature illustrates that patients with early stage disease and favourable tumour features also experience aggressive outcome in the presence of HER2 gene amplification, which makes HER2 a significant player in breast cancer management.

How HER2 is detected in breast cancer?

HER2 can be monitored by immunohistochemistry, real-time PCR and FISH at protein, messenger RNA and DNA levels respectively.

a) For initial screening, immunohistochemistry (IHC) is the choice for relative quantification of HER2 protein (receptors) because it is rapid, easy to perform and has a short turnaround time with the added advantage of reliably excluding 'negative (0 or 1+)' cases from 'strong positive or 3+' cases. Level of surface bound HER2 protein in breast cancer is evaluated in the invasive component by measuring the intensity of membrane staining. HER2 immunostained slides are scored on a scale of 0-3+ based on the proportion of cells staining, completeness and intensity of membrane staining (Fig 2). A score of 2+ is considered as equivocal and require further confirmation by additional tests. Current ASCO guidelines recommend first to examine HER2 protein status by IHC and in case of equivocal staining HER2 gene should be quantified by FISH technique.

b) HER2 gene amplification status in formalin fixed paraffin embedded (FFPE) tissues can be established by FISH assay. It provides direct visual examination of gene copy number

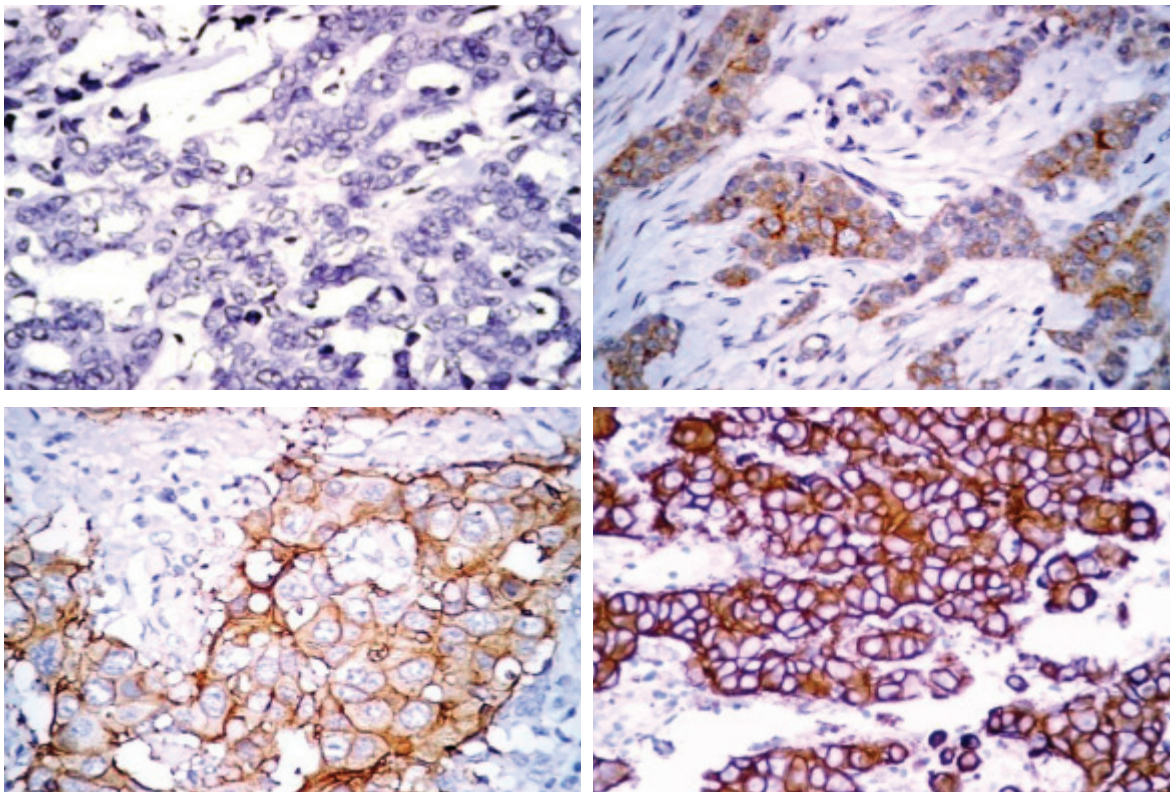

Fig 2. Photographs illustrating immunohistochemical staining of HER2 receptors along with estimated staining scores. Courtesy: AKUH

