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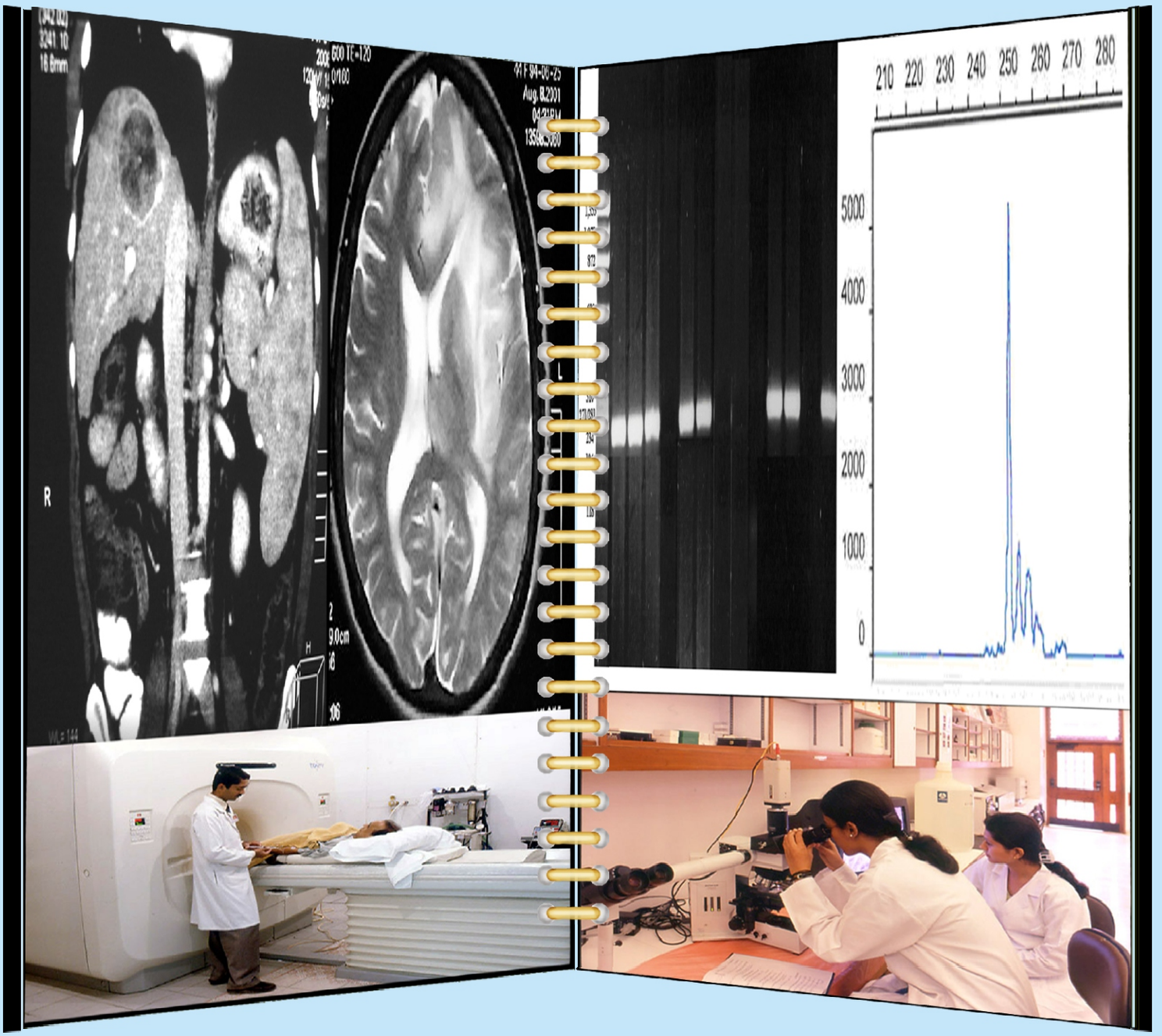
LABRAD : Vol 32, Issue 1 - January 2007

Aga Khan University Hospital, Karachi

Newsletter of Departments of Pathology and Microbiology, and Radiology

Labrad

January 2007 Number 32 Issue 1



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Labrad

A Quarterly Publication of Departments of Pathology and Microbiology, and Radiology

January 2007
Volume 32, Issue 1

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T-Cell Receptor (TcR) Gene Rearrangement Studies: A Useful Adjunct for the Diagnosis of T-Cell Lymphoproliferative Disorders

Dr Shahid Pervez, Histopathology
Dr Tariq Moatter, Molecular Pathology

Background

The molecular diagnosis of lymphoproliferative disorders is based on the assumption that the cells of a malignancy have a clonal origin reflected by the presence of clonally rearranged immunoglobulin (Ig) and T-cell receptor (TcR) genes[1].

T-lymphocytes rearrange their TcR genes to encode a unique antigen receptor expressed on the surface of T cells (Fig 1). TcR is composed of α, β, γ and δ chain genes, which rearrange in the germline genome to give a functional T-cell receptor. Rearrangements involve random assembly of different variable (v), sometimes diversity (D) and joining (J) gene segments (Fig 2).

In diagnostic pathology it is relatively easy to determine clonality of B-lymphoproliferative disorders by immunohistochemical techniques for instance by using monoclonal antibodies to kappa and lambda light chains to look for light chain restriction. However in contrast by routine immunohistochemistry it is difficult to establish clonality (monoclonality) or otherwise of a T lymphoproliferative disorder. The whole idea of T-cell receptor gene rearrangement is based on the assumption that in T-cell Non-Hodgkin's lymphoma (T-NHL) all tumor cells are the progeny of a single transformed T-cell that is, they are monoclonal. Therefore it will be right to believe that all these neoplastic T- cells arrange gene in an identical fashion and therefore come to the detection levels as a single band by PCR (Fig. 3 and 4). In contrast a reactive or in other words polyclonal population will have a heterogenous population of T-cells arranging TcR genes in variable manner and therefore appearing as smear on PCR based gel analysis[2] (Fig. 3 and 4).

T-Cell Receptor Gene Arrangements

TcR genes undergo VDJ or VJ rearrangements in the sequential orders of TcRδ, TcRγ, TcRβ and TcRα. All T-cells undergo rearrangement of at least some of the TcR genes. Approximately 95 per cent of circulating T cells undergo the entire spectrum of rearrangements and are α/β positive but a small population of γ/δ T-cells does not undergo β/α rearrangements.

