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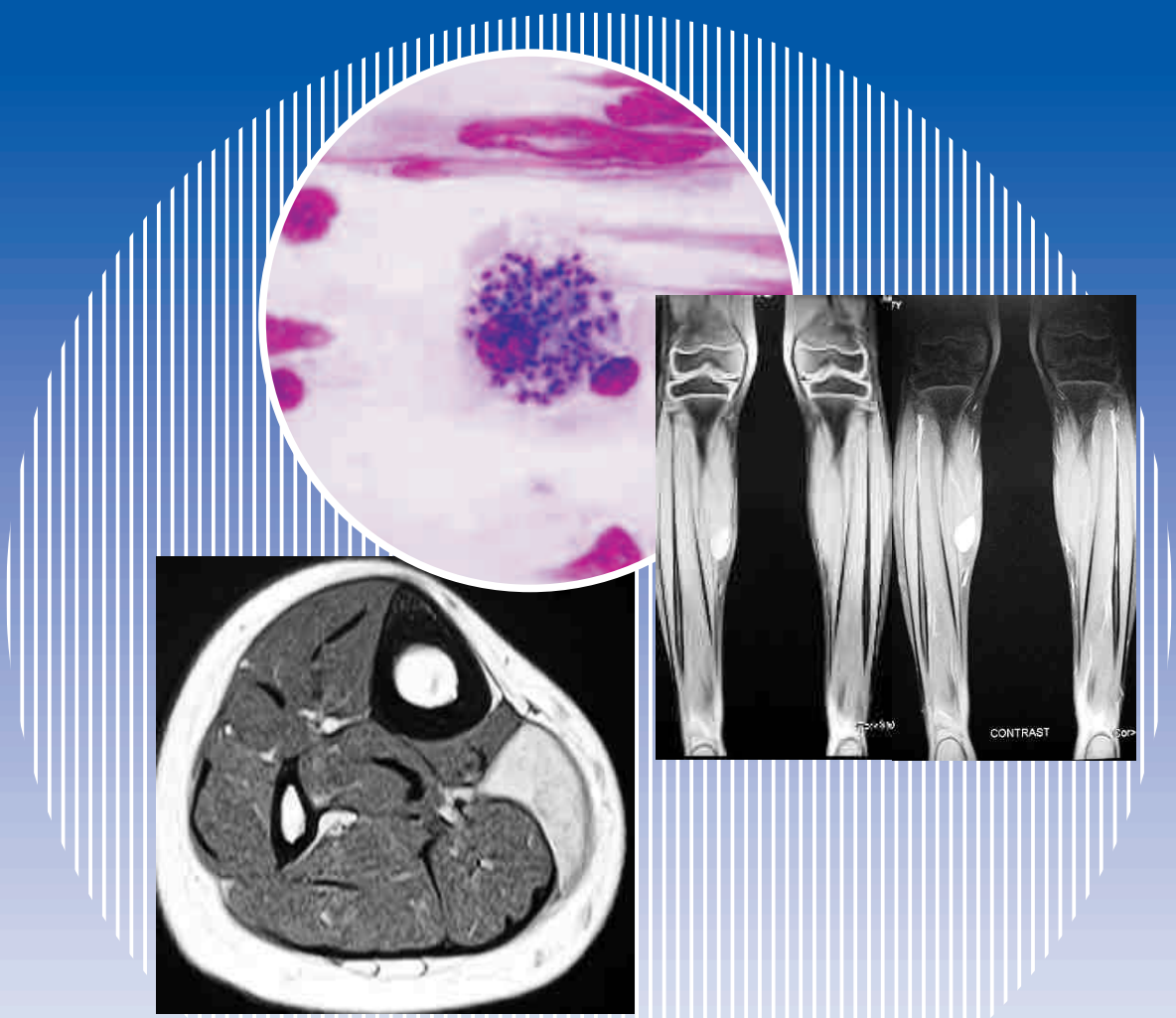

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Contents Page No.

Diagnosis of Malaria

Shahmeena Sadaf, Haematology

Introduction

Malaria is an infectious disease which is most commonly transmitted by the bite of female anopheles mosquito. However, its transmission through blood transfusion and during pregnancy is also documented.

The causative organism for malaria is a protozoan parasite called Plasmodia with four identified species namely, *Plasmodium vivax; P. falciparum, P. ovale* and *P. malariae.* Only two species *vivax* and *falciparum* have been reported from our country.

Malaria is a major public health problem especially in countries like Pakistan. It has emerged as the number one infectious killer and presently a major priority of the World Health Organization (WHO) for prevention. Approximately 1.5-3 million people die of malaria every year (85% of this incidence occurs in Africa), accounting for about 4-5% of all fatalities in the world.

Symptoms and Signs

Malaria can present in any clinical form with fever being the most common symptom. Fever commonly shows three stages viz. cold, hot and sweating stage. The febrile episode starts with shaking chills, usually at mid-day between 11 A.M. to 12 noon, and this lasts from 15 minutes to 1 hour (the cold stage), followed by high grade fever, even reaching above 106^0 F, which lasts 2 to 6 hours (the hot stage). This is followed by profuse sweating and the fever gradually subsides over the next 2 to 4 hours. Headaches, vomiting, delirium, anxiety and restlessness may also accompany. Physical examination of patient may reveal anaemia, jaundice and hepatosplenomegaly.

Diagnosis

Diagnosis of malaria is usually made on clinical grounds. However, laboratory tests are available to confirm diagnosis. Diagnostic investigations are divided into microscopic and non-microscopic tests.

