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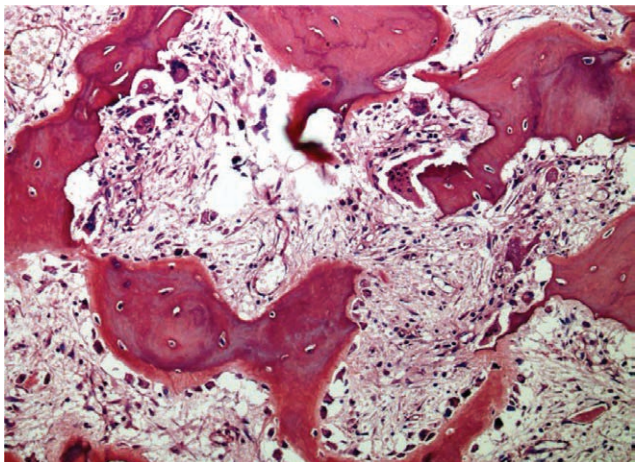
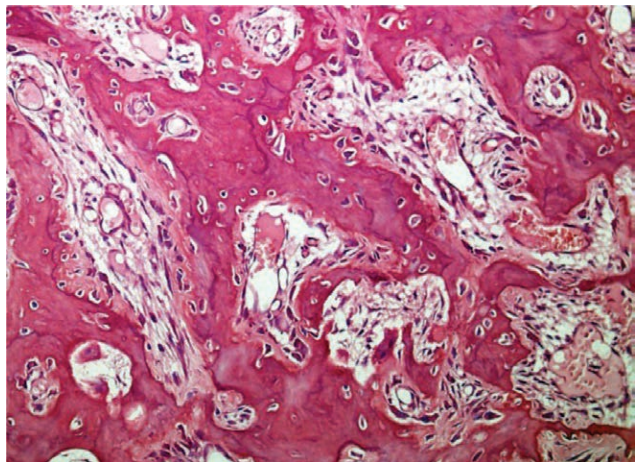
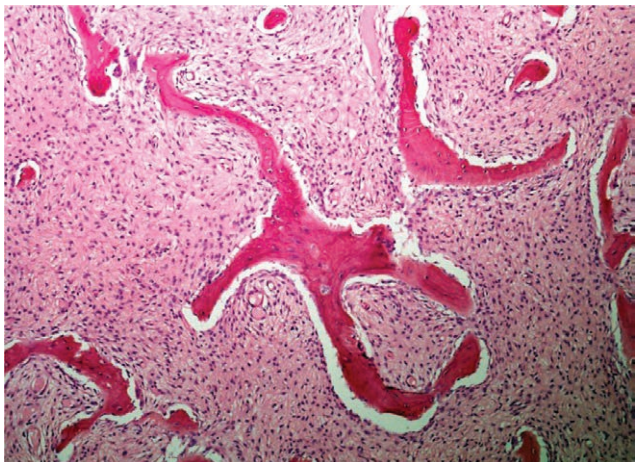
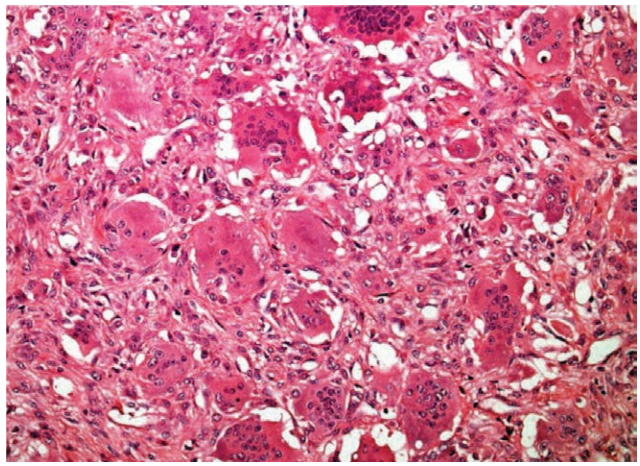
Bone Health









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The Aga Khan University Hospital, Karachi

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From the Editor's Desk

The Department of Pathology & Laboratory Medicine is committed to providing high quality diagnostic services for physicians and hospitals nationwide.

One of the functions of the Department is to update physicians throughout Pakistan about the advancement in laboratory sciences and the services available at the Clinical Laboratory for disease diagnosis, through our Laboratory Updates and this newsletter, LABRAD.

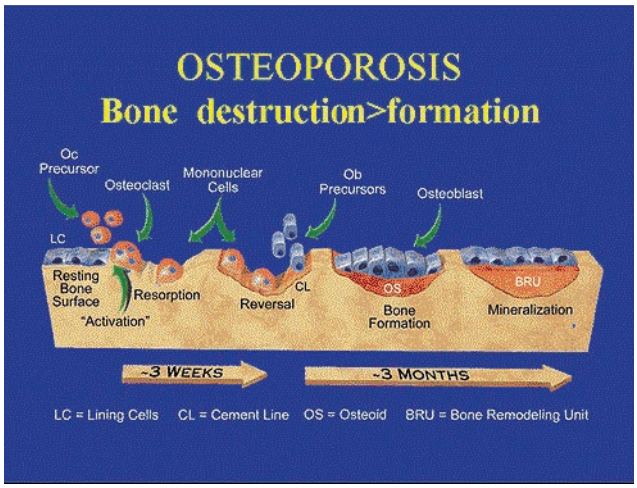
The current issue of the LABRAD focuses on measurements related to bone disorders in an attempt to give readers a better understanding of the diagnostic modalities available at Aga Khan University Hospital Clinical Laboratories.

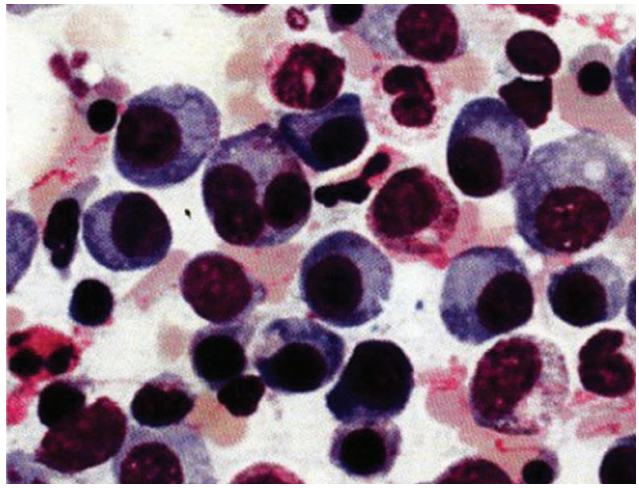
Bone disease is a vast topic which interests a diverse group of medical professionals such as: internists, rheumatologist, orthopaedics, endocrinologist, gynaecologists, paediatricians and geriatrics, researchers in anatomy, physiology, nutrition, biochemistry, pathology and imaging. Diversity of interest is due to a combination of problems ranging from malnutrition in the developing world to the aging populations of the developed world.

Several factors may influence the choice of a method for the measurement of bone disease. A major limiting factor in establishment of a service for measurement of bone diseases is the local availability of equipment and methodology, and also the availability of the required expertise and knowledge for the interpretation of results. Choice of the tests also depends on the type of information required from investigating a patient suspected of having a bone disease and achieving this aim as economically as possible in less time and money.

This issue concentrates on common bone related clinical problems; focusing on practical usefulness of various tests available for diagnosis and management of bone diseases in the field. Biochemical bone profiles have been developed in the section of chemical pathology to help the physician in evaluating a patient suspected of having metabolic bone diseases. Combination of information on different microbiological tools for facilitating management of bone infection along with diagnostic tools available for identifying disorder like ankylosing spondylitis are also presented. On the whole this is a broad sweep of information collected through individual disciplines and we hope it will improve as our newsletter evolves through a cyclic process of learning and sharing with our readers.