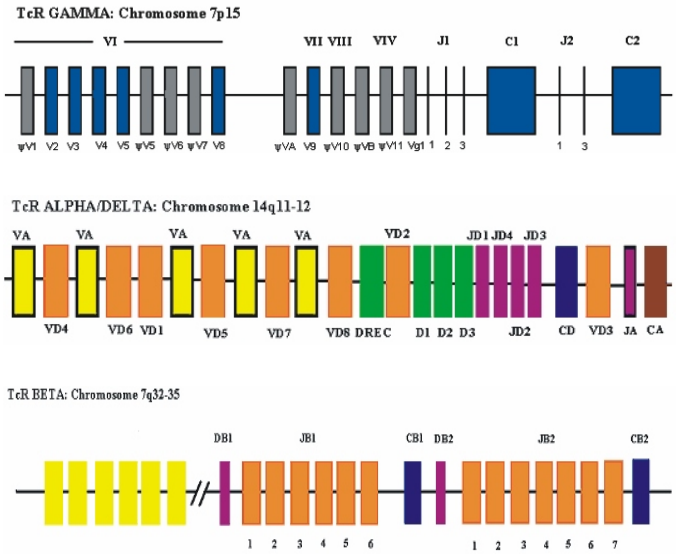


Figure 1:
Germline organisation of T-cell Receptor (TcR) genes

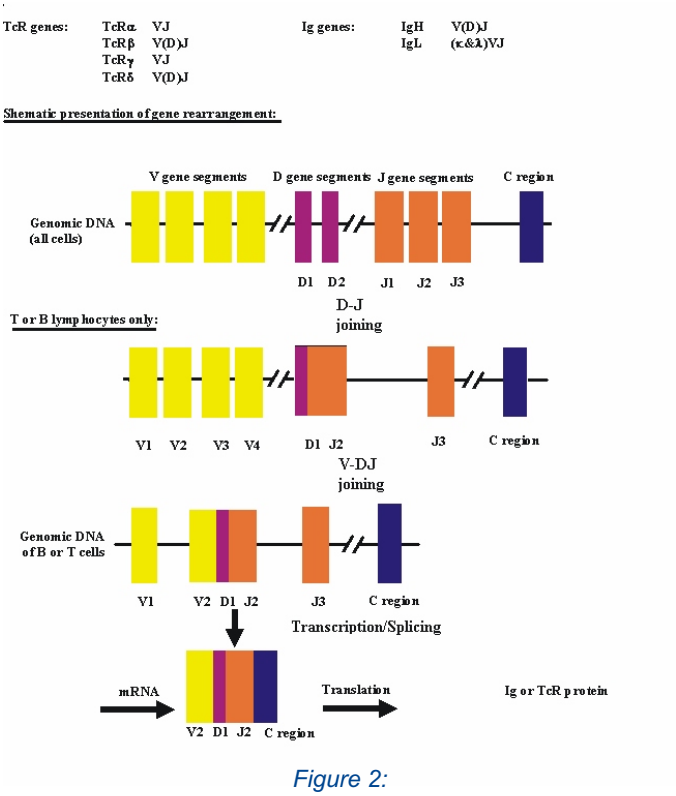


Figure 2:
Schematic illustration of Ig and TcR gene rearrangement

Molecular analysis of the TcRβ and TcRγ genes is commonly used to distinguish monoclonal T-cell neoplasms from polyclonal reactive process. The reason that TcRδ gene is not commonly targeted is because it is deleted during TcRα gene rearrangement. TcRα gene is not targeted because it has so many possible rearrangements that current probe technology cannot handle the diversity.

Most TcR gene analysis nowadays are PCR-based. Because of the complexity of TcRβ locus PCR for these rearrangements require a large number of primers and thus may not be the preferred locus for PCR testing in clinical diagnostic scenario. Nonetheless commercially available standardised Biomed primers for TcRβ appear to work well in practice[3].

The rearranged TcRγ is ideal for PCR amplification for two reasons. First because TcRγ locus is consistently rearranged prior to TcRβ locus. Second, nearly all T-cells harbour rearrangements involving one of eleven variable segments. Eight of these segments are homologous to one another and can thus be targeted by a single consensus primer. In the event that one of the remaining variable segments was used, additional primers are employed in a multiplex fashion, along with several joining region primers to detect virtually all possible TcRγ gene rearrangements. Since this is a PCR based test directed against genomic DNA, TcR PCR can be performed on paraffin embedded tissue.

False Positive and Negative Results

PCR based TcRγ analysis using standardised biomed primers result in very few false negative results provided the sample is representative. Excluding a problem with contamination false positive results are most common when only few lymphoid cells are present in the sample as selective amplification of these few cells may result in an apparent positive result (pseudoclonality). Testing small samples in duplicate will identify these potential false positive cases and will result in an oligoclonal pattern of amplification ie, different sized gene rearrangements on different runs. This should not be interpreted as evidence of a monoclonal population[4].

Lineage Infidelity

A monoclonal TcR gene rearrangement does not always translate into T-cell lineage as many precursor B cell lymphoblastic leukemias and lymphomas are positive for such rearrangements and vice versa. A mature T-cell lymphoma known to show this phenomenon is AILT type T NHL[5].

Clinical Utility

T-cell receptor gene rearrangement studies are used to distinguish benign from malignant T-lymphoid proliferations. Benign reactive T lymphoid proliferations do not harbour monoclonal rearrangements in control to malignant lymphoid tumors. However in spite of its incredible power, results of clonality assays should be interpreted with caution. It is important to recognize that clonality is not always synonymous with malignancy. Therefore, all results must be interpreted with morphological and immunohistochemical clinical data[6].

The test can also be utilised for differentiating T and B cell lineage of a lymphoid proliferation. A complicating factor is lineage infidelity in a minority of cases.

Overall, it has been shown that when diagnostic uncertainty remains after morphologic and immunophenotypic examination, gene rearrangement studies are helpful in 72 per cent of the cases. However, if molecular results appear to be in violation with morphologic diagnosis, time-tested morphologic impression or diagnosis should prevail over molecular results.

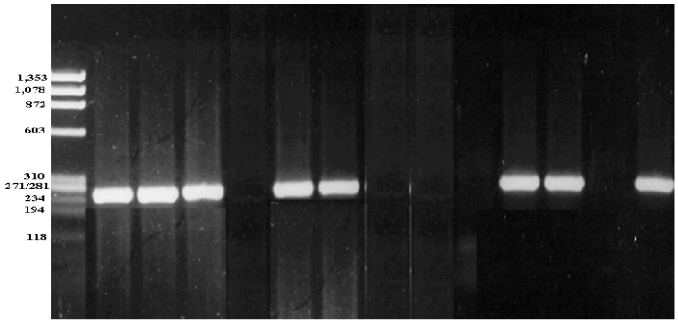
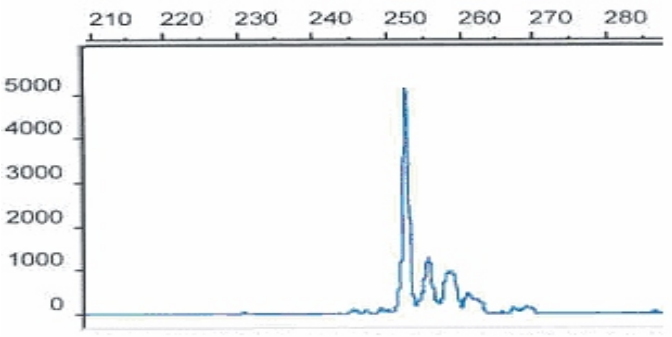
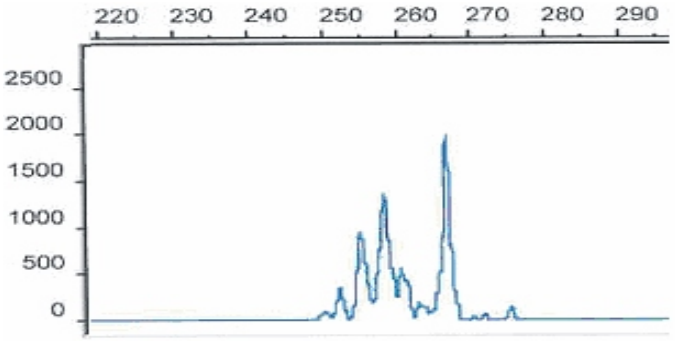


Figure 3:
Ethidium bromide-stained 2% agarose gel showing 240 bp -globin product of different T-NHL samples. M represents the molecular weight marker along with negative (-C) and positive (+C) controls