in the inter-phase nuclei. A DNA probe complimentary to HER2 gene and a second control probe specific to the centromeric region of chromosome 17 are hybridised to the previously processed FFPE tissue. Probe signals are searched in the invasive tumour, which is marked with the help of a corresponding H&E stained slide. In the invasive component of the tumour nuclear morphology is observed using DAPI filter. At least 20 non-overlapping cells from two different regions are scored. A non-amplified tissue shows two HER2 signals and two control probe signals, whereas an amplified HER2 may exhibit two type of signal patterns; a) clusters or patches of signals due to homogenously staining regions, b) individual and discrete signals, which indicate double minute chromosomes. In addition, presence of multiple HER2 and control probe signals that indicate polysomy 17 (Fig 3).

c) Chromogenic in situ hybridisation (CISH) is an alternative to FISH. In CISH assay a HER2 specific DNA probe binds to tumour DNA. The probe is detected followed by multiple treatments concluding with application of antibody linked to horseradish peroxidase enzyme and DAB chromogen (Fig 4). In contrast to FISH, signal detection of CISH is based on bright field microscopy. A major advantage of CISH over FISH technique is that HER2 signals are observed in relationship to the histological architecture. Secondly, as staining is permanent and not sensitive to light, slides can be stored for a longer duration. A recent enhancement in CISH technology is the introduction of a two-colour CISH, which has improved interpretation of borderline cases.

What is the Concordance level between FISH and IHC?

High concordance (> 90 per cent) between IHC and FISH is noted when IHC are 0 or 1+ (negative) and 3+ (positive). Cases with IHC 2+ are considered equivocal and should be tested by FISH assay. In these cases, FISH is considered a gold standard. Among the IHC 2+ cases tested by FISH assay, approximately a quarter of the cases show gene amplification. However, on the basis of current literature, it is concluded that around 20 per cent of the current testing by various methods may be inaccurate resulting in discrepancies between