I. Microscopic Tests

Peripheral Film is still considered to be the gold standard for the diagnosis of malaria.

a. Thick Smear

Thick film should be stained unfixed after drying at 37°C for 15 minutes with Field's stains A and B (Fig 1). It is very useful for detection of infection with low parasite count; however species identification is usually not possible.

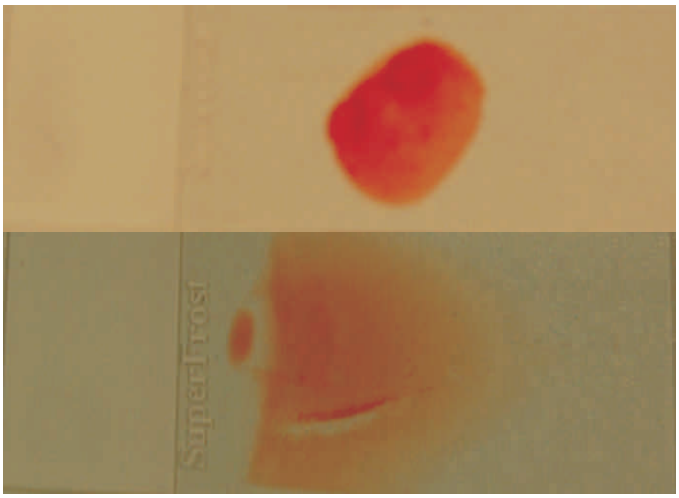

Fig 1: Thick and Thin Films as Used in Microscopy

b. Thin Smear

Thin smears are fixed with methanol and stained with Giemsa or Leishman's stain (Fig 2). It is most suitable for identification of species as it tends to preserve red cell membrane.

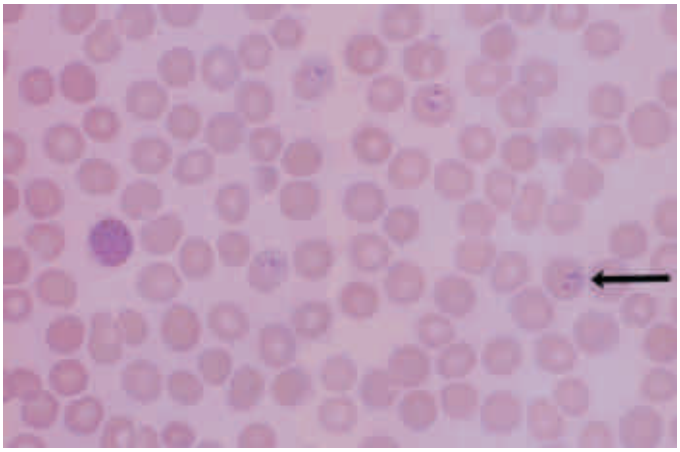

Fig 2: Thin Film Showing Trophozoites of Plasmodium Falciparum

c. Quantitative Buffy Coat (QBC) Test

The test involves staining of centrifuged and compressed red cell layer with acridine orange and observing these under UV light source. The test is user friendly and less technically demanding but has high false positive results and speciation is also not possible.

II. Non-Microscopic Tests

These rapid diagnostic tests (RDT) have recently become very popular because trained microscopists are not required to perform these tests. In emergency cases, especially at night, microscopy may be supplemented by RDT.

a. Immunochromatographic Test (ICT)

The test uses two colloidal gold labeled antibodies targeting plasmodial antigens like histidine rich protein HRP-II, (produced by trophozoites and gametocytes of *P.falciparum)*, and pan malaria antigen (common to all species of plasmodia including falciparum) (Fig 3).

Fig 3: ICT Malaria Test showing a Positive Result for P. falciparum

A potential problem with the dipstick test is that the circulating antigen will be detectable for many days even after the elimination of viable parasites. A positive test therefore may not always indicate an active infection; it may simply be the result of post therapy sero-positivity.

There are new ICT kits based on enzyme detection that could differentiate between active and previous infections.

b. Polymerase Chain Reaction (PCR)

It is possible to detect malaria using the non-isotopically labeled probe following PCR amplification. The PCR test is reported to be 10-fold more sensitive than microscopy. However, it cannot differentiate between viable and non viable parasites and is not routinely available is many clinical laboratories.

Conclusion

Microscopy still remains the gold standard for diagnosis of malaria. It is recommended that peripheral films should be made without delay since morphological alterations of parasites occur with storage of blood sample and parasite may become unrecognisable.

New tests like RDT and PCR are useful additions for diagnosing malaria. A suggested algorithm for the diagnosis of malaria is given in Fig 4. Training of technologists and pathologists and quality assurance practices are the key features that are essential for adequate laboratory diagnosis of malaria.