Test Samples

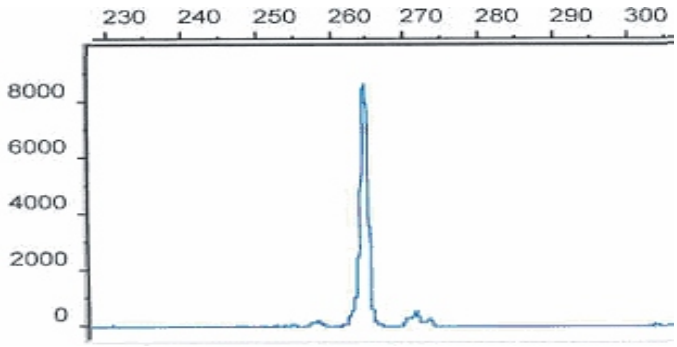


TcR β, sample showing clonal (single) peak

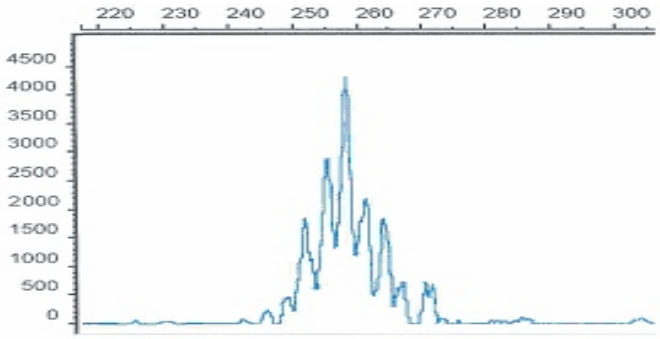


TcR β, sample polyclonal (several) peaks

Positive Control

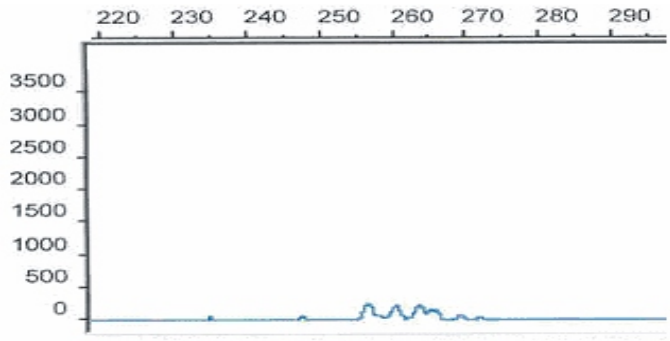


TcR β tube A, positive clonal control showing clonal (single) peak



TcR β tube A, positive polyclonal clonal control showing polyclonal (several) peaks

Negative Control



TcR β tube A, negative control

Figure 4:
Heteroduplex analysis of 6% ethidium bromide-stained polyacrylamide gel illustrates rearranged TcR PCR products for T-malignancy cases, followed with genescan of 2 samples showing clonal and polyclonal peaks with controls

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Vitamin D Measurement

Dr Aysha Habib Khan, Chemical Pathology

The measurement of vitamin D is becoming increasingly important in the management of patients with various disorders of calcium metabolism associated with rickets, osteomalacia, neonatal hypocalcaemia, pregnancy, nutritional and renal osteodystrophy, hypo and hyperparathyroidism as well as osteoporosis.

As calciferol from the skin enters the circulation, it is metabolised to several forms, the major form being 25-hydroxycalciferol (25-OHD) in the liver. Only a small amount is metabolised in the kidney to other dihydroxy vitamin D metabolites in man. Since 25-OH D is the predominant circulating form in the normal population, it is the most reliable index of vitamin D status.

Factors such as ultraviolet exposure, season, race, and dietary intake are all known to effect levels of 25-OHD in humans. A decrease in vitamin D levels with advancing age has been demonstrated in the literature. Anticonvulsants have been shown to cause a decrease in 25-OHD levels. A high prevalence (30-90 per cent) of sub clinical 25-OHD deficiency among normal apparently health populations has been observed in many countries, particularly in winter months. Studies show high prevalence of vitamin D deficiency among Pakistani immigrants in western countries. Vitamin D deficiency was also reported among pregnant women in Karachi, Pakistan. Our clinical laboratory data at AKUH suggests a high prevalence of vitamin D deficiency in patient population (unpublished data).

In Aga Khan Clinical Laboratory, 25-OHD test is performed by a radioimmunoassay using ^{125}I. The calibrators are serum based. The assay is FDA approved because of overall simplicity and its strong correlation with values obtained from HPLC analysis. The assay recognizes both plant and animal vitamin D with equal affinity as well as a number of other circulating vitamin D metabolites like 24, 25 dihydroxyvitamin D, 25, 26 dihydroxy vitamin D, 25-OHD3-26-23-lactone. However, as these metabolites only account for about 6 per cent of the overall circulating concentration of vitamin D and its metabolites, their concentration can be ignored under this assay format.

QUIZ

Dr Naveen Naz Syed, Haematology

82-year old male presented with history of progressive fatigue and shortness of breath on exertion since three months. Physical exam showed pallor with mild scleral icterus, and palpable liver 2 cm below the left costal margin.

Complete blood count revealed Hemoglobin 2.7g/dl, hematocrit 7.7 per cent, MCV 129 fl, MCH 34.8 pg, white blood cell count 2600/cmm and platelets 20,000/cmm.

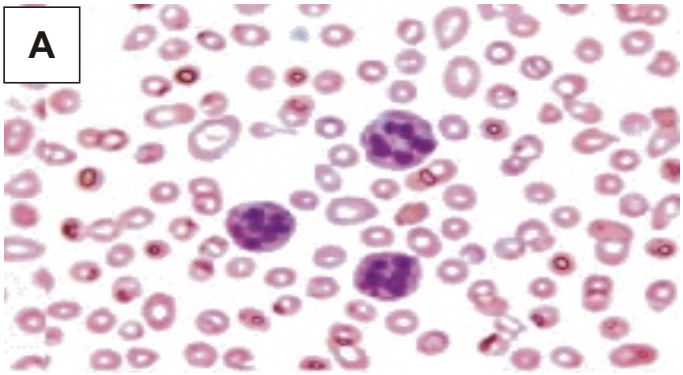

Figure A: Identify red blood cell abnormality.

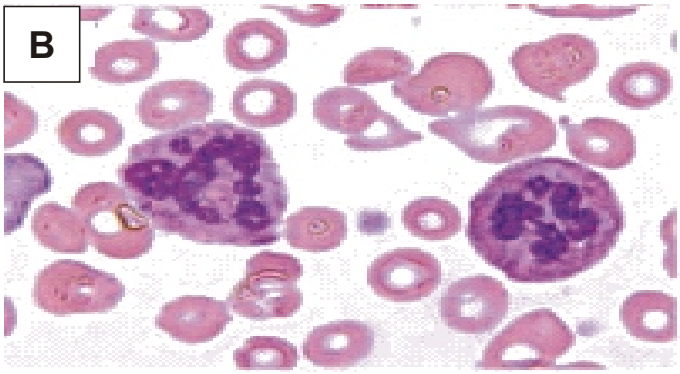

Figure B: Identify white blood cell abnormality.

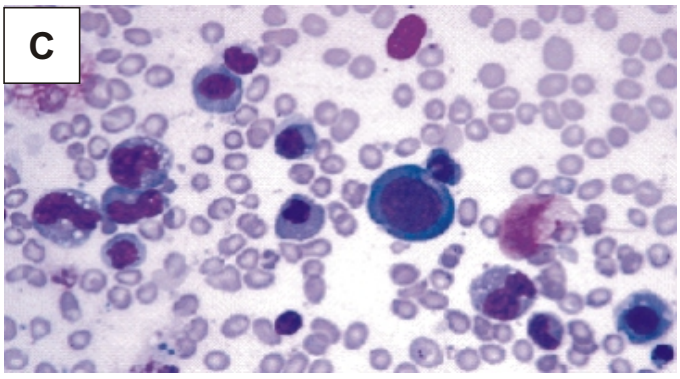

Figure C: what is your most likely diagnosis after reviewing bone marrow aspirate?

Ristocetin Cofactor Activity

Dr Safoorah Khalid, Haematology

The functional property of von Willebrand factor (vWf) that promotes platelet aggregation by the drug ristocetin is called ristocetin cofactor activity. Ristocetin induces an activation of vWf such that it then binds platelets via the platelet glycoprotein (GP) 1b receptor[1]. Ristocetin is an antibiotic that was taken off the market because it caused thrombocytopenia in individuals with normal vWf.[2]

Ristocetin cofactor activity is adversely affected by loss of larger molecular weight vWf multimers. The discrepancy between ristocetin cofactor activity and vWf antigen that is observed in qualitative defects characterised by loss of both the intermediate and high molecular weight multimers is thought to be on this basis. Ristocetin cofactor activity is also generally used to follow a von Willebrand's disease (vWD) patient's response to therapeutic interventions.[1]

In practice, a standard curve for the ristocetin cofactor test is constructed by adding serial dilutions of a standard plasma preparation (the source of vWf) to fresh or formalinized washed platelets and then adding ristocetin to a final concentration of approximately 1.2mg/ml (figure 1). The time to platelet aggregation is measured and graphed against the concentration of vWf. The standard plasma used for the initial curve should be related to the WHO standard. The results are reported as per cent of factor activity as determined by the intersection of the test sample slope on the best-fit line of the standard dilutions (figure 2). 100 per cent activity equals 100 IU/dl or 1 IU/ml. When using Chrono-log Cofactor Assay kit, results of less than 40 per cent are considered abnormal and indicative of vWD. However, the individual laboratory utilising its particular instrument or reagent combination should establish a normal range.