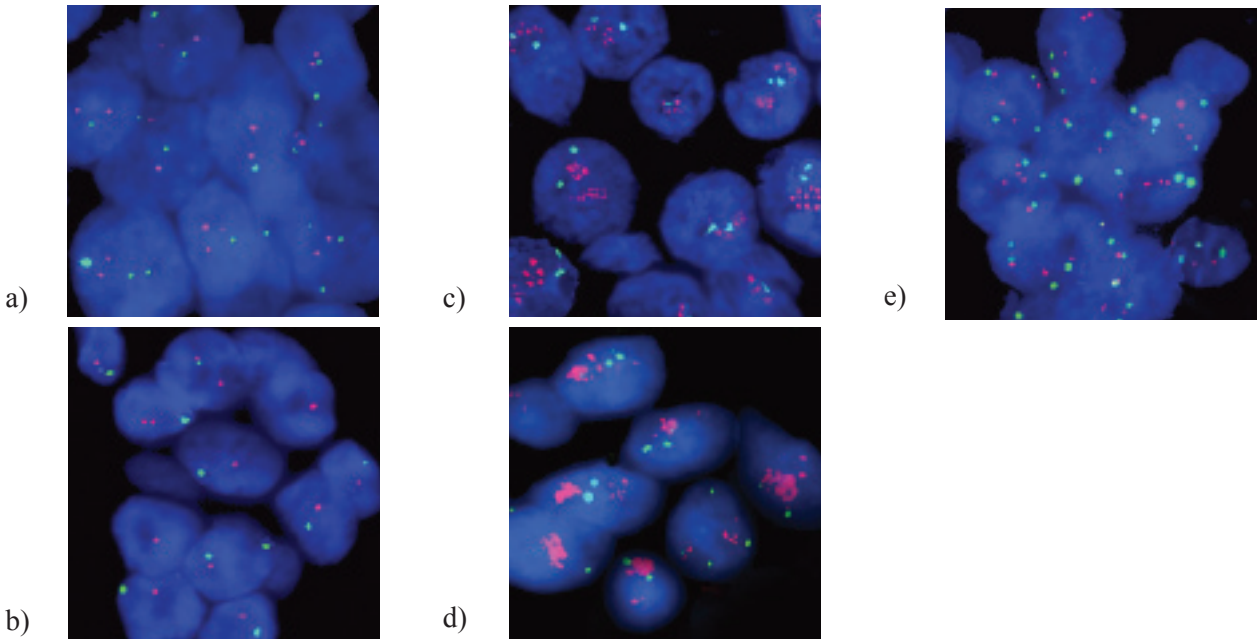

Fig 3. Representative photographs of FISH assay results. (a) two red signals of HER2 and two green signals of chromosome 17 (CEP) so normal with no amplification; (b) one red signal of HER2 and one green signal of chromosome 17 (CEP) so monosomy with no amplification.; (c) multiple red signals (mean of 22) of HER2 and two green signals consistent with strong amplification; (d) multiple red signals (patches) of HER2 and two green signals consistent with strong amplification; (e) multiple red signals (mean of 4) of HER2 with multiple green signals (mean of 4) represent polysomy. Courtesy: AKUH

IHC and FISH results. Following are the possible reasons for this discrepancy usually resulting in IHC 3+ with negative FISH.

a) Heterogeneity of antigenic expression as in otherwise IHC 3+ cases areas of IHC 2+ may also be present focally. It is important therefore to do FISH on strong positive tumour foci.

b) Over and under fixation of the tissue resulting in masking or loss of antigens. Optimum fixation time is between 6-24 hours in 10 per cent buffered formalin depending on the size of the tumour.

c) HER2 protein over expression can occur without gene amplification because of increased transcriptional or post transcriptional activity, mRNA stabilisation or the presence of chromosome 17 polysomy.

d) Selection of HER2 antibody and use of standardised or nonstandardised assays.

e) Interpretation by the reporting pathologist as current score given on morphologic assessment alone is largely subjective.

Tips and Tricks

a) HER2 assessment shall only be done on infiltrative component.
b) No grade 1 carcinoma breast like tubular carcinoma should be HER2 positive.
c) No HER2 positivity in Medullary carcinoma of breast.
d) No HER2 positivity in classic lobular carcinoma breast.
e) Changing of magnification from low to high power precludes 3+ IHC score.
f) In case of polysomy if absolute copy number on FISH is > 6 may qualify for amplification.

What is Polysomy 17?

Polysomy 17 is defined as the presence of three or more copies of chromosome 17 in a cell. It is a frequent feature of breast carcinoma. According to a report, polysomy 17 is seen in 20-40 per cent of invasive breast carcinomas. However, the association between over expression of HER2 protein and polysomy 17 is controversial. Some reports link polysomy 17 to HER2 over expression, whereas other publications show no influence on HER2 protein expression. A

scientific report has suggested that a high HER2 copy number observed in polysomy 17 in invasive breast carcinomas is a significant contributing factor in HER2 protein over expression. However, currently polysomy 17 positive tumours are ineligible for trastuzumab therapy.

What is Trastuzumab and its Mechanism of Action?