We hope this edition would help increase your knowledge banks and would answer many of your questions. We are hopeful for making this issue a useful document for future reference in the bone disease area. Happy reading!





Biochemical Bone Profiles Available at Clinical Laboratory of Aga Khan University Hospital

Dr Farhan Javed Dar
Chemical Pathology

The utility of bone profiles in screening and diagnosis of bone diseases have a profound effect upon bone health. Choosing any screening profile helps to rule out metabolic bone diseases such as vitamin D deficiency, osteoporosis. Panels are made to make clinical interpretation easy for physicians and economical for patients.

Following profiles are available at Clinical Laboratory AKU for diagnosis and monitoring of bone disorders. Only 5 ml of blood is sufficient for any one of the panel as shown in the Table.

Commonly encountered bone diseases include those related to vitamin D deficiency (rickets in children and osteomalacia in adults), osteoporosis, osteogenesis, imperfecta and Paget's disease of bone. Bone disease can lead to fragile and brittle bones, which break easily.

AKUH clinical laboratory is now providing Bone Health Panel, (please refer to the table below) which helps in assessment of bone health. This profile is intended to screen, investigate and monitor patients with disorder of calcium and bone metabolism. It provides answer to the potential clinical questions that a physician seeks when investigating for metabolic bone diseases for example; does my patient have hypocalcaemia or hypercalcaemia? If there is hypercalcaemia, is it due to hyperparathyroidism or a parathyroid hormone independent cause? If it is hypocalcaemia, is it due to parathyroid failure, vitamin D deficiency or renal failure? Does my patient have hypomagnessemia? If the patient has aches and pains or unexpected fracture then is it due to metabolic bone disease?

Biochemical Bone Profile	
Bone Health Panel	**Osteoporosis Panel**
Calcium	Calcium
Phosphorus	Phosphorus
Albumin	Albumin
Magnesium	Alkaline phosphatase (ALP)
Alkaline phosphatase (ALP)	25-hydroxy vitamin D (25-OHD)
Creatinine	Intact parathyroid hormone (iPTH)
Intact parathyroid hormone (iPTH)	N-telopeptide of type I collagen (NTx)
25-hydroxy vitamin D (25-OHD)	

Bone Health Panel

Bone health is important throughout the lifetime of an individual. Bones protect our internal organs from damage, they are the factory of our blood cells and are the storehouse for minerals and nutrients (such as calcium and phosphorus) needed in the body. With all of these critical functions for our health, understanding how to maintain and build healthy bones is an important part of investigating for a healthy future.

It is especially important to build strong and healthy bones during childhood and teen years to avoid osteoporosis and other bone problems later in life. After the mid-30s, bone loss slowly begins to occur. Women lose bone quickly after menopause. Importantly, healthy habits can help to limit the bone loss that occurs. Taking care of our bones when we are young will help prevent problems in the future.

Osteoporosis Panel

Osteoporosis is a systemic skeletal disorder characterised by low bone mass and micro architecture deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture.

According to WHO by 2025, almost 3 million of the global population will suffer from osteoporosis which will be expected to rise to 6.3 million by 2050. About 75 percent of this 6.3 million hip fractures will occur in the developing countries, mainly due to projected large increase of aged population. Osteoporosis is a costly disease due to its chronic nature, severity of its complications and means required to treat it. Osteoporosis is diagnosed late as patient remains asymptomatic until fracture occurs. AKUH Clinical Laboratory is providing Osteoporosis Panel, mentioned in the above table, which helps in assessment of osteoporosis.

It is to be noted that diagnosis should be made in correlation with clinical picture and Dual-energy X-ray absorptionetry (DXA) results.

Ionized Calcium Determination in Clinical Labortory

Dr Lena Jafri
Chemical Pathology

Plasma calcium exists in three forms ionized (iCa) (45 to 50 per cent), protein bound (40 per cent) and calcium complexed with anions (10-15 per cent). Although all the forms are in equilibrium with each other, only the plasma iCa has been reported to be active at cellular level. This parameter has also been called 'free' or 'ionic' calcium. It is considered the best indicator of calcium status because it is biologically active and tightly regulated by parathyroid hormone (PTH) and 1, 25 dihydroxy vitamin D. Total calcium level does not give an indication of what is available at the cellular level. Only disturbances in iCa are physiologically relevant.

Analysis of iCa is technically demanding. The sound analytical performance of today's iCa analyzers using ion selective electrode (ISE) technology have made measurements accurate and precise. iCa recently introduced in Aga Khan University Clinical Laboratory after thorough research and understanding of Clinical Laboratory Standard Institute Guidelines (CLSI) for sample handling, storage and transportation. Table 1 shows the precautions to be taken prior to blood sampling for iCa determination and collection.

The clinical usefulness of measuring iCa rather than total calcium is more in disorders with plasma

Table 1. Recommendations for Specimen Collection of iCa as per CLSI Guidelines

Precollection Variables and Recommendations	Collection Techniques
Have the patient rest for 5-10 min before collecting blood	If a series of tubes must be collected, fill gel tube for iCa first
Ensure that the patient has not eaten for at least 4 hours	Do not leave the tourniquet on for more than 3 minutes
Collect specimens under consistent conditions ideally the patient should be seated	Do not allow the patient to exercise the forearm or make a fist
	Fill gel tubes completely
	Handle specimen anaerobically (do not open the tube until analysis)

protein abnormalities like liver failure, protein losing nephropathy, burns, cardiac failure or malnutrition. iCa determination is more useful in those undergoing major surgery who have received citrated blood/ platelets, heparin, or intravenous calcium.

It should be the test of choice when neonatal hypocalcemia is suspected, especially if bicarbonate is given to neonates with hypocalcemia.

Rapid measurement of iCa in intensive care units is helpful in cases with sepsis and acid base disturbances. It has been documented that the use of total calcium is unreliable in cases where there is a change in the protein-calcium binding characteristics as in patients with hypergammaglobulinemia or a decrease/increase in pH. An increase in blood pH is associated with a decrease in iCa a decreased blood pH is associated with an increased iCa.

Ionized calcium is also useful in cardiopulmonary bypass and during hemodialysis where maintenance of good cardiac function is essential. During dialysis monitoring a slight positive calcium balance is important for maintaining good cardiac contractility. Ionized calcium is the best means to monitor this.

Literature shows that iCa rather than total calcium is more useful in malignancies, hypo/ hyperparathyroidism and pancreatitis.

Parathyroid Hormone Disorders and Issues of Testing

Dr Syed Talha Naeem
Chemical Pathology

Parathyroid hormone (PTH) is secreted by the parathyroid glands and regulates serum calcium (Ca) through its effects on the bone, kidney and intestine. PTH secretion is stimulated by decrease in serum Ca and magnesium and an increase in serum phosphate, which in turn raises serum ionized Ca levels through direct action on bone and the kidneys. Long-term regulation of total body Ca by PTH occurs through its stimulation of vitamin D metabolism.

PTH can be measured in the blood in several different forms: intact PTH; N-terminal PTH; mid-molecule PTH, and C-terminal PTH. An intact PTH (iPTH) provides a better index of parathyroid function and is the test available in AKUH clinical laboratory for clinical use.

PTH is secreted in episodic or pulsatile fashion with an overall circadian rhythm characterised by a nocturnal rise. Measurement of iPTH on more than one occasion should minimise the effect of episodic secretion and circadian rhythm. Because of the nocturnal rise in iPTH levels, samples should be collected in the morning, preferably after an overnight fast.