Abnormal tests should be repeated for confirmation. However, complete diagnosis and determination of the variant forms of vWD requires other factors such as family history, bleeding time, factor VIII Ag and VIII:C activity.

The ristocetin cofactor test has been difficult to standardise from laboratory to laboratory, but it is the most useful in vitro assay for the diagnosis of vWD.

The test will be shortly available at Clinical Laboratory, Aga Khan University Hospital.

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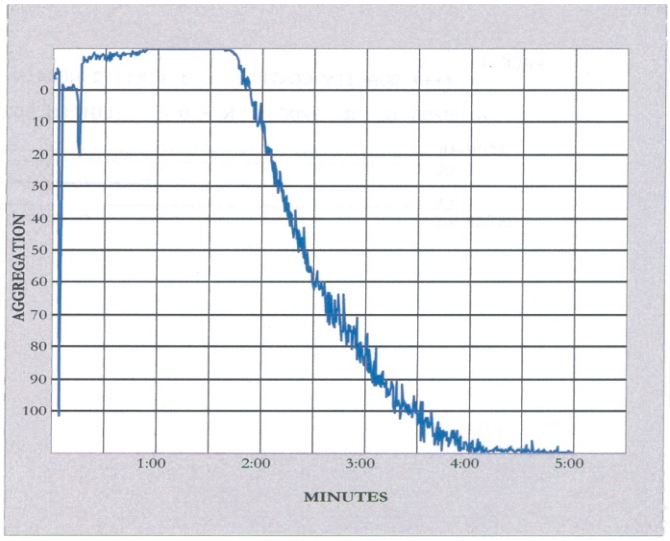

Figure 1:
Aggregation of Platelets in Response to Ristocetin

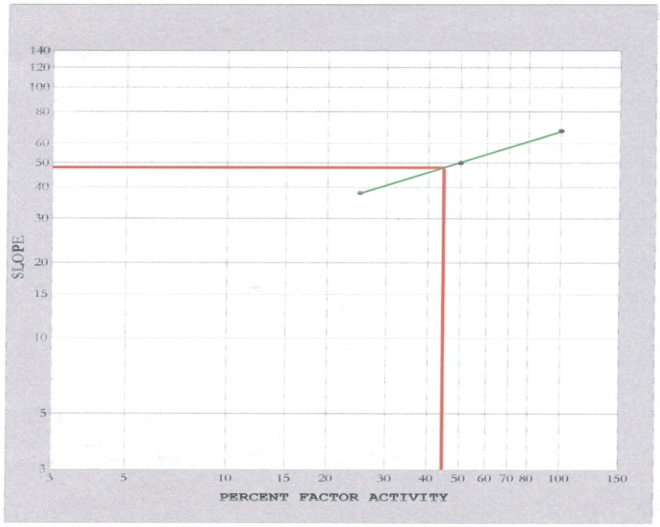

Figure 2:
Measurement of ristocetin cofactor activity of test plasma (red Line) against standard plasma (green line)

Portal Biliopathy- An Uncommon Cause of Biliary Obstruction

Dr Zishan Haider, Dr Ishtiaq A Chishti, Radiology

Benign bile-duct strictures are usually caused by bile-duct injury during cholecystectomy. Less common causes include sclerosing cholangitis, chronic pancreatitis, congenital abnormalities, and nonsurgical trauma. Portal hypertension related bile duct changes represent another group of uncommon causes of benign stricture, which can result in biliary obstruction, choledocholithiasis, and cholangitis.

Portal biliopathy is a rare condition that is usually not diagnosed. It is associated with presence of varix around the bile duct with concomitant ischemic damage and structural alterations of bile duct wall. This produces obstructive phenomena (Fig 1). There are scarce reports in the literature about this entity.

In patients with portal hypertension, particularly with extrahepatic portal vein obstruction, portal biliopathy producing biliary ductal and gallbladder wall abnormalities are common. Portal cavernoma formation, choledochal varices and ischemic injury of the bile duct have been implicated as causes of these morphological alterations. While a majority of the patients are asymptomatic, some present with a raised alkaline phosphatase level, abdominal pain, fever and cholangitis. Choledocholithiasis may develop as a complication and manifest as obstructive jaundice with or without cholangitis. The knowledge of portal biliopathy is very important because it is a less commonly seen entity in radiology, so accurate recognition can make prompt diagnosis.

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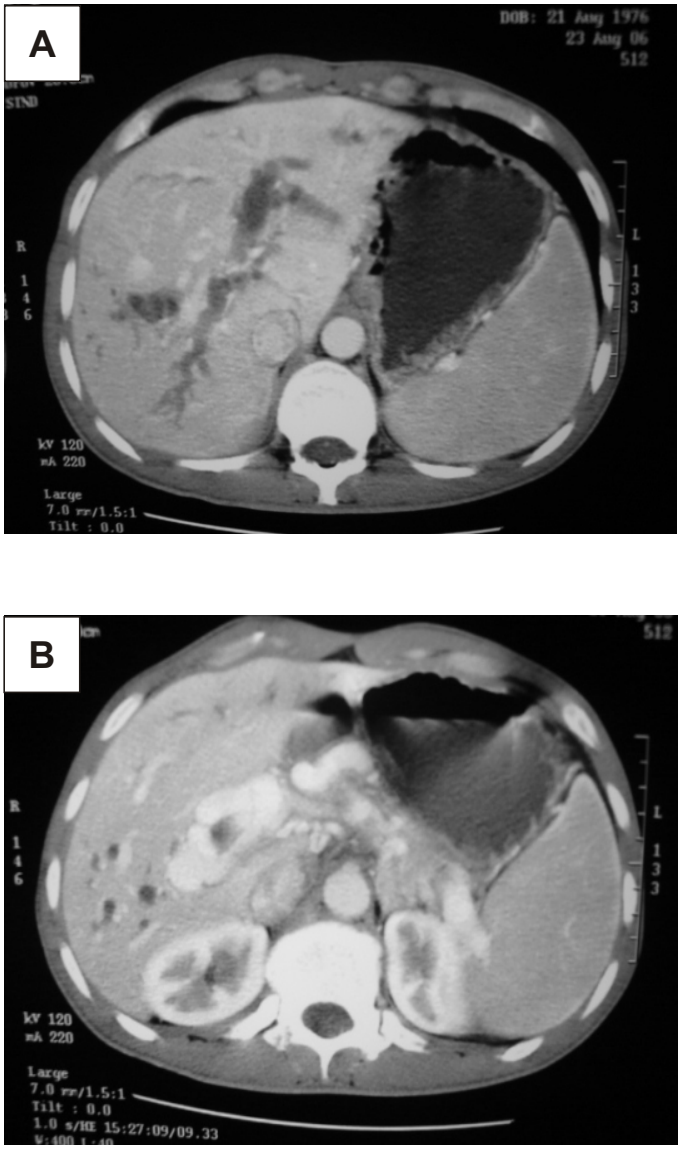

Fig 1:

(A) Axial post contrast CT scan of abdomen showing moderate intrahepatic biliary dilatation without any focal lesion in the liver

(B) Same patient in lower section of abdominal CT showing distal common bile duct is encased by varices leading to its narrowing and obstruction suggestive of portal biliopathy

Laboratory Diagnosis of Hepatitis C: Transition from Serological to Molecular Methods

Dr Aqsa Nasir, Dr Tariq Moatter,
Molecular Pathology

Chronic Hepatitis C (HCV) Virus infection is a leading cause of cirrhosis, hepatocellular carcinoma, gastrointestinal tract haemorrhage and hepatic insufficiency. Evolution of latest antiviral therapies has reduced the risk of disease progression but unfortunately, deaths due to HCV are increasing due to inadequate and therefore late detection and treatment. However, major breakthroughs have been achieved in its diagnosis and treatment. Timely diagnosis and an effective treatment plan is very important to reduce the high mortality rate among patients with HCV infection. Several diagnostic tests are available in this regard. They are grouped into:

- Serological Assay for Antibodies;
- Molecular Tests for Viral Particles.