The knowledge of molecular mechanisms that operate in breast cancer has enhanced significantly in the recent years leading to refinement of therapies that target signalling molecules. Breast cancer patients who over express HER2 gene can benefit from one such treatment that is a recombinant monoclonal antibody called trastuzumab (Herceptin). This antibody is developed by linking the antigen binding regions of mouse anti-HER2 monoclonal antibody into a human IgG. Trastuzumab binds to the extracellular domain of the HER2 receptor (Fig 1). Although, the mode trastuzumab's action is not completely worked out but current literature suggests that it can cause HER2 receptor down regulation, inhibit intracellular signalling or even block receptor cleavage. Trastuzumab has also been shown to inhibit angiogenesis and decrease microvessel density in vivo. In patients with early stage disease, trastuzumab has significantly improved disease free survival and overall survival rates. According to available reports, trastuzumab can produce response rates up to 35 per cent in selected metastatic breast cancer patients.

Can HER2 Levels Predict Disease and Treatment Outcome?

HER2 gene amplification is an independent marker of inferior prognosis in breast cancer and is associated with high-grade tumours, lymph node metastasis and shorter disease-free and overall survival. Over expression of HER2 predicts success of trastuzumab therapy, which is manifested by lower recurrence rate and improved survival. On the other hand, the role of HER2 expression in predicting chemotherapy response is still debatable. So far, in several studies resistance to CMF (cyclophosphamide, methotrexate, and fluorouracil) chemotherapy has been reported in patients over expressing HER2. Conversely, HER amplification predicted a better response to anthracyclenes. Moreover, patients over expressing HER2 gene demonstrated a favourable response to taxanes. In estrogen receptor positive breast cancer patients, HER2 over expression has been implicated in resistance to tamoxifen treatment as well, highlighting the predictive value of HER2 in breast cancer.

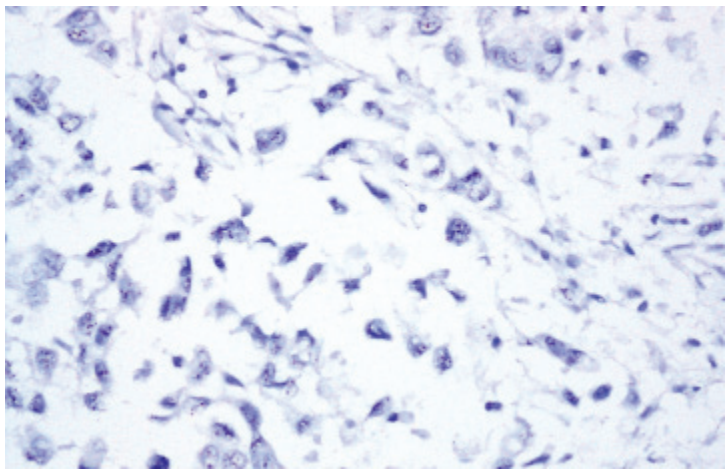

Fig 4. Detection of HER2 gene amplification by CISH. Multiple dark brown dots in the nuclei indicate multiple HER2 gene copies consistent with gene amplification. Courtesy: AKUH

Role of Flow Cytometry in the Evaluation of Minimal Residual Disease of Acute Lymphoblastic Leukemia

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In our flow cytometry practice at AKUH, bone marrow and/or peripheral blood samples from patients with anaemia, leukopaenia, thrombocytopaenia, or leukocytosis are routinely submitted for immunophenotypic evaluation of a possible hematolymphoid neoplasm. Lymph node and extranodal tissue biopsies can also be evaluated in patients with lymphadenopathy whose differential diagnosis includes lymphoma. Body fluids are also frequently submitted for the diagnosis or staging of patients with suspected lymphoproliferative disorder.

The first step is to establish a diagnosis of a hematolymphoid neoplasm (e.g. actue lymphoblastic leukemia/lymphoma) and then flow cytometry can be used to monitor status of disease and/or response to prior therapy and most importantly evaluate for minimal residual disease (MRD) once the disease is in clincal remission.