Determination of iPTH is useful in the differential diagnosis of disorders of bone and mineral metabolism, including hypocalcaemia and hypercalcaemia, renal failure and secondary hyperparathyroidism; when PTH increases long before Ca becomes abnormally low.

PTH is elevated in the majority of patients with primary hyperparathyroidism. It is below normal or in the lower half of the reference interval in most patients with nonparathyroid hypercalcemia including hypercalcemia-associated malignancy. In cases with hypercalcemia, PTH estimation should be performed before initiating any therapy to reduce serum Ca; as PTH secretion may be stimulated by declining but still elevated levels of serum Ca. This can complicate the differential diagnosis of hypercalcaemia. In the majority of patients with hypercalcaemia associated with malignancy, iPTH is suppressed to levels below normal or at the lower end of the normal reference interval. Elevated levels of iPTH in patients with hypercalcemia and malignancy suggest coexisting hyperparathyroidism and malignancy, because ectopic PTH production appears to be extremely rare.

In secondary hyperparathyroidism, iPTH is increased before total or free calcium becomes abnormally low, a consequence of homeostatic

mechanisms for maintenance of serum Ca. Consequently, PTH is more sensitive than Ca for identifying secondary hyperparathyroidism. Subnormal or normal PTH is observed in the majority of patients with hypoparathyroidism; such concentrations are inappropriately low for patients with hypocalcemia.

In patients with end-stage renal disease, measurement of iPTH is helpful in assessing parathyroid function, in estimating bone turnover, and in improving management. Patients with high turnover bone disease because of secondary hyperparathyroidism (advanced osteitis fibrosa) have the highest concentrations of PTH, whereas patients with low-turnover, adynamic bone disease, including osteomalacia, have the lowest concentrations. Intermediate levels are found in patients with low-turnover adynamic (aplastic) disease and early osteitis fibrosa. Considerable overlap in iPTH levels is apparent between the various forms of renal osteodystrophy.

iPTH is also useful intraoperatively, for assessing the completeness of parathyroidectomy and facilitating minimally invasive parathyroid surgery, thereby improving cost-effectiveness and cosmetic outcomes.

PTH levels may be altered in some patients with hyperthyroidism and hypothyroidism and may increase after treatment with lithium carbonate. PTH concentrations are inversely correlated with T3 levels in hyperthyroid patients, increase in patients who become hypothyroid after radioactive iodine treatment, and decrease with replacement therapy; changes apparently mediated by serum Ca. Chronic lithium carbonate therapy has been reported to increase parathyroid gland size and circulating intact PTH. Drugs that may increase PTH levels include phosphates, anticonvulsants, steroids, isoniazid, lithium, and rifampicin.

Because of the physiological relationship between circulating Ca and PTH, it is always important to interpret PTH results in the light of total or ionized Ca levels. Indices of renal function, measurements of albumin, as an adjunct to measurement of total calcium levels and determinations of phosphorus, chloride, and magnesium levels may also aid in the interpretation of PTH and Ca results. It should also be remembered that hypercalcemia and hypocalcemia may be secondary to disordered vitamin D metabolism. For diagnostic purposes PTH results should always be used in combination with the clinical examination, patient medical history, and other findings.

Tubular Maximum Reabsorption Rate of Phosphate to Glomerular Filtration Rate (TmP/GFR)

Dr Noreen Sherazi
Chemical Pathology

Phosphate filters entirely through the glomeruli but is then largely reabsorbed in the proximal part of the proximal renal tubule. Several factors influence the tubular reabsorption of phosphate (Table 1) by acting on the sodium/phosphate co transporters. Tubular reabsorption of phosphate depends on plasma phosphate and glomerular filtration rate and is not a satisfactory indicator of tubular phosphate handling. This has led to increasing use of 'tubular maximum for phosphate corrected for GFR (TmP/GFR)', a factor independent of plasma phosphate and renal functions for assessment of renal phosphate handling. TmP/GFR (Normal 2.8-4.4 mg/dL) is an index of renal threshold for phosphate which can be determined directly by the formula TmP/GFR = Plasma phosphate - (urine phosphate x serum creatinine)/urine creatinine.

Assessing renal reabsorption of phosphorus is needed in a variety of pathological conditions associated with hypophosphatemia including hypophosphatemic rickets, tumour-induced osteomalacia and tumoral calcinosis. It is also used in adjusting phosphate replacement therapy in severe deficiency states; like conditions that cause phosphate redistribution (e.g. glucose infusion, respiratory alkalosis), so if

Table 1. Factors Affecting Tubular Reabsorption of Phosphate

Factors that decrease renal phosphate absorption	Factors that increase renal phosphate absorption
Phosphate-free diet	High dietary phosphate intake
Respiratory acidosis, metabolic alkalosis	Respiratory alkalosis, metabolic acidosis
PTH, PTHrP, EGF, glucocorticoids, catecholamines, calcitonin, $1,25(OH)_2$ D_3	GH, IGF1, thyroid hormones (T3), insulin
Diuretics	
Phosphatonins	

hypophosphataemia persists, a low (fasting) TmP/GFR indicates the need for phosphate replacement. In the treatment of severe phosphate deficiency, TmP/GFR can be used as an indicator of intracellular repletion.

The measurement of TmP/GFR along with other parameters is required for diagnosis of X-linked hypophosphataemic rickets and hereditary hypophosphataemic rickets with hypercalciuria.

Measurement of TmP/GFR is central to the diagnosis of oncogenic osteomalacia in addition to other parameters

We, at AKUH clinical laboratory, are starting this test which will required at least 6-8 hours fasting serum sample for phosphate and creatinine and simultaneously taken spot urine sample for phosphate and creatinine. TmP/GFR will then be calculated from above mentioned parameters.

LABRAD Quiz

Dr Hafsa Majid
Chemical Pathology

Question #1

A three-week-old girl was brought to the emergency room because of intermittent twitching of her left limbs for 4 days. The girl had been born normally at 39 weeks of gestation and was being breast-fed. Examination was unremarkable. Her biochemical investigations were:

Na^+ : 125mmol/l (135-145 mmol/l)
Cl^- : 100mmol/l (101-111 mmol/l)
K^+ : 2.8mmol/l (3.6-5.0 mmol/l)
HCO^3 : 30mmol/l (23-28 mmol/l)

Ca^+ : 4.5mg/dl (8.4-10.2mg/dl)
Albumin : 2g/dl (3.2-5.5g/dl)
PO_4 : 10mg/dl (2.5-4.6 mg/dl)
PTH : <4pg/l (16-87pg/ml)

1.1: What is the diagnosis?

1.2: Which other bone mineral is required for optimal parathyroid function?

1.3: How would this be treated?

Question #2

A 60-year-old female came to ER due to fracture neck of femur after a minor fall. Her past complaints were of generalised body aches and bone pains with difficulty in rising from sitting position since two year. She was a known case of hypertension for the past 20 years and taking antihypertensive since then. She was diagnosed to be osteoporotic and was considered for bisphosphonate therapy.

2.1: What is the most likely cause of body aches in the patient?

2.2: What biochemical test can be performed to confirm diagnosis?

2.3: How will you monitor the response to bisphosphonate therapy?

Renal Osteodystrophy: A Disturbed Metabolic Aspect of Renal Failure

Dr Sahar Iqbal
Chemical Pathology

Renal osteodystrophy is a common complication of chronic kidney disease (CKD). It is part of a broad spectrum of disorders of mineral and bone metabolism that develop in this clinical setting and result in both skeletal and extra skeletal consequences. The term CKD-Mineral and Bone Disorder (CKD-MBD) has been recommended to be used to describe a broader clinical syndrome that develops as a systemic disorder of mineral and bone metabolism in CKD. The manifestations include abnormalities of calcium, phosphorous, parathyroid hormone (PTH) or vitamin D metabolism; abnormalities in bone turnover, mineralisation, volume, linear growth or strength; and vascular or other soft tissue calcification.