Serological Assays for Antibodies

Diagnosis of the infection is based on detection for serum antibodies against HCV. The virus has an incubation period of 2-26 weeks (average 6-7 weeks). Seroconversion occurs at an average of 2-3 months.

Enzymes immunoassays are very sensitive and very specific and detect antibodies within 4-10 week of infection. Immunosuppressed patients can have false negative results, including those with HIV infection, renal failure, and HCV associated essential mixed cryoglobulinaemia. Antibodies against HCV are still detectable during and after treatment, irrespective of therapeutic response.

Once seroconversion occurs, these tests usually remain positive. However, the concentration of HCV antibodies decreases gradually over time in few patients in whom infection spontaneously resolves.

Molecular Tests for Viral Particles

Qualitative PCR Tests

Qualitative HCV RNA tests are based on PCR techniques and can detect viral copies as low as 100 copies of HCV RNA per ml of serum (50 IU per ml). This is the most specific test of infection. It is especially useful when transaminase concentrations are normal, when other causes of liver disease are present (alcohol consumption), in immunosuppressed patients (graft recipients, HIV co-infected patients), and in acute hepatitis C before antibodies have developed.

While a single qualitative positive test for HCV RNA confirms active viral replication, a single negative assay does not exclude viremia and may reflect only a viral load below the detection limit of the assay. Therefore, a follow-up qualitative HCV RNA is required to exclude active HCV replication. Once HCV infection is confirmed repeating the test does not help in managing untreated patients, except for determining whether an acute infection has resolved.

HCV Quantification

It gives the exact quantity of HCV RNA in the given sample. The HCV RNA level can be quantified by means of:

- Target Amplification Technique (PCR)
- Signal Amplification Technique (Branched DNA Assay)

Quantification of HCV RNA has no prognostic value in predicting the severity of HCV-induced liver injury. Viral load is an independent predictor of treatment response when assessed before therapy, and measurement of viral load is useful in monitoring the efficacy of the treatment. In most studies, high base line viral load has been defined as greater than $2x10^6$ HCV RNA copies / ml, but there is variation in the results of the HCV RNA quantification used by the different assays. The role of HCV RNA quantification affects the treatment plan in the following two ways:

- Baseline HCV RNA viral load
- The genotype of the HCV

Patients with high baseline viral loads are less likely to achieve a sustained viral response (SVR) than those with low viral loads. For example, 7-13 per cent of highly viremic patients in two large trials achieved SVR after receiving standard interferon therapy alone for 48 weeks, compared with 29-31 per cent with low baseline viral loads. Patients with high viral loads responded well (36-40 per cent) when treated with combination treatment (interferon plus ribavirin) for 48 weeks as compared to 24 weeks regimen (27-28 per cent). However, the results were same in patients with

low HCV RNA levels at baseline who were treated for 24 weeks as compared to those who got the therapy for 48 weeks. Regarding treatment with peg interferon alpha 2a, patients with pretreatment low viral load (a baseline HCV RNA level of < $2x10^6$ copies per ml) responded well and were most likely to be associated with a SVR than those with high viral load.

The role of the measurement of HCV RNA levels for therapy differs in various genotypes. Baseline viral load is not necessary in patients with genotype 2 or 3. However, in monitoring of patients in genotype 1 measurement of HCV RNA before treatment and again after 12 weeks of treatment is very useful and, according to the present knowledge, also for those with genotype 4, 5 and 6. A 2-log drop at least or undetectable HCV RNA at week12 is defined as early virological response (EVR) which is now believed to have a poor positive predictive value but an excellent negative predictive value to SVR. These results will help in deciding whether to stop or continue the treatment as early as 12 weeks after the start of therapy. However, the clinical reliability of serial HCV viral load testing in the same patient depends on the use of the similar quantitative assay each time. Therefore, treatment could be stopped in patients with low SVR after 12 weeks of therapy if there is no extensive fibrosis. By contrast with HIV infection the viral load does not correlate with the severity of hepatitis on progression of fibrosis

HCV Serotyping

The advent of serotyping systems has made study of the clinical significance of the different types of HCV possible. There are six serotypes of HCV, 1, 2, 3, 4, 5 and 6.

Serological methods are used for the detection of antibodies directed to genotype specific HCV epitopes. The assay identifies only the types but not the sub types of HCV. The concordance with molecular assays is around 95 per cent and is higher for genotype1 than for the others. Mixed serologic reactivity is sometimes observed. This test cannot distinguish between true mixed infection and cross reactivity or recovery from one genotype infection and persistence of viremia with another.

HCV Genotyping

On the basis of variations in the nucleotide sequence of HCV, six genotypes (numbered 1 to 6) and more than 100 subtypes (identified by lower case letters, for example, 1a and 1b) have been identified. The HCV genotype and subtype are intrinsic characteristics and do not change during the course of the infection. The clinical course, including long-term complications of chronic HCV, is genotype independent. There is a consensus on the fact that the HCV genotypes are predictors to the antiviral therapy response. HCV has the following genotypes/subtypes identified by reverse transcription methods:

1a-1c
2a-2d
3a-3f
4a-4k
5a
6a,

new subtypes are continuously being discovered.

Clinical Significance of HCV Genotyping

Chronic HCV has historically been a difficult to treat disease. The primary goal of treatment for HCV is eradication of HCV. Clinically this is defined as SVR and is evidenced by undetectable HCV RNA in serum on sensitive molecular tests performed 24 weeks after completion of treatment. SVR rates have improved over the past decade as new treatment strategies have revolutionised the treatment plan but despite these improvements in overall SVR rates, less satisfactory results have been observed in certain patient populations. One of the most important related factors is HCV genotypes 1 or 4 and a high HCV RNA level before treatment.

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Answer to Quiz
Laboratory Diagnosis of Pernicious Anaemia

Dr Naveen Naz Syed, Haematology

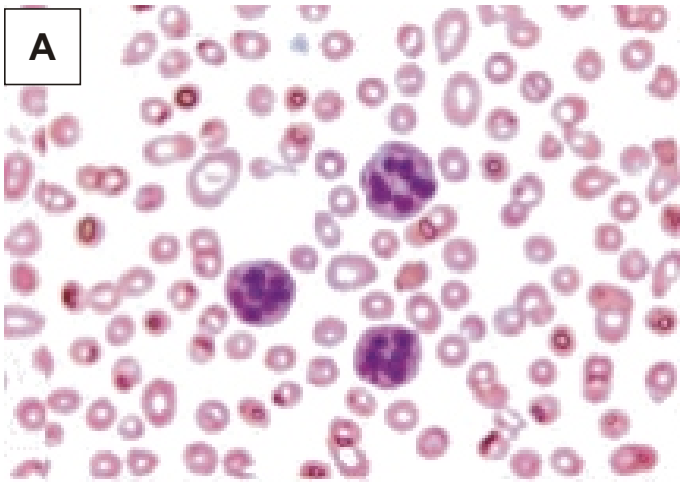
Patient History

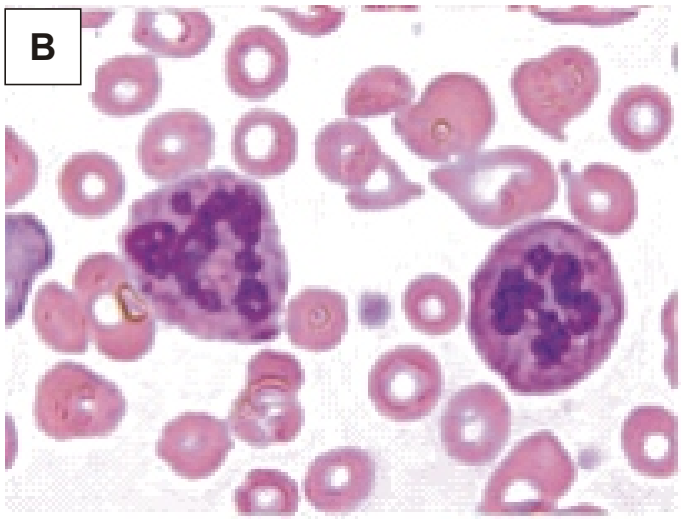
The patient is a 82 year old male who presented to the emergency department with complains of progressive fatigue and shortness of breath on exertion over a two-to-three month period. He had no history of drug intake, infections, bleeding or bruising in the past. However, he had recurrent episodes of acid peptic ulcer disease. Physical exam showed pallor with mild scleral icterus, lungs clear to auscultation, regular heart rate with no audible murmurs. Abdomen examination revealed, palpable liver 2 cm below the left costal margin, and spleen was not enlarged.