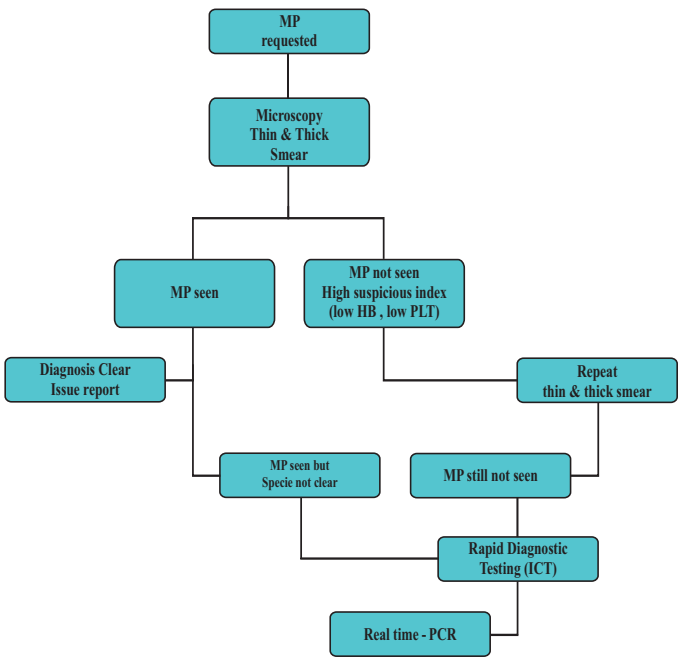

Fig 4: A Suggested Algorithm for the laboratory diagnosis of malaria by Clinical Lab, AKU

Imaging Feature of Synovial Sarcoma in Paediatrics Patients

Drs Fatima Mubarak and Naila Nadeem, Radiology

Introduction

Synovial sarcoma is an uncommon malignant mesenchymal tumor that occurs in the vicinity of joints, bursae, and tendon sheaths. The unique diagnostic histological feature of these tumors is their biphasic nature with the coexistence of two types of neoplastic cellular elements, the epithelial cell component and the spindle cell component (1). It is a rare tumor in all age groups and constitutes 5-10% of adult and 5-6% of childhood malignant soft-tissue sarcomas (1).

Case Report

We report a case of an eight years old child who presented with painless swelling on medial aspect of right leg for two to three months. Size of swelling remained unchanged. Not associated with fever or any other symptoms. Systematic examination was unremarkable. On local examination swelling was firm, well defined, 4-5 cm, mobile and non tender.

Plain films of right tibia and fibula were normal. Enhanced MRI examination revealed benign looking sharply defined mass lesion. This lesion was iso-intense on T1WI and high on T2WI and showing moderate enhancement (Fig 1 & 2).

Excision biopsy was submitted for histopathology which revealed spindle cell neoplasm with immunohistochemical features favoring synovial sarcoma. The sections were positive for VIMENTIN, MIC-2, BCL-2.For staging enhanced CT-scan, chest and bone scans were performed which were negative. Re-exploration of the primary site was done for complete excision; however histopathology only revealed inflammatory changes and margins were negative for the tumor. As the tumor on imaging appeared to be less than 5 cm, no further treatment was given.

Discussion

This tumor is characteristically seen in adults, and most cases present in the third to fifth decades of life (2). In review of 24 children with synovial sarcoma, Lee et al. (3) noted that one-half of their patients were 13-15 years old. Radiographic findings are nonspecific and usually show a soft-tissue mass of water density. Extensive calcification has been suggested to indicate a more favorable prognosis (4). Bone involvement is reported in about 20% of cases and may be due either pressure erosion with sharp margins and reactive sclerosis or to direct invasion causing cortical destruction (2).The efficacy of MR imaging for staging soft-tissue tumors has been established; however, its ability to show differences between benign and malignant lesion remains controversial (5,6). In a study of synovial sarcoma, Mahajan et al. (7) also noted a frequent relative clarity of tumor margins, and Benquist et al. (8) found that synovial sarcoma was the malignant tumor most frequently misdiagnosed as benign.

Conclusion

MR findings cannot be considered specific for synovial sarcoma, an awareness of the typical morphologic appearance may aid in the preoperative recognition of these lesions. The finding of inhomogeneous, septated mass with infiltrative margins located close to a joint, tendon, on bursae should suggest the presence of synovial sarcoma, especially if soft-tissue calcification can be identified on CT scans or plain radiographs.

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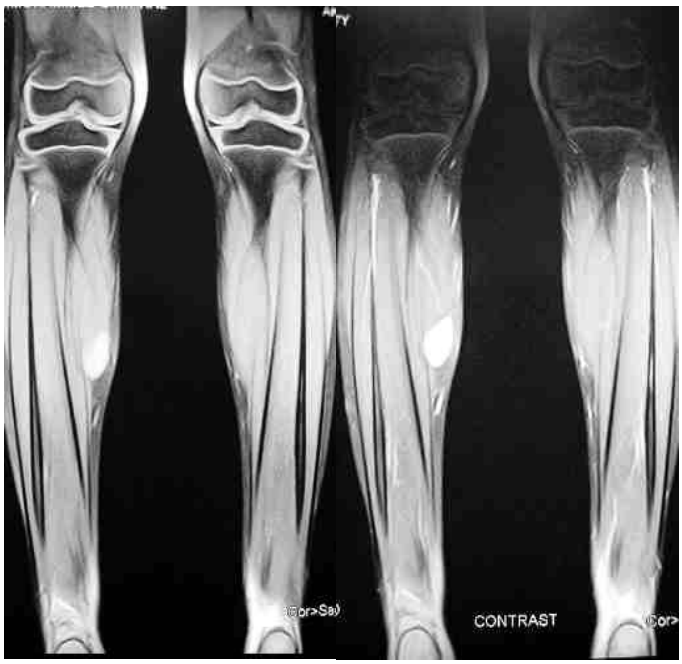

Fig 1: Synovial sarcoma with intermuscular origin coronal T2- & post gad fat sat image show heterogeneous Soft tissue mass with signal intensity higher with T2 & enhancement with Gadolinium.