The term renal osteodystrophy should be used exclusively to define alterations in the bone morphology associated with CKD. This is a single measure of the skeletal component of the systemic disorder of CKD-MBD that is quantifiable by histomorphometry of bone biopsy. The abnormalities of the bone in the setting of CKD may manifest as high turnover bone disease (ostietis fibrosa or secondary hyperparathyroidism), dynamic bone disease, osteomalacia and mixed renal osteodystrophy.

Biochemical markers for diagnosing renal osteodystrophy are intact PTH (iPTH), vitamin D (25OHD), blood calcium and phosphorus levels and bone turn over markers such as alkaline phosphatase and N-telopeptide of type I Collagen (NTx).

The pathogenesis of osteodystrophy is complex. The cycle starts with the renal failure and retention of phosphate, inhibiting calcitriol (1,25 dihydroxy vitamin D) synthesis and decrease in calcium through a reduction in intestinal calcium absorption. Hypocalcaemia stimulates PTH secretion causing high turnover renal osteodystrophy.

The biochemical picture of high turnover renal osteodystrophy usually is an increase in blood PTH, bone alkaline phosphatase and phosphate concentration and low calcium and 25OHD levels.

Low turnover bone disease is commonly observed in dialysis patients, but has been described in some cases even before dialysis. Adynamic bone disease is associated with the over suppression of parathyroid gland activity due to high calcium intake (from diet, dialysate or calcium-containing phosphorus binders) and/or administration of vitamin D analogs in excess.

Patients with adynamic bone disease have lower blood concentrations of iPTH that may fall below the recommended target range of 150-300 pg/ml of iPTH, resulting in a bone turnover rate that is below normal. In these circumstances, the bone may not take up calcium for incorporation into new bone and any excess calcium may predispose to calcification in soft tissues. Currently in the United States, approximately 25 per cent of patients undergoing dialysis have a iPTH concentration above the target range (high-turnover disease), approximately 25 per cent have a PTH concentration within the target range and 50 per cent have a PTH concentration below the target range (adynamic bone disease).

The ultimate goal of drug therapy in the management of CKD is to prevent complications of CKD, specifically bone disease and extra skeletal calcification. Need for the evaluation, treatment and monitoring of bone metabolism is to prevent secondary hyperparathyroidism. At stage 3 CKD, serum calcium, phosphorus and iPTH concentrations should be evaluated. After initial assessment, routine monitoring should be performed (see Table 1). Appropriate management requires a balanced diet, phosphorus binders and active vitamin D analogs. The recommended ranges for corrected calcium, phosphorus and iPTH for different stages of CKD for kidney diseases outcomes quality initiative are shown in Table 2.

Table 1. Monitoring of frequency of calcium, phosphorus and intact PTH in different stages of CKD kidney diseases
National kidney foundation. Clinical practice guidelines for bone metabolism and disease. Am J Kidney Dis 2003; 42(4 supp 3): S12

Stage of CKD	Measurement of iPTH	Measurement of Calcium and Phosphorus
3 (30-59 ml/min/1.73 m^2	Every 12 months	Every 12 months
4 (15-29 ml/min/1.73 m^2	Every 3 months	Every 3 months
5 (<15ml/min/1.73 m^2	Every 3 months	Every month

Table 2. Recommended ranges for phosphorus, corrected calcium and PTH for different stages of CKD.
National kidney foundation. Clinical practice guidelines for bone metabolism and disease. Am J Kidney Dis 2003; 42(4 supp 3): S12

Stage of CKD	Phosphorus (mg/dl)	Corrected calcium (mg/dl)	Serum PTH (pg/ml)
3 (30-59 ml/min/1.73 m^2	2.7-4.6	Normal range	35-70
4 (15-29 ml/min/1.73 m^2	2.7-4.6	Normal range	70-110
5 (<15ml/min/1.73 m^2	3.5-5.5	8.9-9.5	150-300

Osteopenia and Osteoporosis in Beta Thalassemia Major

Dr Huma Mansoori and Dr Shabina Sikandar
Haematology

Beta thalassemia major (TM) is an inherited autosomal recessive disorder in which synthesis of beta globin chains is compromised due to mutation of beta globin gene culminating in ineffective erythropoiesis.

The anaemia manifests at 6 to 9 months after birth as haemoglobin switches from HbF to HbA with features like failure to thrive, poor feeding, recurrent infections, pallor and enlarged spleen. In untransfused patients haemoglobin level ranges from 3 to 6 gm/dl. However, regular blood transfusion and compliance with prompt iron chelation therapy has markedly improved life expectancy of thalassemics, but morbidity due to its chronic complications like osteopenia and osteoporosis remains there.

Osteopenia refers to bone mineral density (BMD) that is lower than normal peak BMD but not low enough to be classified as osteoporosis while osteoporosis is characterised by low bone mass and disruption of bone architecture, resulting in decreased bone strength with an increased risk of fracture.

The frequency of osteopenia or osteoporosis in well-treated patients of TM is approximately 40-50 per cent which clearly depicts that despite the normalisation of haemoglobin levels, adequate hormone replacement and effective iron chelation; thalassemics continue to show an unbalanced bone turnover and an increased resorption resulting in seriously diminished BMD. Bone disease in thalassemia is manifested by diffuse bone pain, spinal deformities like scoliosis, nerve compression and various degrees of osteopenia, osteoporosis and spontaneous fractures.

Factors contributing to osteoporosis and osteopenia in thalassemics include iron overload resulting in endocrinopathies; which directly or indirectly leads to decreased bone mass. There is marrow expansion due to increased erythropoiesis causing mechanical

interruption of bone formation leading to cortical thinning of bones. In addition, osteoblasts are also affected by direct iron toxicity as well as due to liver disease. Chelation therapy with desferrioxamine inhibits DNA synthesis, osteoblast and fibroblast proliferation, osteoblast precursor's differentiation and collagen formation, whereas in high doses it enhances osteoblast apoptosis.

The serum biochemistry is usually unhelpful in diagnosis of osteoporosis because the levels of calcium, phosphate and PTH are generally normal. However, markers of bone turnover such as serum alkaline phosphatase, serum osteocalcin, and N telopeptide of Type I Collagen (NTx) may be raised in osteoporosis in TM. Dual energy x-ray absorption (DXA scan) is also commonly used to assess bone mineral density but it should be kept in mind that it may fail to provide accurate and precise information on osteoporosis in thalassemics as they have spinal degenerative skeletal changes, which can be detected only by MRI and is likely to interfere with BMD values, resulting in false diagnosis of bone disease. Vitamin D deficiency can also co-exist.

Following therapeutic strategies should be undertaken to prevent and treat osteopenia/ osteoporosis in TM.

- lifestyle measures should be encouraged such as physical activity and smoking quitting.
- calcium and vitamin D intake during skeletal development increases bone mass in adult life with the final goal to prevent bone loss and fractures.
- induction of puberty at a proper age and treatment of hypogonadism with hormone replacement therapy shows quite promising results to prevent osteoporosis and other bone deformities.
- calcitonin, a potent inhibitor of osteoclasts, in combination with calcium and vitamin D has shown to decrease bone pain and radiological signs of osteoporosis.
- alendronate, pamidronate, and zoledronic acid have shown efficacy in osteoporotic patients with TM either with normal or impaired gonadal function but further research in the therapeutic trials with bisphosphonates is needed to allow definite conclusions especially in our setting where vitamin D deficiency is widely prevalent. However, they may be used as a second line treatment if the above strategies have failed.