A number of laboratory investigations were requested including complete blood count, bone marrow examination, cytogenetics and various biochemical tests. The results are as follows:

Complete Blood Count

	Patient Value	Normal Range (Male)
Hemoglobin	2.7 g/dL	12.9-16.9
Hematocrit	7.7%	38.0-48.8
MCV	129.7 fL	82.6-97.4
MCH	34.8 pg	27.8-334
WBC	2.6 x 10^9/L	3.8-10.6
POLYMORPHS	68%	
LYMPHOCYTES	19%	
PLATELETS	20 x 10^9/L	156-369





Figures A and B: peripheral smear 40 and 100 X objective showing hyper segmented neutrophils and macrocytes

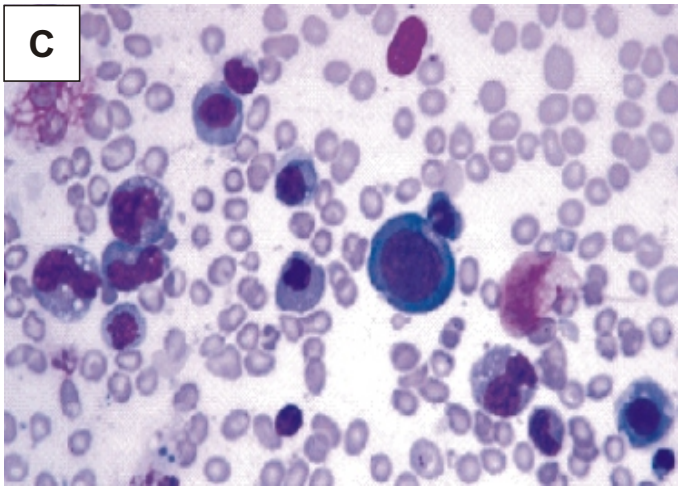


Figure C: Bone marrow aspirate 100 X objective, showing megaloblastic erythropoiesis

Other Laboratory Values on Admission Included the Following:

	Patient Value	Normal Range (Male)
Reticulocytes	9.2%	0.8-2.0
LDH	6338 IU/L	<170
T. Bilirubin	8.8 mg/dL	0.3-1.5
D. Bilirubin	<0.1	
B12	<150 pg/mL	211-911
RBC Folate	805 ng/mL	293-809
Intrinsic factor Antibody	Positive	Negative

Cytogenetics
Normal, 46 X, Y.

Based on the above labs, diagnosis of megaloblastic anemia secondary to B12 deficiency was made. As the patient was sero-positive for intrinsic factor antibodies (IFA), pernicious anemia was a likely possibility.

Pernicious Anaemia
Pernicious anaemia is a rare disorder characterised by the inability of the body to properly utilise vitamin B12 and, results from the lack of the gastric protein known as intrinsic factor, without which vitamin B12 cannot be absorbed. The symptoms of pernicious anaemia may include weakness, fatigue, palpitation, and/or progressive neuropathy. Recurring episodes of anaemia (megaloblastic) and an abnormal yellow coloration of the skin (jaundice) are also common.

Pernicious anaemia is thought to be an autoimmune disorder, and certain people may have a genetic predisposition to this disorder. The disease is associated with human leucocyte antigen (HLA) types A2, A3, and B7 and type A blood group.

Anti-parietal cell antibodies occur in 90 per cent of patients with pernicious anaemia but in only 5 per cent of healthy adults. Similarly, binding and blocking antibodies to IF are found in most patients with pernicious anemia. A greater association than anticipated exists between pernicious anemia and other autoimmune diseases, which include thyroid disorders, type I diabetes mellitus, ulcerative colitis, Addisons disease, infertility, and acquired agammaglobulinemia. An association between pernicious anaemia and *Helicobacter pylori* infections has been postulated but not clearly proven.

Combined system disease: a history of either paresthesia in the fingers and toes or difficulty with gait and balance should prompt a careful neurological examination. Loss of position sense in the second toe and loss of vibratory sense for a 256-Hz but not a 128-Hz tuning fork are the earliest signs of postero-lateral column disease. If untreated, this can progress to spastic ataxia from demyelination of the dorsal and lateral columns of the spinal cord.

Lab Studies
1. **Peripheral Blood:** The peripheral blood usually shows a macrocytic anemia with a mild leucopenia and thrombocytopenia. The mean cell volume (MCV) and mean cell hemoglobin (MCH) are increased, with a mean corpuscular hemoglobin concentration (MCHC) within the reference range. The leucopenia and thrombocytopenia usually parallel the severity of the anaemia. The peripheral smear shows oval macrocytes, hyper segmented granulocytes, and aniso-poikilocytosis. In severe anaemia, red blood cell inclusions may include Howell-Jolly bodies, cabot rings, and punctate basophilia. The macrocytosis can be obscured by the coexistence of iron deficiency, thalassemia minor or inflammatory disease.
2. **Serum B12 Levels:** low.
3. **A Bone Marrow Aspirate and Biopsy:** Hypercellular and show trilineage differentiation.

   Erythroid precursors are large and often oval; nucleus is large and contains course mottled chromatin clumps, providing a checkerboard appearance. Nucleoli are visible in the more immature erythroid precursors. An imbalance in the rate of maturation of the nucleus relative to the cytoplasm exists, such that disassociation between the maturity of the nucleus and the hemoglobinization of the orthochromic megaloblastic normoblasts occurs.

   Giant metamyelocytes and bands are present, and the mature neutrophils and eosinophils are hyper segmented.

   Imbalanced growth of megakaryocytes is evidenced by hyperdiploidy of the nucleus and the presence of giant platelets in the smear.

   Lymphocytes and plasma cells are spared from the cellular gigantism and cytoplasmic asynchrony observed in other cell lineages.
4. **Intrinsic Factor Antibody:** Enzyme linked immunosorbent assay (ELISA) is utilised for qualitative and semi-quantitative detection of antibodies to intrinsic factor in patient serum.

Treatment:
Treatment in our patient included a blood transfusion due to the severity of his anaemia, then therapy with intravenous cyanocobalamin supplements, resulting in stabilization of his laboratory values. An important aspect of treatment for pernicious anaemia is that it will need to be continued lifelong.

Dengue Fever

Drs Afia Zafar, Erum Khan, Microbiology

Dengue is a mosquito-borne disease that is caused by Dengue virus which is usually endemic in tropics. Dengue virus family consists of four related species (DEN1-DEN4). Infection by one type confers immunity to that particular type only, but does not protect against other types. In fact; second infection by another serotype is more serious.

Clinical Feature

Dengue virus infection may be asymptomatic or may lead to dengue fever (DF) or dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).

Dengue Fever

The clinical features of DF frequently depend on the age of the patient. Infants and young children may have a nonspecific febrile disease, often with a maculopapular rash. Older children and adults may have either a mild flu like illness or the classic incapacitating disease with high fever of abrupt onset, sometime with two peaks, severe headache, pain behind the eyes, muscle and bone or joint pains, nausea, vomiting and rash. Leucopenia (low white blood cells) and thrombocytopenia (low platelets) are frequently associated with it. DF is a self limiting disease and recovery may be associated with prolonged fatigue and depression, especially in adults.