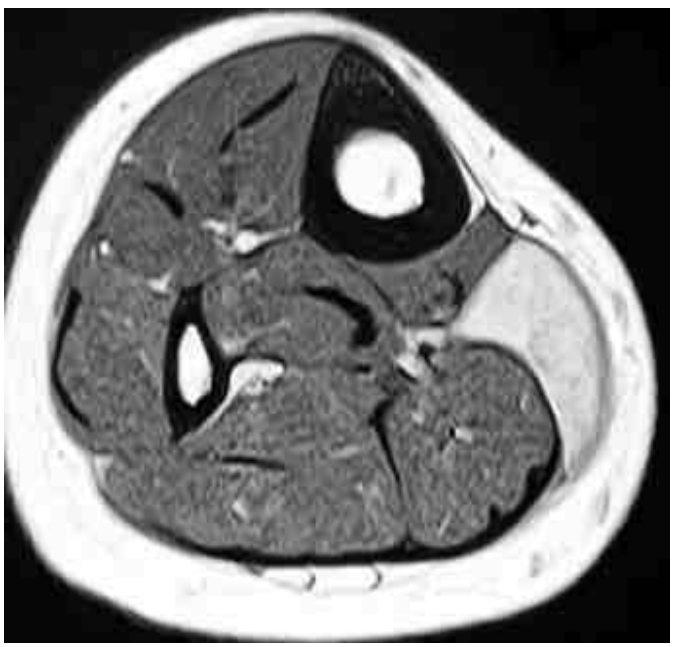

Fig 2: Axial post contrast image enhancing intermuscular Soft tissue mass

Fine Needle Aspiration Biopsy: The Best Test to Differentiate Benign from Malignant Thyroid Nodules

Dr Syeda Samia Fatima, Histopathology

It is usually not possible to distinguish between benign and malignant thyroid nodules by noninvasive procedures as any thyroid disease can present as a nodule. A wide variety of benign nodules but relatively few cancers further compound the diagnostic dilemma.

Although solitary nodules are considered more suspicious clinically, thyroid cancer occurs in a multinodular gland as often as in a solitary nodule.

Fine needle aspiration (FNA) biopsy is the best test apart from surgery for the evaluation of a thyroid nodule. It has become an extremely popular technique as it is quick, inexpensive, can be carried out in the physicians office, and the risk of complications (including tumor implantation) is minimal. Furthermore the material is suitable for immunohistochemical evaluation also. Published results report a sensitivity and specificity of over 90% therefore currently FNA is recommended as the initial test in the evaluation of any thyroid nodule. Most papillary carcinomas and other types of malignancy other than follicular carcinoma can be identified with ease. The same is true for various types of thyroiditis.

The principal reason for false-negative biopsies is inadequate sampling. Less than 5% to 10% of thyroid aspirates are unsatisfactory if performed by an experienced person. Because of the high false negative rate of FNA biopsy, even patients with benign lesions must be carefully followed up, e.g., by another biopsy in 6 to 12 months, provided the lesion is not clinically suspicious. If the FNA biopsy is consistently benign, observation may be safely discontinued.

HLA-B27 and Disease Association

Nazneen Islam, Molecular Pathology

Introduction

The human leukocyte antigen (HLA) system was discovered by analogy with the human red cell blood groups in the search for polymorphic antigens to match for transplantation. HLA complex is located on the short arm of chromosome 6, band p21.3 and consist of three non-overlapping segments called class I, II, III and encodes cell surface heterodimeric glycoproteins. The genes for these antigens are highly polymorphic and play a central role in the immune response. Since, HLA antigens are intimately involved in immune response, one's HLA genotype can influence susceptibility to disease that have an immunopathogensis. There are dozens of autoimmune diseases that fit into this description and of all the HLA disease associations; perhaps the strongest is between HLA-B27 and ankylosing spondylitis. HLA-B27 is one of the allele at HLA-B locus; it represents a family of 28 closely related alleles or sub types. These sub types are designated as HLA-B2701 to HLA-B2728.

Historical Background

The effect of HLA-B27 on susceptibility to spondyloarthritis was reported in 1973. The actual role of HLA-B27 in triggering an inflammatory response causing disease is still not precisely known. The oldest theory is that of molecular mimicry, in which an autoimmune response initially is mounted against a peptide from an infectious agent, and is subsequently directed against HLA-B27 itself due to epitopic similarities. A second theory that takes into consideration arthritogenic peptide, postulates that HLA molecules act as a peptide-binding molecule for infectious agents. The third theory suggests that the T-cell antigen is the true susceptibility factor, and a fourth theory implicates an innate etiology unrelated to HLA. Finally, HLA-B27 may simply represent a marker locus, closely linked to the as yet unidentified true immune response gene responsible for the inflammatory response.

Ankylosing spondylitis is a form of chronic inflammation of the spine and the sacroiliac joints which are located in the low back where the sacrum meets the iliac bones. Chronic inflammation in these areas causes pain and stiffness in and around the spine leading to complete cementing together of the vertebrae, a process referred to as ankylosis, eventually causing loss of spine mobility. The tendency for developing ankylosing spondylitis is believed to be genetically inherited. HLA-B27 is found in 88% of patients with ankylosing spondylitis. It is inherited in a Mendelian fashion, and it is found in 50% of first-degree relatives of those patients with spondylo arthropathies who are HLA-B27 positive. The chance that an HLA-B27 patient will develop spondyloarthritis or eye disease is 1 in 4. Molecular epidemiological studies have confirmed the association of HLA-B*2702, HLA-B*2704 and HLA-B*2705 alleles with spondyloarthritis. However molecular studies of other subtypes have produced some what conflicting results.