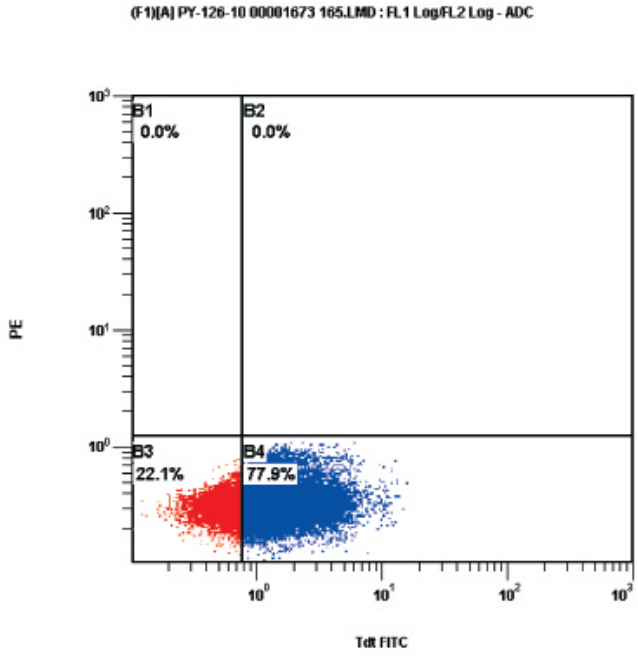

Fig 1. Precursor B-cell ALL (prior therapy). 78% lymphoblasts show positive expression for TdT (quadrant B4). The B-cell lineage had been determined which is not shown.

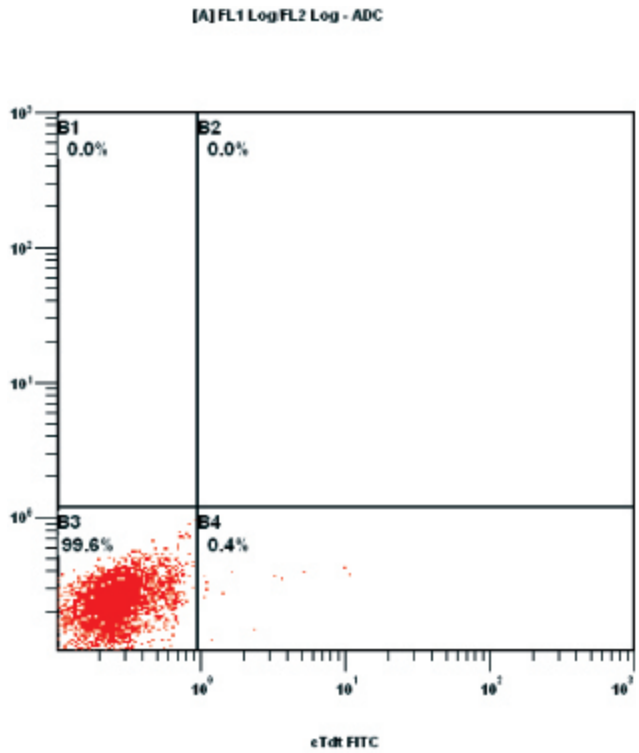

Fig 2. Minimal Residual Disease (MRD) following therapy (day 28) from same patient (Fig 1). <0.01% lymphoblast present showing positive expression for TdT (quadrant B4).

Acute lymphoblastic leukemia which includes precursor-B cell ALL and precursor-T cell ALL are routinely diagnosed at AKUH. Precursor B-cell ALL comprises of 85 per cent of childhood ALLs and 75 per cent of adult ALLs with the remainder being precursor T-ALL. Precursor B-cell ALL are lymphoblasts which are committed to the B-cell lineage. On flow cytometric studies, the B lineage is established by the positive expression of CD19, CD22 and CD79a. CD20 which is a highly specific marker for B-cell lineage is expressed in one-third to one-half of precursor B-cell ALLs. Surface immunglobulins are generally absent. CD34 and TdT (Fig 1) which are expressed in 80 per cent and 98 per cent of precursor B-cell ALLs respectively are markers of immature phenotype, but do not distinguish between B and T lineage. Finally, CD10, although not a reliable discriminator between B and T lineage, has

a positive expression which carries a prognostic significance. In Precursor T-cell ALL, cytoplasmic CD3 is the single most sensitive and specific marker of T-lineage differentiation. Surface CD3 is specific, but less sensitive. Surface CD7 is expressed in virtually in all cases of T-cell ALL, however it is not specific and its positive expression is seen in Acute myeloid leukemia. Immaturity in precursor T-cell ALL is established by expression of Tdt and CD34.