Dengue Haemorrhagic Fever

Typical cases of DHF are characterised by four major clinical manifestations: high fever, haemorrhagic manifestations, enlarged liver and circulatory failure.

Dengue Shock Syndrome

The condition of patients who progress to shock suddenly deteriorates after a fever of 2-7 days duration. This deterioration occurs at the time of, or shortly after a fall in temperature between the third and the seventh day of the disease. There are typical signs of circulatory failure: the skin becomes cool, blotchy and congested, the pulse becomes rapid and patient enters into a critical stage of shock.

Transmission

These viral illnesses are mosquito borne and by and large its transmission is dependent upon mosquito bite. The occurrence of DF outbreaks is linked to a number of factors such as density of vector (*Aedes aegypti*), source (an infected febrile person) and presence of susceptible hosts in the community. The *Aedes aegypti* mosquito is commonly found in urban dwellings, indoors and outdoors. Therefore it is easy to speculate that fast urbanisation and dense population facilitates the progress of outbreak.

Diagnosis

Since clinical features are indistinguishable from other common diseases, laboratory confirmation of dengue virus is important from management and epidemiological point of view. Detection of antibodies against dengue virus in the blood of the infected patient is the simplest method of diagnosis. This test is available at the Aga Khan University laboratory. However, in some patients (30 per cent) antibodies may not be detectable during the acute phase of the disease in such cases special test like PCR is required to confirm the diagnosis.

Treatment

There is no specific anti-viral drug available for the treatment of patients with dengue fever. Early diagnosis and symptomatic care is the mainstay in the treatment. It is important that patients are kept well-hydrated. Mild cases managed at home are encouraged to take excess fluid and bed rest. Severe cases admitted to hospitals are managed by intravenous drips and blood /platelet transfusion if required.

Prevention

Personal Protection from Mosquito Bite

Use of mosquito repellent and protective clothing is an important measure to prevent the viral transmission from mosquito bite. Windows and doors should be lined with the thin nets to prevent entry of mosquitos in the house. Frequent insecticide use inside the house is encouraged

Measures to Prevent Mosquito Breeding

Since there is currently no vaccine available; prevention is mainly targeted in the eradication of

mosquito breeding points in the community. Prevention is only possible if collective effort is made by all members of the community. Proper disposal of household waste is first and foremost. Water in the containers should be stored properly with covering lids. Water in flower vases and fridge drains should be changed frequently, at least on weekly basis.

References

Dengue hemorrhagic fever: diagnosis, treatment, prevention and control. 2nd edition. WHO. Geneva.

Bone Densitometry (DEXA Scanning) in the Diagnosis of Osteoporosis

Dr Riffat Hussain, Radiology

Osteoporosis has been called the “silent disease” since damage occurs over many years without any evident symptoms until a fracture occurs. A broken bone, for some women, may be the first warning sign that osteoporosis has occurred.

Among the available modalities for the diagnosis of a low bone mass or osteoporosis bone density scanning, also called dual-energy x-ray absorptiometry (DXA or DEXA) or bone densitometry, is today's established gold standard for measuring bone mineral density (BMD). It is an enhanced form of x-ray technology that is used to measure bone loss. It is generally performed on the lower spine and hips. DEXA bone density testing is the most accurate method available for the diagnosis of osteoporosis and is also considered an accurate estimator of fracture risk Bone mineral density tests help in diagnosing osteoporosis and are more sensitive than ordinary x-rays by virtue of detecting bone loss at an earlier stage.

The advantages that this technique has to offer are that:

- It uses minimal amounts of radiation;
- It has a high degree of precision (reproducibility). In addition, each time a patient returns for a follow-up scan, the prior analysis can be used to determine if a change has occurred;
- It measures both the lumbar spine and femoral neck (the precision is around 1 per cent for the spine and 2 per cent to 3 per cent for the femoral neck region.)
- It allows for standardisation of data based on age, weight, height, and ethnic background.

Aga Khan University Hospital acquired its DEXA machine (Hologic Discovery) two years back. Since its inception, it has proved itself in helping establish osteoporosis in individuals as well as identifying patients who have a low bone mass and are in need of specific treatments to help in the control of further deterioration of their bony mineral content.

The examination is done on an outpatient basis. The technique involved is simple with no special preparations except the withholding of oral intake of Calcium supplements for a day. However if a recent barium examination has been done or a patient has been injected with a contrast for a CT scan or radioisotope scan, a wait of 10 to 14 days is advised before undergoing a DEXA test.

The test involves a central DEXA examination, which measures bone density in the hip and spine as the patient lies on a padded table. An x-ray generator is located below the patient and an imaging device, or detector, is positioned above.

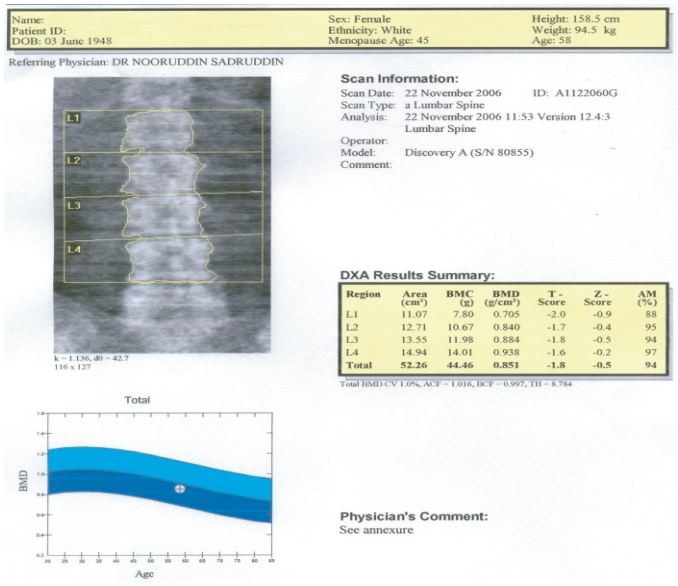
To assess the spine, the patient's legs are supported on a padded box to flatten the pelvis and lower (lumbar) spine. To assess the hip, the patient's foot is placed in a brace that rotates the hip inward. In both cases, the detector is slowly passed over the area, generating images on a computer monitor.

The test is completed in about 10 minutes and the results of the test are reported the next day.

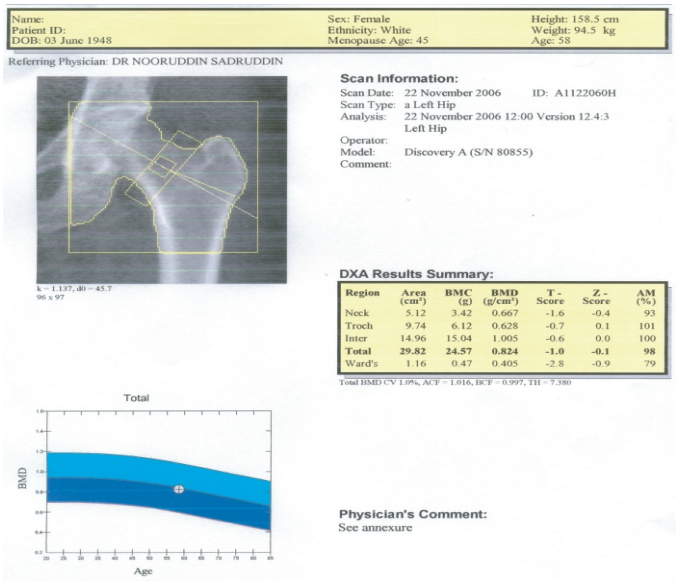
Bone density testing is strongly recommended in the following:

- Post-menopausal females;
- Personal or maternal history of hip fracture or smoking;
- Tall (over 5 feet 7 inches) or thin (less than 125 pounds) post-menopausal females;
- Patients on medications that are known to cause bone loss, including corticosteroids such as Prednisone, various anti-seizure medications such as Dilantin and certain barbiturates, or high-dose thyroid replacement drugs;

- Type 1 (formerly called juvenile or insulin-dependent) diabetics, liver disease, kidney disease or a family history of osteoporosis;
- Patients with high bone turnover, which shows up in the form of excessive collagen in urine samples;
- Hyperthyroidism;
- Previous history of fracture after mild trauma;
- X-ray evidence of vertebral fracture or other signs of osteoporosis.