DNA analysis for the identification of HLA-B27

At the AKUH, PCR technique is used for the detection of HLA-B27 alleles which is based on DNA extraction from patient blood sample and amplification of HLA-B27 using *Taq* polymerase in a PCR buffer containing primers specific for the sequence of HLA-B27. Each reaction also includes control primers for the conserved region of human growth Harmon gene. This serves as an internal control and monitors PCR inhibition. After PCR amplified DNA fragments are separated by agarose gel electrophoresis and visualized by staining with ethidium bromide and exposure to ultraviolet light (Figure). Interpretation of PCR result is based on the presence or absence of specific amplified DNA fragment of specific size. Between January to July of 2007, blood samples of 384 patients suspected of ankylosing spondilytis were received in Molecular Pathology section and out of those 149 (39%) were positive. This data when compared with the frequency of HLA-B27 in the Pakistan population which is approximately 1.3%, illustrated a strong association between HLA-B27 and ankylosing spondilytis.

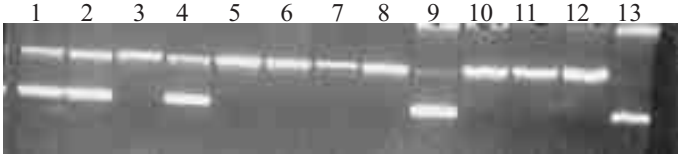

Figure: A representative photograph of PCR products separated on an agarose gel. Lanes 1,2,4 and 9 are samples positive for HLA-B27; Lanes 3,5,6,7,8,10,11 are samples negative for HLA-B27 allele; Lane 12 is a negative and Lane 13 is a positive control.

Bacterial Contamination of Blood Components

Dr Saba Qaiser, Microbiology

A serious complication of transfusion therapy is transmission of infection by various microorganisms. Apart from transmission of blood borne viruses (HBV, HCV, HIV etc.) bacterial contamination of transfusion products especially platelets is a significant concern in current modern medical practice. Unlike viruses, bacteria proliferate rapidly in nutrient rich blood product environment during storage. This is particularly true for platelet products that are stored at room temperature. This transfusion reaction that is due to contaminated blood products has high morbidity and mortality and is dependent on amount of bacteria transfused, virulence of bacteria, rate of transfusion, age and immune status of the patient. However even healthy individuals can have rapidly fatal outcome when transfused with large load of endotoxin producing gram negative organisms.

Predominance of gram-negative bacilli in blood for transfusion is related to the ability of these organisms to proliferate at 0 to 6°C, the temperatures at which blood is stored. *Yersinia spp.* and *Pseudomonas spp*. are examples of such organisms. Some gram negative bacteria produce endotoxin whose presence in the bloodstream may cause rapid and sometimes irreversible shock. Even low levels of bacterial contamination can result in a blood product that contains high concentrations of endotoxin and/or bacteria after 2 or 3 weeks of storage. Gram-positive organisms grow poorly in the cold and rarely contaminate blood stored under these conditions. On the other hand, platelets are routinely stored for long periods at room temperature (22°C) and thus provide an excellent medium for the growth of these bacteria.

Clinical Manifestations

Clinical manifestations are same as those of a hemolytic transfusion reaction unrelated to infection. Therefore, bacterial contamination of blood products should be considered in any transfusion reaction after excluding other causes.

Bacterial Agents Associated with Transfusion

Blood Component	Bacterial Agents
Packed Red Cells	*Yersinia enterocolitica,* Gram negative organism including *Pseudomonas spp.*
Whole blood	Gram negative organisms
Platelets	Skin flora eg: *Staphylococcus epidermidis, Streptococcus species, Corynebacterium spp,* Propionibacterium spp.
Plasma	*Salmonella spp., E.coli, Enterococcus spp., Clostridium spp., Serratia spp., Staphylococcus aureus, Pseduomonas spp.*
Others	*Treponema pallidum, Ehrlichia chaffeenisis, Rickettsia rickettesiae, Borrelia burgdorferi, Ehrlichia cytophagophilia*

Sources of Bacterial Contamination for Cellular Blood Products

- Asymptomatic bacteremia in a healthy donor
- Contamination during whole blood collection with skin flora
- Environmental organism introduction at the time of collection or during process
- Water bath in which frozen blood products are thawed
- Contaminated of blood bags

Diagnosis

If there is a suspicion of bacterial contamination of blood products, visual inspection for any change of color or blood clots should be done. Afterwards Gram stain and cultures should be performed. A negative culture will rule out significant contamination with bacteria at the time of transfusion. However a positive culture does not confirm the isolated agent as the source of transfusion reaction since it may be a minor contaminant. Moreover it also does not determine the source of contamination unless both blood bank and patient's blood yield identical organisms. It is important to note that a simultaneous culture of the patient's blood should also be sent for culture. The supplier of blood and blood products should be notified in case of a positive culture of their products.