Role of β_2 Microglobulin in Multiple Myeloma

Dr Mehreen Imran
Haematology

Multiple myeloma is a B cell malignancy characterised by monoclonal accumulation of abnormal plasma cells in the bone marrow (Fig. 1). The clinical signs and symptoms of myeloma are heterogeneous and include bone complications, symptoms of impaired formation of blood cells and hyperviscosity, renal dysfunction, infections, peripheral neuropathy and extra medullary disease.

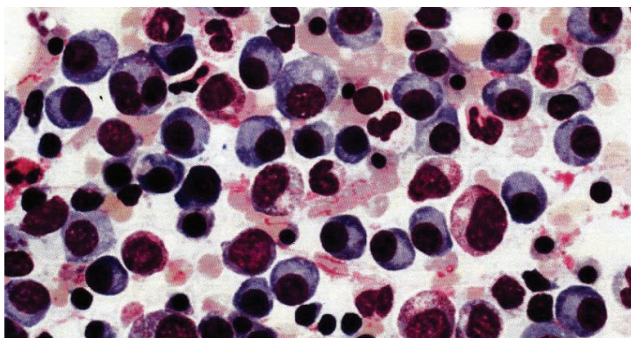


Fig. 1. Bone marrow aspirate demonstrating plasma cells of multiple myeloma

Multiple myeloma constitutes about 15 per cent of the haematological malignancy. The disease develops in one to four per 100,000 people per year. It is more common in men, and is twice as common in blacks as it is in whites. With conventional treatment, the prognosis is three to four years, which may be extended to five to seven years or longer with advanced treatments.

Diagnostic work up for myeloma is shown in Table 1. One of the laboratory parameter is β_2 microglobulin

Table 1. Laboratory and Radiological Work ups for Multiple Myeloma Available at AKUH

Laboratory Tests
Full blood count, peripheral film and ESR
Evaluation of kidney function, serum calcium, CRP, β2 microglobulin, LDH, uric acid levels and liver function tests
Protein electrophoresis and paraprotein quantification
Quantitative analysis of normal immunoglobulins, 24-hours urine collection for light chain (Bence Jones protein) excretion, coagulation screen
Bone marrow aspiration and trephine biopsy for morphology, immunophenotyping (CD138, CD79a, kappa, lambda, CD20) and cytogenetic
Radiological Tests
Complete skeletal survey
Computerised tomography (CT)

Magnetic resonance imaging (MRI)
(also known as β2M) is a component of MHC class I molecules, which are present on all white blood cells (including plasma cells). In humans, the β2 microglobulin protein is encoded by the β2 microglobulin gene (Fig. 2). Normal urinary excretion of β2 microglobulin is less than 370 micrograms per 24 hours. Elevated serum concentration in the presence of normal glomerular filtration rate suggests increased β2 microglobulin production or release, which is seen in lymphoproliferative diseases such as multiple myeloma.

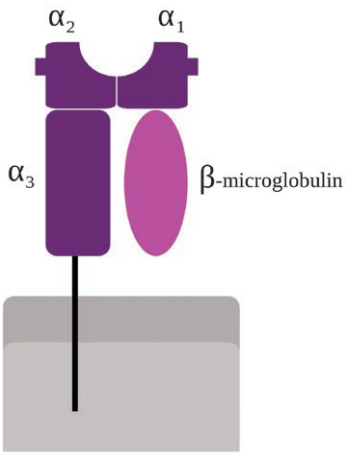


Fig. 2. Structure of MHC class I β_2 microglobulin

The increase is seen in cancers involving white blood cells, but it is more meaningful in newly diagnosed multiple myeloma patients. Increased production or destruction of plasma cells causes β2 microglobulin level in the blood to increase. Pre-treatment values of β2 microglobulin are thus useful in confirming tumor mass grade, and in assessing response to chemotherapy; marked reductions following chemotherapy correlated well with the onset of remission. The serum β2 microglobulin level is one of the prognostic factors incorporated into the International staging system. Patients with high values have inferior survival.

The following levels of β2 microglobulin correlate with stages of multiple myeloma:

Stage I multiple myeloma

- β2 microglobulin level is lower than 3.5 mg/mL
- albumin level is 3.5 g/dL or higher

Stage II multiple myeloma

- β2 microglobulin level is lower than 3.5 mg/mL and the albumin level is lower than 3.5 g/dl
- β2 microglobulin level is between 3.5 and 5.5 mg/mL

Stage III multiple myeloma

- β2 microglobulin is 5.5 mg/mL or higher.

The prognostic value of serum β2 microglobulin level in myeloma is probably due to correlation of levels with tumour burden. High levels are also associated with renal failure, which carries an unfavourable prognosis in multiple myeloma. The reference ranges are shown in Table 2.

Table 2. Reference Ranges for β_2 Microglobulin as Reported in AKU-Laboratory

		ng/mL			
Group	**Median**	**2.5%ile**	**95%ile**	**97.5%ile**	**N**
Males	1,556	604	2,157	2,284	424
Females	1,473	607	2,295	2,454	370

β2 microglobulin test is performed by a chemilumniscent assay in Clinical Laboratory AKUH. It is done on clotted blood sample of the patient; however, it can also be performed on patient's urine. Urine may be a single collection or collected throughout a 24 hour time period. The urine should be refrigerated until it is brought to the laboratory and must not become acidic.

Update on Microbiological Diagnosis of Bone and Joint Disorders

Dr Kauser Jabeen, Dr Salima Qamar
Microbiology

Prosthetic Joint Infections; How to Effectively use the Available Diagnostic Modalities?

Prosthetic joint infections (PI) are associated with substantial morbidity and costs; therefore early and accurate diagnosis is crucial. The clinical presentation may be non-specific and vary between different patient populations. Effective use of the currently available diagnostic modalities for PI results in improves outcomes. PI occurring within three months of joint replacement are categorized as early and 3-12 months after surgery as delayed and >12 months as late.

A widely accepted case definition for PI includes:

- purulence around a prosthesis at arthrotomy or arthroscopy
- presence of one or more sinus tract communicating with the joint
- histological features of infection
- isolation of similar organism from at least two deep culture samples. Isolation of virulent organisms, such as *Staphylococcus aureus*, *Escherichia coli*, or *Candida spp*., in one deep tissue sample may be considered as significant to confirm the diagnosis.

Gram positive organisms, especially Staphylococci (commensal skin organisms), are most commonly involved and in early infection, pathogens are usually more virulent (for example, Staphylococcus aureus), whereas more indolent organisms predominate later on (for example, coagulase negative staphylococci, Propionibacterium acnes) (Table 1).

Types: *PJI can either be acute, chronic or secondary to hematogenous seeding.*

Etiologies of Prosthetic Joint Infection		
Type of Infection	*Etiology*	*Time of Onset*
Early Postoperative Infection (<3 weeks)	S. aureus B-hemolytic Strep Gram-negatives	Symptoms within days to weeks of surgery
Chronic Infection (>3weeks)	Coagulase-negative Staph Corynebacteria Gram-negatives	Symptoms several months to 2 years after prosthesis placement
Hematogenous seeding	Inciting event from prior infection in other area of the body	Within days of inciting event

Laboratory Investigations

Detection of inflammatory markers:
Baseline blood tests for inflammatory markers including C reactive protein (CRP), erythrocyte sedimentation rate (ESR), leukocyte count should be performed for any suspected case. These parameters may be elevated up to two weeks after surgery and cannot be relied upon in early infection. Serial measurements of these markers are recommended and persistently or progressively high inflammatory markers most likely reflect infection. It is important to note however, that a normal result does not exclude joint infection especially if the pathogen is of low virulence.

Cultures:
Blood for culture should be taken in every case before starting antibiotics as even a single dose is known to decrease the diagnostic yield. In most cases however, it is difficult to take deep cultures before starting antibiotics in patients with systemic sepsis or rapidly evolving local infection.