Bone Mineral Density Scan of lumbar spine showing osteopenia



Bone Mineral Density Scan of Left Hip showing osteopenia

Western Blot for HIV 1 Virus

Ms Naureen Niaz Ali and Dr Jawaid Jabbar,
Chemical Pathology

Human immunodeficiency virus (HIV) is a retrovirus that causes acquired immunodeficiency syndrome (AIDS). In AIDS, there is failure of immune system so that the patients are prone to life-threatening opportunistic infections.

There are 2 types of HIV; 1 and 2. Ninety per cent of AIDS patients are affected by HIV-1 worldwide, The basic viral proteins of HIV-1 are: (as shown diagrammatically in fig. 1 and listed in Table 1)

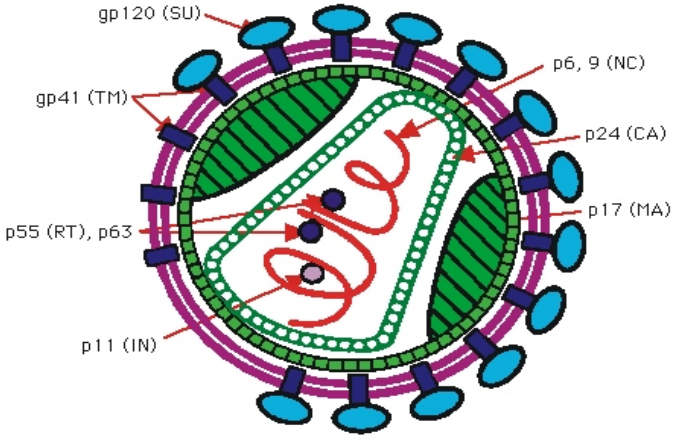


Figure 1
Schematic Representation of the Structure of HIV-1

DENOMINATION	GENOME	NATURE
GP 160	ENV	Glycoprotein precursor of GP 110/120 and GP 41
GP100/120	ENV	Envelope Glycoprotien
P68	POL	Reverse transcriptase
P55	GAG	Precursor of core protein
P52	POL	Reverse transcriptase
GP41	ENV	Transmembrane Glycoprotein
P40	GAG	Precursor of core protein
P34	POL	Endonuclease
P24/25	GAG	Core protein
P18	GAG	Core protein

Table 1

Diagnostic Approach

Screening

The initial approach is to screen the suspected individuals for the presence of antibodies to both

HIV-1 and HIV-2. At Aga Khan University Clinical Laboratory a micro particle enzyme immunoassay is use to detect antibodies to both HIV-1 and HIV-2.

False positive results may occur due to the following:
1- Hyperlipimic samples;
2- Hemolysed or plasma samples;
3- Multiparous women;
4- Recent recipients of influenza vaccine;
5- Recent recipients of Hepatitis B vaccines;
6- Patients with autoimmune disease.

Confirmatory Testing (Western Blot)

The confirmation for the presence of the virus requires testing with western blot technique to identify the basic viral proteins.

The test is based on indirect ELISA technique on a nitrocellulose strip containing all the HIV-1constituent proteins and an internal anti-IgG control. Firstly, strip is rehydrated and samples are incubated on the strip along with controls. If anti-HIV1 antibodies are present, they bind to the identified viral proteins present on the strip. After washing, the alkaline phosphates-labeled anti-human IgG antibodies are incubated. The conjugate binds to Anti-HIV1 antibodies captured on the solid phase. After washing, the colour development solution allows demonstrating the enzymatic activity of the complexes bound to nitrocellulose. The appearance of specific coloured bands allows demonstrating the presence of anti- HIV1 antibodies in the sample.

Interpretation

1- Positive:
Presence of 2 ENV protein and or GAG and or POL protein.

2- Indeterminate:
Presence of 1 ENV protein and or GAG and or POL, Presence of a GAG and POL and only presence of GAG and POL protein.

3- Negative:
No bands present on the strip.

Indeterminate results may reflect one of the following alternatives:
a) -Seroconversion
b) -HIV-2
c) -Cross reactivity due to the retrovirus.

Eighth Sheffield International Conference - Diagnostic Imaging

UK based radiologists, Sheffield University collaborate with AKU for radiology meeting

Reported by Ms Nida Husain, Radiology

Mr. Hamish Daniel, Deputy High Commissioner, UK

The two-day eighth Sheffield International Conference on Diagnostic Imaging was held at Aga Khan University on January 13 and 14, 2007. This biennial conference was organised by Sheffield Diagnostic Imaging, a group of UK-based physicians and Sheffield University UK, and was hosted by Aga Khan University (AKU). 21 invited speakers from the UK, one from Netherlands and four from Aga Khan University (AKU) made presentations on various sub-specialties and findings from latest research. The conference's inaugural session was attended by radiology professionals, physicians and surgeons from all over Pakistan, and was opened by Mr Hamish Daniel, Deputy High Commissioner, British High Commission who also inaugurated a concurrent 'Scientific Poster Exhibition' and a display of radiology-related equipment.

Mr. Hamish Daniel and Prof. Sameh Morcos Inaugurating the Scientific Poster Exhibition with members of the organising committee

Dr Nadeem Ahmad, Chairman, Department of Radiology, at the inaugural session

Dr Nadeem Ahmed, Chairman Department of Radiology, AKUH welcomed the guests and recognised the Sheffield Group's contribution to promote global education through such conferences, and especially thanked the invited international speakers and participants. Professor Dr Sameh Morcos, head of Sheffield Diagnostic Imaging provided an overview of the conference. The chief guest Mr Hamish Daniel, stressed upon the importance of country-to-country linkages in health care, as well as highlighting the sheer significance of the human factor not withstanding technological innovations.

A unique feature of the Sheffield International Conference was that it is held in developing countries, enabling concerned medical practitioners and technicians in these countries to familiarise themselves with state-of-the-art technologies and innovations, and the existing knowledge base.

Presentations made were focused on common diseases in Pakistan with emphasis on both basic and advanced imaging techniques. Topics covered during the conference included radiology in trauma and haemorrhage, oncology, women imaging, imaging in diabetes, vascular imaging, cardiothoracic imaging, neuro-radiology, nuclear imaging, etc.

Prof. Sameh Morcos, Chairman, Sheffield Diagnostic Imaging, addressing the inaugural session

The conference was designed to provide an opportunity to radiologists, technicians and other members of Pakistan's medical community to expand and build upon their knowledge and understanding of the dynamic field of radiology, presently undergoing very rapid change with new technology transforming this specialty into specific subspecialties. Available facilities in Pakistan are fast increasing and the organisers saw the necessity of expanding the pool of appropriately-educated radiologists. It is important that not only radiologists but all health care providers keep abreast of latest trends and technologies.

Dr Zafar Sajjad, Associaate Professor, Chairman Organising Committee, giving his welcome address at the inaugural session

The conference is a step towards initiating collaborative research projects and understanding at institutional, national and international levels. Over 400 participants attended the conference from Pakistan and abroad. The conference was accredited 11 Category-1 CPD credits by the Royal College of Radiologists of the United Kingdom.

As a part of its social responsibility and commitment to the advancement of health research and continuing education, AKU regularly holds seminars, symposiums and events to raise awareness about health and education related issues which are of national importance and interest.

Mr Nadeem Mustafa Khan, DG and CEO, AKUH, Captain (R) Habib Khan, Manager Radiology, Dr Nadeem Ahmad, Chairman Radiology, Mr Hamish Daniel, Deputy High Commissioner, UK, Dr Sameh Morcos, Chairman Sheffield Diagnostic Imaging, and Dr Zafar Sajjad, Associate Professor, Chair Organising Committee

آغا خان یونیورسٹی

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