Proposed Strategies to Reduce Transfusion Associated Infections

1. Reducing risk of blood product contamination
- Improved donor screening
- Proper venipuncture site disinfection
- Removal of first aliquot of donor blood

2. Blood component processing and storage
- Optimize storage temperature
- Limit storage time

3. Pre transfusion bacterial detection
- Visual inspection of components before issue
- Direct staining of bacteria
- Assay for bacterial endotoxin
- CO_2 production and oxygen consumption measure produced by bacteria
- Direct bacterial culture

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Pathology Quiz

Dr Tariq Mahmud, Pathology & Microbiology

Case History

A 2 years old girl presented to ER with loss of consciousness after fever and vomiting for 4 hours. She was admitted in the intensive care unit with septic shock. She had confluent petechial and purpuric lesions on her face, arms, and legs. Her hands and feet were cool and cyanotic. She had no signs of meningeal irritation. She was intubated, placed on cardiopulmonary support, and given large doses of catecholamines. A single dose of dexamethasone (3 mg per kg of body weight) was given immediately, and treatment with massive doses of Penicillin G was started. Meanwhile, the petechiae & purpura spread rapidly (Fig. 1), and four hours later peripheral pulses could not be detected in the arms or legs. The patient's condition deteriorated; she went into coma, and died. Autopsy was done and Fig. 2 shows her adrenal gland.

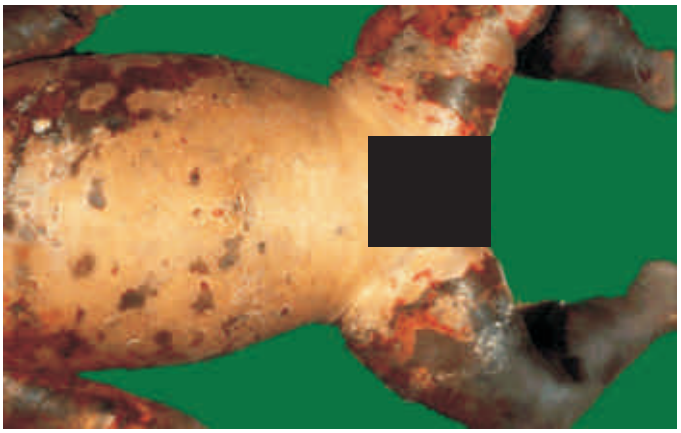

Fig 1:

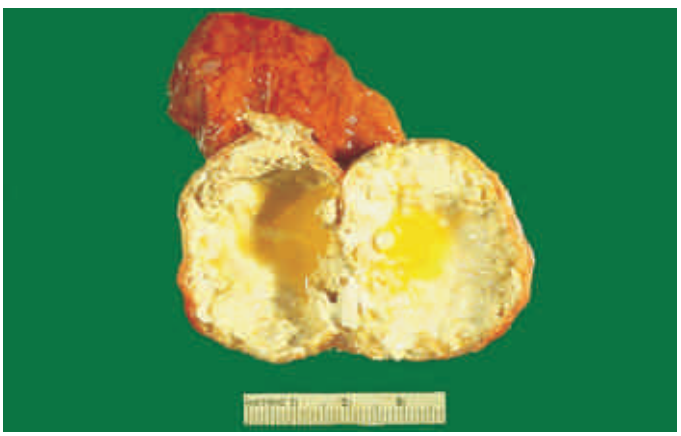

Fig 2:

Questions

1. What is your diagnosis?
2. What clinical syndrome did the patient have?
3. Name the most important etiological agent responsible for this disorder.
4. What are the other possible etiologies?
5. What is the characteristic lesion found in the adrenal glands in this disorder?
6. Enlist the laboratory tests which should be done to assist diagnosis.

Biochemical Investigations

Dr Aysha Habib Khan, Chemical Pathology

Investigations of Male Infertility

Laboratory investigations may detect early hormonal deficiency or distinguish between testicular and pituitary causes. Blood testosterone, leutinizing hormone (LH) and follicle stinutating hormone (FSH) determinations along with a semen analysis are the first line biochemical tests for investigating male infertility.

Raised plasma LH with a low testosterone concentration indicates leydig cell failure, while a raised plasma LH with a normal testosterone concentration suggests a lesser degree of damage with a compensatory increase in LH secretion. Low plasma LH and testosterone concentrations suggest pituitary or hypothalamic disease.

A raised plasma FSH concentration indicates seminiferous tubular failure, irrespective of the plasma testosterone concentration. There is usually oligospermia. Oligospermia with a low plasma FSH concentration suggests pituitary or hypothalamic disease and the patient should be investigated for the condition.

Markedly raised prolactin level is suggestive of pituitary tumor. A combined pituitary stimulation test may be performed to assess pituitary function, if clinically indicated.

Neonatal Thyroid Function

The fetal hypothalamic-pituitary-thyroid axis develops independently of maternal hormones. Immediately after birth plasma TSH concentration rise rapidly, in response to the stress of birth, to about 15 times the upper adult reference limit. They reach a peak within the first hour before falling, rapidly at first, during the next week. Plasma total thyroxin concentrations peak within the first 24 hours and then fall gradually. Screening tests for neonatal congenital hypothyroidism should be delayed for about a week after birth to allow the plasma TSH concentration to stabilise.

There is a similar pattern of secretion in healthy preterm infants, but the peak concentrations of both hormones are lower, this reduced response is even more marked in ill preterm infants, in whom it may be very difficult to interpret results of thyroid function tests: plasma total thyroxin concentrations are low, and those of TSH 'normal'. This pattern resembles that in ill adults. Treatment with thyroxin is usually not indicated.

Thyroid function tests should be repeated in infants found to have a positive screening test after birth; if the diagnosis is confirmed thyroid replacement should be started immediately. Thyroid function should be reassessed, after withdrawal of treatment, at the age of one year because neonatal hypothyroidism is sometimes transient. Results of screening tests may be misleading in ill, premature infants and may need to be repeated before discharge from hospital.