Response to therapy in patients with acute leukemia has traditionally been evaluated morphologically; less than 5 per cent blasts in the bone marrow are considered to have achieved morphologic complete remission. Minimal residual disease (MRD) refers to the presence of small numbers of residual neoplastic cells following therapy, at levels below the threshold of detection of conventional morphologic anaylsis. Flow cytometry is an analytic method (Fig 2) which has a sensitivity of at least one leukemic cell among 10,000 normal cells in ALL. The detection of MRD in a patient who is in morphologic remission following therapy, may be an adverse prognostic factor. The strategy is to analyze for antigenic combinations or isolated antigens that are not detectable in normal bone marrow e.g. cytoplasmic CD3 and TdT in precursor T-ALL. In cases of acute lymphoblastic leukemias, we have introduced a panel in the flow cytometric immunophenotyping (which includes Tdt) which will be useful in detecting MRD following therapy.

Meeting Reports: Clinical Laboratory Continuing Medical Education Seminar

Reported by Dr Raihan Sajid
Haematology

Professor Mohammad Khurshid addressing the audience at the CME Seminar

Participants at the CME seminar

The CME seminar titled Recent advances in Blood banking was organised by the section of Haematology, Department of Pathology and Microbiology on April 28, 2010. Prof. Mohammad Khurshid introduced the objective of the CME and highlighted the importance of advances in blood banking like Nucleic acid amplification testing (NAT) and cryopreservation of umbilical cord stem cells. A lucid presentation on NAT, emphasising the importance of this technique in the screening of blood donors was given by Dr Bushra Moiz. This technique which will shortly be available at Aga Khan University clinical laboratories promises the enhanced safety of blood products.

Moderator, Dr Salman Adil along with speakers Dr Mohammad Kashif, Dr Mohammad Usman, Dr Bushra Moiz and Dr Raihan Sajid.

Dr Raihan Sajid explained the concepts of cryopreservation of stem cells and the setting up of this facility at Aga Khan University in the near future. Dr Mohammad Usman Shaikh elaborated the usage of irradiated blood products in the clinical setting. He further stressed that all the patients undergoing bone marrow transplantation should receive irradiated red cells and platelets. Luckily, the AKU blood bank offers this facility to all patients who are under treatment outside AKU hospital. The proceedings were concluded by Dr Salman Adil who also moderated the question answer session at the end. The seminar was well attended and more than 430 participants from different hospitals and institutions of Karachi participated.

World Blood Donor Day at AKU Blood Bank

The World blood donor day was celebrated on June 14, 2010 under the auspices of Aga Khan University Blood bank to appreciate voluntary blood donors for their selfless sacrifice in saving the lives of needy patients. The function started with Prof. Mohammad Khurshid giving the welcome address. This was followed by an interesting presentation by Dr Bushra Moiz on New blood for the world which highlighted the significance of recruitment and retention of young voluntary donors. Later, Mr Anwar Ajani talked about the activities of Karachi Lion's club for setting up blood procurement camps. One of the patients, who had benefitted from the bank, thanked the voluntary blood donors for their contribution to the society. The event ended with an interesting quiz program. Dr Natasha Ali and Dr Raihan Sajid hosted the prize distribution ceremony.

Prof Naila Kayani , Chairperson of the Department of Pathology and Microbiology presented a shield of appreciation to Mr Anwar Ajani

Dr Natasha Ali giving the prize to one of the participants

www.aku.edu/akuh/hs/cs/pathology.shtml