Superficial swabs should not be collected as they most likely reflect colonising flora and results must therefore be interpreted with caution. Deep samples of synovial fluid and tissue taken during arthrotomy/arthroscopy, or by joint aspiration are needed for definitive diagnosis. Organisms should be identified accurately as treatment options differ for different organisms (Fig.1 and Fig.

2). Identification of similar organisms from at least three culture samples is highly predictive of infection and to increase sensitivity and specificity multiple

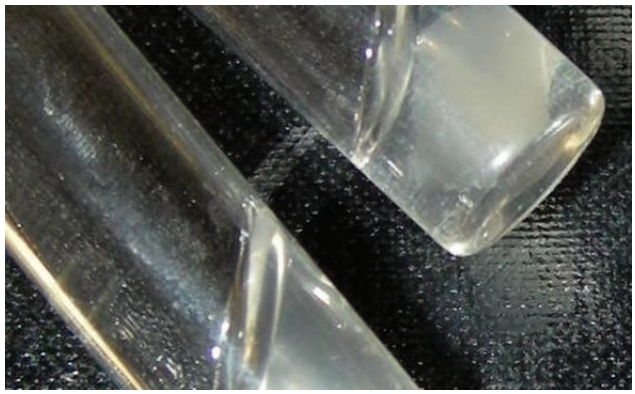


Fig. 1. Coagulase test that is required to differentiate between Staphylococcus aureus and other Staphylococcus species; two most common organisms responsible for prosthetic joint infections. Upper tube is coagulase positive confirming the identity of this organisms as Staphylococcus aureus

(five to six) samples should be sent for culture (Box 1). Organisms associated with prosthetic-joint infection usually form biofilms thus culture of prosthesis by vortexing and sonication than conventional peri-prosthetic tissue culture is more sensitive and specific especially in patients with prior antibiotic treatment. Cultures should also be held for longer duration if fungi or mycobacteria are suspected as causative organisms.

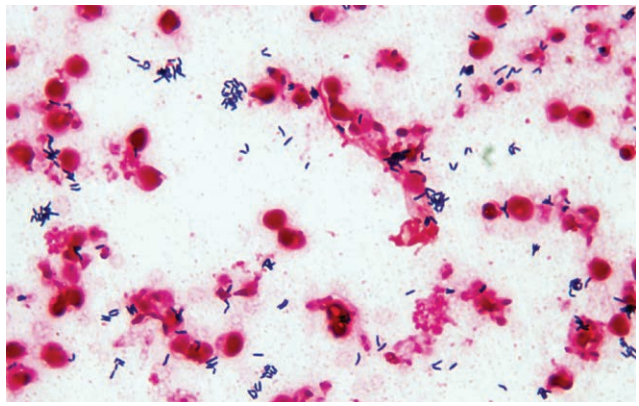


Fig. 2. Photomicrograph on the left showing Gram stain of Propionibacterium species; this organisms requires anaerobic conditions

Other diagnostic modalities:
Apart from culture histopathology is an essential adjunct to microbiology in the diagnosis of infection. An elevated white cell count and neutrophil differential of the synovial fluid are also suggestive of infection. Serial plain radiographs may help in detecting loosening or bone loss in chronic PI. Ultrasound should be performed to confirm effusion and to facilitate aseptic aspiration. Computed tomography and magnetic resonance imaging may be useful in the evaluation of complex cases, but metal inserts interfere with these tests, and abnormalities may be non-specific.

Box 1. Recommendations to improve yield of cultures for diagnosis of PJIs

- Intraoperative histopathological examination of periprosthetic tissue obtained during surgery
- At least 3 and optimally 5 or 6 periprosthetic intraoperative tissue samples or the explanted prosthesis
- Withholding antimicrobial therapy for at least 2 weeks prior to collection of samples

Bone and Joint Infections: Laboratory Diagnosis

Bone and joint infections occur as a result of haematogenous seeding, contiguous spread of infection to bone from adjacent soft tissues, or direct inoculation of infection as a result of trauma or surgery. Haematogenous spread is more common in children while contiguous spread is commoner in adults. Contiguous infection may be either polymicrobial or monomicrobial while haematogenous infection is usually monomicrobial. Staphylococcus aureus, coagulase-negative staphylococci, and aerobic gram-negative bacilli are the most common organisms; less common pathogens include streptococci, enterococci, fastidious gram negative organisms and anaerobes. Mycobacteria, Brucella spp. and fungi have also been reported in appropriate endemic and clinical settings.

Diagnosis; General Considerations

Cultures: Several factors should be considered while investigating for the diagnosis of these infections. The most important factor is specimen collection as it is extremely important to prevent contamination by normal flora. The samples should be collected as tissue or fluid rather than swabs because:

- they are more likely than tissue or fluid to be contaminated
- the amount sent for culture is often insufficient
- they may inhibit the growth of certain pathogens
- fastidious organisms survive less well in swabs than in aspirated fluid or pus
- bacteria may adhere to swabs and give a false-negative microscopy result

Ideally, culture specimens should be taken before antibiotics are commenced. In practice, however, empirical antibiotics may be commenced before culture specimens are taken.

Bone and Joint Infections: Laboratory Diagnosis

If Mycobacteria or fungi are suspected as etiologic agents; specimens should be sent for mycobacterial and fungal culture in addition to culture for routine organisms.

Histopathology: Histopathology and cytology not only provide rapid results than culture but also guide in assessing the significance of cultures. However the tissue Gram stain is an insensitive test for detecting bacteria as fixing and processing including chemical decalcification, adversely affects the Gram stain characteristics.

Nucleic acid amplification tests: There is little published information regarding the role of nucleic acid amplification based methods for skin, bone, joint, and soft-tissue infections. Therefore, routine use of nucleic acid amplification is not recommended for the diagnosis of these infections. Although these techniques may prove useful in selected cases where fastidious organisms are suspected as cause of infection.

Bone Infections

Close communication between clinician, radiologist and pathologist/microbiologist is required for optimum diagnosis. Excisional bone biopsy is required in cases in which clinical and radiographic features are not diagnostic. In all cases however, culture is essential for identification and antimicrobial susceptibility testing. For that an adequate amount of tissue should be sent to the microbiology laboratory. For chronic osteomyelitis, culture of an infected bone specimen is necessary to establish the diagnosis. Blood culture should also be sent to aid the diagnosis if the patient is septic. A positive culture precludes the need for more invasive procedures if the organism isolated from blood is a likely pathogen to cause osteomyelitis.

Cultures of superficial wounds and sinus tracts are of no value because the results do not correlate reliably with the pathogen in the underlying bone except if Staphylococcus aureus or Salmonella spp are isolated (Fig. 1).

Fig. 1. Swabs from superficial wounds and sinus tract should not be sent as the organisms isolated do not correlate with the pathogen in bone

Serial measurements of serum C-reactive protein levels and erythrocyte sedimentation rates are also commonly used to monitor response to therapy. The CRP is a more sensitive parameter than the ESR.