Hypothyroidism may be suspected clinically, for example, because of failure to thrive or persistent jaundice. In such cases thyroid function should be fully investigated.

Ferritin Determination

Circulating ferritin is usually in equilibrium with that in stores. However, it is an 'acute phase' protein and its synthesis is increased in many inflammatory conditions. The normal plasma ferritin concentration is about 100 ug/L. A plasma concentration below about 10ug/L almost certainly indicates iron deficiency, although the assay is rarely necessary to make the diagnosis. Results can be misleading if there is coexistent inflammatory disease, since accelerated synthesis may lead to normal or even high plasma concentrations despite very low iron stores, in this situation result of plasma iron and transferring assays are also difficult to interpret, haematological parameters remain the most reliable diagnostic indicators of iron deficiency.

High concentrations of plasma ferritin always occur in significant iron overload, but may also be due to inflammatory conditions, malignant disease; liver disease.

Thus the finding of a normal or low plasma ferritin concentration almost certainly excludes the diagnosis of iron overload, but a high one does not necessarily confirm it.

Leishmaniasis

Dr Bushra Moiz, Haematology

A 20 month-old female child from Hazara District presented with six months history of recurrent fever, weight loss and repeated blood transfusions. On examination, the patient was strikingly cachexic, febrile with massive splenomegaly. There was no family history of similar illness in other siblings. Complete blood counts showed: hemoglobin 100 g/L, MCV 76.6 fl, MCH 26.6 pg, total white cell count 3.6 $X10^9$/L (absolute neutrophil count of 0.4 $X10^9$/L) and platelet count 46 $X10^9$/L. Peripheral film showed pancytopenia with dimorphic red cells. A bone marrow examination was performed which showed histiocytes loaded with amastigotes form of *leishmania.* The diagnosis was later confirmed with ICT leishmania test and with PCR.

Leishmaniasis is a protozoal infection caused by several pathogenic species of *Leishmania.* Infection usually follows the bite of a sand fly of genus phlebotomus. Three major clinical manifestations are recognized which are visceral, cutaneous and muco-cutanoeus leishmaniasis. As many as 7 species have been identified today causing human infection including *Leishmania donovani, major, tropica, aetiopica, mexicana complex, brazilensis and peruviana*. In Pakistan, cutaneous and visceral leishmaniasis are mostly prevalent.

Symptoms of visceral leishmaniasis include low grade fever with malaise and sweating. This is accompanied by gross hepatosplenomegaly and cutaneous hypo pigmentation in later stages. Muco-cutanoeus disease starts as a pustular swelling in mouth or nostrils with a tendency to become ulcerative and extend into naso-pharyngeal mucous membrane. Cutaneous type usually begins as a painless papule on exposed skin which therefore is commonly seen on face. This may become ulcerative and spread further.

In visceral Leishmaniasis, histiocytes with round parasitic forms called amastigotes can be demonstrated by staining bone marrow (as seen in our patient), lymph node fluid, nasal scrapings, and liver biopsy. Rarely, amastigotes can be demonstrated in buffy coat preparations from peripheral blood. Similarly it is possible to demonstrate the parasite in stained film from skin smears of cutaneous ulcers and mucosal scrapings in cutaneous and muco-cutanoeus leishmaniasis respectively. Culture is used for all types of material for diagnosis. Polymerase chain reaction (PCR) can be used for diagnosis and can readily identify the species of *leishmania* present in biopsy or culture material. Serological tests like IFAT, Elisa, direct agglutination test (DAT), latex agglutination test for IgG antibodies. An immunochromatographic test (ICT) for rk39 antibody detection is also available.

Answers to Quiz

1. Fulminant meningococcaemia
2. Waterhouse-Friderichsen syndrome (WFS)
3. *Neisseria meningitidis*
4. WFS can also be caused by *Streptococcus pneumoniae* and *Haemophilus influenzae* type b infections, common bacterial pathogens typically associated with meningitis in the children and adults. *Staphylococcus aureus* has recently also been implicated in pediatric WFS. Septicaemia caused by *Pasteurella multocida*, complicated by Waterhouse-Friderichsen syndrome without skin haemorrhages has also been reported. Skin and mucosal haemorrhages are, therefore, not an essential feature of Waterhouse-Friderichsen syndrome and this condition should be suspected in all patients presenting with sudden illness and fulminant septicaemia.
5. There is extensive, usually bilateral, haemorrhagic necrosis of the adrenal glands.
6. Laboratory investigations include:
    a. CSF for D/R, Antigen detection & PCR
    b. Cultures of Blood, CSF & Skin lesions
    c. Blood complete picture
    d. Coagulation profile

CD55 and CD59 for the Diagnosis of PNH

Dr. Mohammad Kashif, Hematology

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal hematopoietic stem cell disorder characterized by chronic intravascular hemolysis, venous thrombosis, defective hematopoiesis, frequent episodes of infection and rarely, leukemic transformation.

Pathophysiology of PNH

The basic mechanism of hemolysis is due to unregulated complement activation on the abnormal red cell surface. Because of absence of regulatory molecules such as CD55 (decay accelerating factor, DAF) and CD59 (membrane inhibitor of reactive lysis, MIRL). In normal circumstances, these molecules attach on the plasma membrane via GPI (glycosylphosphatidylinositiol) anchor. Patients with PNH have a somatic mutation in the X-linked gene PIG-A (glycosylphosphatidylinositol complementation group A) which encodes a protein required in a step in the biosynthesis of GPI molecules. More specifically this protein transfer N-acetyl-glucosamine to phosphatidylinositol in order to synthesize N-acetyl glucosamine phosphatidylinositol .This mutation occurs in an early hematopoietic stem cell clone and results in absent or decreased cell surface expression of all GPI-anchored proteins in all types of blood cells.