Joint Infections

Synovial fluid analysis is useful in establishing diagnosis and presence of pus suggest the possibility of infection (Fig. 2). Joint fluid

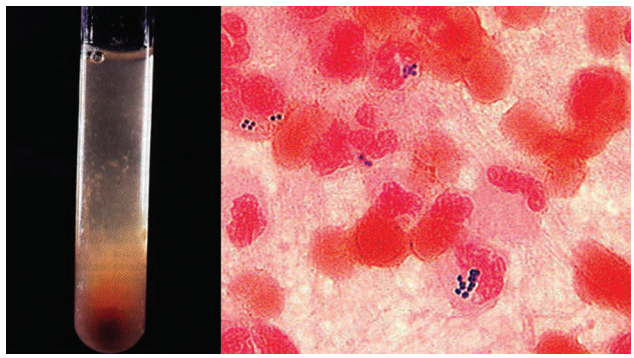


Fig. 2. Pus sample aspirated from infected knee joint highly suggestive of infection. Photomicrograph on the right is showing Gram stain of same sample revealing numerous pus cells and Gram positive cocci in clusters

should be cultured for both aerobic and anaerobic bacteria. Blood culture bottles should not be used to culture joint fluid as they have not been evaluated for yield for culturing joint fluid (Fig. 3). Fluid aspirated from joints should be collected aseptically, transferred to a sterile container (or

Fig. 3. Blood culture bottles should not be used to culture joint fluid; fluid aspirated should be transferred to a sterile container

left in the syringe), and sent immediately to the laboratory. Cultures for Neisseria gonorrhoeae should be transported immediately to the laboratory. Specimens to be submitted for mycobacterial culture do not require special handling.

Mycetoma: Microbiological Diagnosis

Mycetoma is a chronic inflammation of skin and subcutaneous tissue caused by either fungi (eumycetoma) or filamentous bacteria (actinomycetoma). This infection most commonly presents as a progressive, subcutaneous swelling with development of multiple nodules. These nodules later develop into sinuses with discharging grains (Fig. 1).

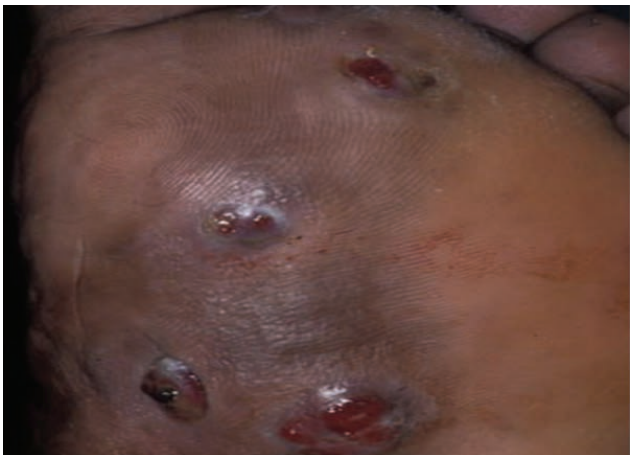


Fig. 1. Mycetoma of the foot

As the disease progresses involvement of bones and in advanced cases osteoporosis and bone destruction can occur. The organisms responsible for mycetoma are present in the soil and enter the subcutaneous tissue by traumatic inoculation. This infection is mainly seen in tropical regions and Pakistan is one of the endemic countries. Mycetoma commonly affects adults aged 20 to 40 years, predominantly males. The foot is the most commonly affected site (80 per cent of the cases).

Diagnosis

Diagnosis is usually determined clinically; however radiology is required to assess the extent of spread and involvement of bone and joints. Visual examination of colour of discharging grains is crucial for identification of possible etiologic agent (Fig. 2). In addition to that microscopic examination after crushing the grains between two slides should be performed to visualise fungal or bacterial elements (Table 1). Specimen should also be cultured for fungi and aerobic actinomycetes (Fig. 3). If granules are not present, an excisional biopsy for culture and histopathology or cytology is essential. Histopathology is also important for correlation with

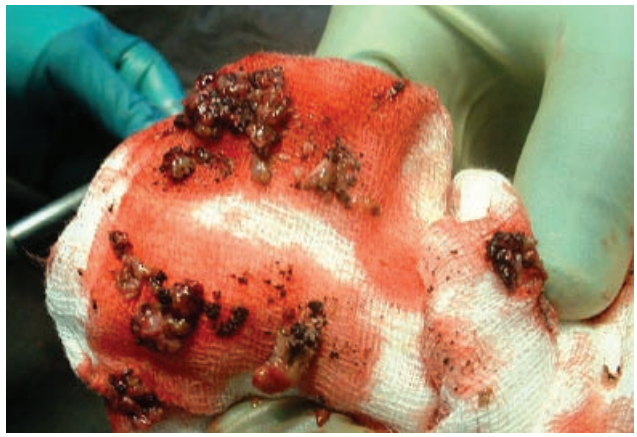
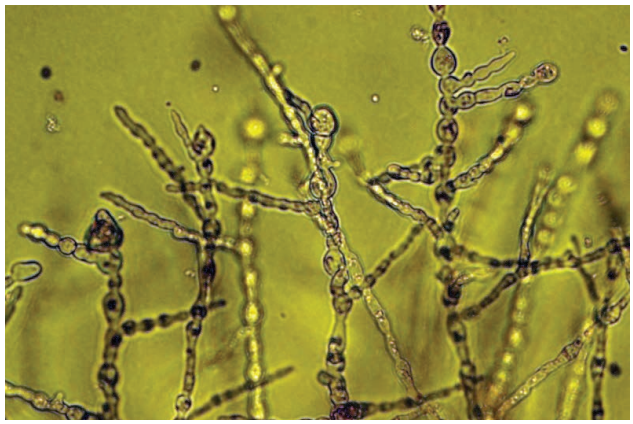


Fig. 2. Discharging grain from a case of mycetoma

cultures as the specimens may become contaminated with bacteria or fungi from skin or sinus tracts. The superficial material from sinus tracts should never be sent for culture as it will likely grow mixed flora that does not represent the actual cause of infection.



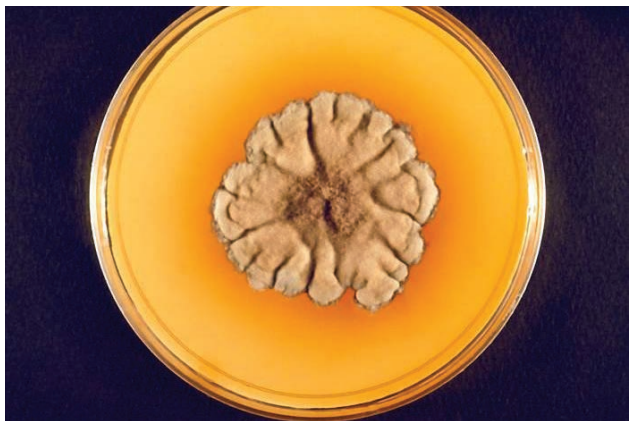


Fig. 3. Culture plate and photomicrograph showing Madurella spp.; commonest agent of eumycetoma

Table 1. Common Etiologic Agents of Mycetoma

Etiologic Agents	Colour of the Grains
Eumycetoma Species	
Madurella mycetomatis	Black
Madurella grisea	Black
Fusarium spp	White
Acremonium spp	White
Actinomycetoma Species	
Nocardia brasiliensis	White
Nocardia asteroides	White
Streptomyces somaliensis	White
Actinomadura madurae	White
Actinomadura pelletieri	Red

Microbiological Diagnosis of Diabetic Foot Infections

Dr Tazeen Fatima
Microbiology

Diabetic foot infection (DFI) is a frequent clinical problem. DFIs typically begin in a wound, most often a neuropathic ulceration or traumatic wound.
The presence of infection is defined by the presence of more than two signs of inflammation or purulence or secondary signs (Table 1). Most DFIs are polymicrobial with Staphylococcus aureus and beta hemolytic streptococci the most common causes of infection (Table 1). Gram-negative bacilli are commonly seen in chronic wounds or in those patients who have received prolonged prior antibiotic treatment. Anaerobes may be co-pathogens in ischemic or necrotic wounds.

- **Severity**

Infections are classified into mild (superficial), moderate (deeper or more extensive), or severe (accompanied by systemic signs). This classification system helps to direct the management of patient, requirement of imaging procedures, surgical interventions or amputation. Accurate and timely diagnosis and proper management is crucial to avoid unnecessary amputations, morbidity and mortality.