PNH is not inherited. It often occurs as an isolated disorder. However, it also arises in an estimated 30% of aplastic anemia cases and in some patients with myelodysplastic syndrome (MDS). So all patients with a diagnosis of aplastic anemaia and MDS should be screened for PNH clone. In addition, PNH may evolve into acute myelogenous leukemia.

There are certain diagnostic tests which are used in the diagnosis of PNH including Ham's test, sucrose lysis test and, CD55 and CD59 analysis by flowcytometry and by using gel card technique. However, flowcytometry is the gold standard for PNH diagnosis. But is not currently available at Aga Khan University Hospital.

The complement lysis sensitivity test of Rosse and Dacie is a more precise method. In this test, RBCs are sensitized with a potent lytic anti-i antigen and hemolyzed with limiting amounts of normal serum as a source of complement. This demonstrates three groups of RBCs in PNH patients including the following:

- PNH I cells are normal in sensitivity to complement.
- PNH II cells are moderately more sensitive than normal cells.
- PNH III cells are markedly sensitive, requiring one fifteenth to one twentieth of complement for an equal degree of lysis. This group is increased in patients with more severe PNH and is associated with a mean life span of 10-15 days.

CD55 and CD59 Determination by Gel Card Technique

This is a simple method for screening red cells for deficiency of GPI linked protein. One study showed that this technique has 100% specificity and 92.8% sensitivity when compared with flowcytometric analysis of CD55 and CD59.

For optimal results, the determination should be performed using a freshly drawn blood sample preferably into EDTA, citrate or CPD-A anticoagulant.

Gel card tubes contain antibodies against CD55 and CD59; if cells express both antigens the cell agglutinates by forming antigen antibody complexes and form a red line on the surface of the gel matrix. PNH cells lack these antigens so they pass from gel matrix and form a solid button on the bottom of microtube (Figure). Positive test indicates that cells posses these antigens and patient does not have PNH.

This test has recently been introduced at Clinical Laboratories Aga Khan University Hospital.

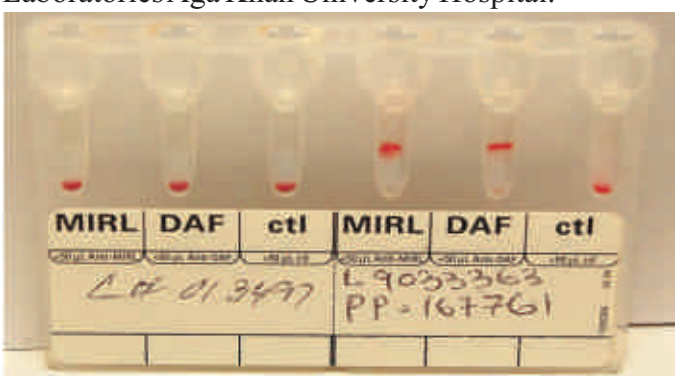

Figure: PNH gel card showing absent GPI linked proteins (MIRL and DAF) in a PNH patient. On the right side is a normal individual with both proteins present

References

1. R Gupta et al. A prospective comparison of four techniques for diagnosis of paroxysmal nocturnal hemoglobinuria. Int J Lab Hematol. 2007 Apr; 29 (2):119-26.
2. Dacie and Lewis Practical Haematology 10th Ed.
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Meeting Report

Continuous Medical Education Seminar (CME) in Multan

The CME was attended by 143 local Physicians which included Consultants from various specialties as well as GPs. Renowned faculty members of pediatrics, gastroenterologists and oncologists from local academic institutions also attended the CME.

Dr Asghar Javaid, Staff Pathologist Multan Stat Lab welcomed the guests and introduced the speakers. **Dr Farooq Ghani** Associate Professor and Consultant Chemical Pathologist at AKU presented an **"Overview of Clinical Laboratory Services and Quality Assurance Programmes"** in which he highlighted the service and academic aims of the Pathology Department. He also explained the value of performing cutting edge research, utilising state of the

art technology and participation in international quality assurance programmes as the key elements that have made AKUH laboratories a center of excellence in this region.

Dr Shahid Pervez Professor and Section Head of Histopathology was invited to give his valuable presentation on "**Specialised Staining of Biopsy Specimens for Diagnosis, Disease prognosis and Selection of Therapy"** He highlighted the importance of immunohistochemistry in particular for the precise diagnosis of various types of cancers by citing various examples.

Dr Usman Skeikh Assistant Professor and Consultant Haematologist delivered his presentation on **"Interpretation of Complete Blood Count"** in which he explained the importance of peripheral blood film in the diagnosis of haematological and non haematological disorders. He emphasised that a proper examination of peripheral blood smear is not only diagnostic but also cost effective and really helps the physicians in the management of their patients.

Dr Erum Khan, Assistant Professor and Consultant Microbiologist gave her presentation on **"Hepatitis B and C, Interpretation of Serological and PCR Tests"** in which she discussed the clinical and pathological aspects of common forms of hepatitis in our region. She highlighted different diagnostic tests available and interpretation of these test reports in light of clinical findings. The audience took keen interest with vibrant question answer session at the end.

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