- **Sample collection**

Microbiological cultures play an inevitable role in management of DFIs. Whenever there is suspicion of infection, appropriately obtained specimens for culture prior to starting empiric antibiotic therapy should be sent as therapy may be for a prolonged duration. Specimen from deep tissue is obtained by curettage or scraping of tissue from the ulcer base using a dermal curette or sterile scalpel blade after the wound is properly cleansed and debrided. Aspirate and purulent secretions collected using a sterile needle can also be used. Swab specimens should be avoided as results are less accurate. Collected specimens should be immediately sent in a sterile container and appropriate transport media to microbiology lab.

- Management and follow-up

Clinically uninfected wounds do not require antibiotic therapy. Empiric antibiotic therapy should only be commenced when there is suspicion of infection: it should target the likely etiologic agent. A combined surgical and medical approach, with

Table 1. Diagnosis of Diabetic Foot Infections

Clinical Signs of Infected Diabetic Foot	
Inflammatory signs (>2) Purulent secretions Secondary signs Other signs Systemic signs of infection	Redness, warmth, swelling, tenderness, or pain Non-purulent secretions, discolored granulation tissue, undermining of wound edges, foul odor Positive probe-to-bone (PTB) test, ulceration for >30 days, recurrence, peripheral vascular disease or a previous lower limb amputation and loss of protective sensation Fever, tachycardia, hypotension, and metabolic abnormalities (acidosis, dysglycemia, electrolyte abnormalities, worsening azotemia), and deranged laboratory markers (leukocytosis, elevated ESR, CRP or procalcitonin)
Etiology (Mostly Polymicrobial)	
Gram positive	Staphylococcus aureus (most common), beta-hemolytic streptococci (groups A, F, B, C, G), enterococci, coagulase negative staphylococcus (especially with osteomyelitis), corynebacteria
Gram negative	Pseudomonas aeruginosa, Enterobacteriaceae (E.coli, Klebsiella and Enterobacter species, Proteae), Aeromonas species etc.
Anaerobes	Peptostreptococcus species, Bacteroides species (fragilis group), Prevotella species and Clostridia
Fungal (rare)	Mucoraceous (Rhizopus, Mucor, Absidia species etc.), Fusarium species

either oral or parenteral antibiotics, is recommended according to severity of the lesion. Per oral coverage for aerobic gram-positive cocci (GPC) is sufficient for one - two weeks for mild to moderate infections in patients without recent antibiotic exposure. For more severe infections, two – three weeks of broad-spectrum parenteral empiric antibiotic therapy with gram positive, anti-pseudomonal and anaerobic coverage is recommended. Definitive therapy should be based on the results of an appropriately obtained culture and sensitivity testing of a wound specimen as well as the patient's clinical response to the empiric regimen. Bony abnormalities (deformity, destruction, soft tissue gas) should be ruled out by plain radiographs or MRI scans of the affected foot. The therapy should be at least 4 weeks when there is accompanying osteomyelitis unless all infected tissue has been resected. A close follow-up of wound and limb condition is necessary to identify when re-culturing and re-debridement is required.

Classification of Tumours of Bone: an Update Based on the 2013 World Health Organization Classification of Tumors of Soft Tissue and Bone

Dr Nasir Ud Din
Histopathology

Introduction

The 4th edition of World Health Organization (WHO) Classification of Tumours of Soft Tissue and Bone was published in 2013 almost 11 years after the prior volume of 2002. Many changes have taken place in bone tumor classification during this period predominantly based on the identification of new genetic findings in different tumor types. Also, several new morphologically distinct tumor types have been described, often along with their novel genetic changes. The advances in classifying and understanding the pathogenesis of bone Tumours based on the correlation of histologic and genetic findings have been particularly significant in the field of bone oncopathology, perhaps more so than in many other areas of pathology, with the exception of hematolymphoid neoplasia. Although many interesting molecular genetic findings have been described in bone tumours, this article will focus on changes in the classification of bone

sarcomas as well as bone tumours of intermediate biologic potential (ie, locally aggressive or rarely metastasizing tumours), new molecular insights into these tumours, and associated surgical and clinical implications. The changes are reviewed according to the categorization of Tumours in the WHO volume. These changes, along with those in bone Tumours, are summarized in Table 1.

Table 1. WHO Classification of Tumours of Bone

CHONDROGENIC TUMOURS
Benign
Osteochondroma
Chondroma
  Enchondroma
  Periosteal chondroma
Osteochondromyxoma
Subungual exostosis
Bizzare parosteal osteochondromatous proliferation
Synovial chondromatosis
Intermediate (locally aggressive)
Chondromyxoid fibroma
Atypical cartilage tumour/Chondrosarcoma grade I
Intermediate (rarely metastasizing)
Chondroblastoma

Malignant
Chondrosarcoma
  Grade II, grade III
Dedifferentiated chondrosarcoma
Mesenchymal chondrosarcoma
Clear cell chondrosarcoma

OSTEOGENIC TUMOURS
Benign
Osteoma
Osteoid osteoma
Intermediate (locally aggressive)
Osteoblastoma
Malignant
Low –grade central osteosarcoma
Conventional osteosarcoma
  Chondroblastic osteosarcoma
  Fibroblastic osteosarcoma
  Osteoblastic osteosarcoma
Telangiectatic osteosarcoma
Small cell osteosarcoma
Secondary osteosarcoma
Parosteal osteosarcoma
Periosteal osteosarcoma
High grade surface osteosarcoma

FIBROGENIC TUMOURS
Intermediate (locally aggressive)
Desmoplastic fibroma of bone
Malignant
Fibrosarcoma of bone

FIBROHISTIOCYTIC TUMOURS
Benign fibrous histiocytoma/Non-ossifying fibroma

HAEMATOPOIETIC NEOPLASMS
Malignant
Plasma cell myeloma
Solitary plasmacytoma of bone
Primary non-Hodgkin lymphoma of bone

OSTEOCLASTIC GIANT CELL RICH TUMOURS
Benign
Giant cell lesion of the small bones
Intermediate (locally aggressive, rarely metastasizing)
Giant cell tumour of bone
Malignant
Malignancy in giant cell tumor of bone

NOTOCHORDAL TUMOURS
Benign
Benign notochordal tumour
Malignant
Chordoma

VASCULAR TUMOURS
Benign
Haemangioma

Intermediate (locally aggressive, rarely metastasizing)
Epithelioid hemangioma
Malignant
Epithelioid hemangioendothelioma
Angiosarcoma

MYOGENIC TUMOURS
Benign
Leiomyoma of bone
Malignant
Leiomyosarcoma of bone

LIPOGENIC TUMOURS
Benign
Lipoma of bone
Malignant
Liposarcoma of bone

TUMOURS OF UNDEFINED NEOPLASTIC NATURE
Benign
Simple bone cyst
Fibrous dysplasia
Osteofibrous dysplasia
Chondromesenchymal hamartoma
Rosai-Dorfman disease

Intermediate (locally aggressive)
Aneurysmal bone cyst
Langerhans cell histiocytosis
Erdheim-Chester disease

MISCELLANEOUS TUMOURS
Ewing sarcoma
Adamantinoma
Undifferentiated high grade pleomorphic sarcoma of bone

Chondrogenic Tumours

Osteochondromyxoma is a new addition to this category, which is a benign but locally aggressive tumour exhibiting both osteoid and chondroid production. This rare tumor arises in approximately one percent of patients with Carney complex. Sites of involvement include the tibia and sinonasal bones, and destructive growth with extension into soft tissue can occur. Disease recurrence is more likely at sites where complete resection is difficult; metastases have not been reported.

Chondrosarcoma is now separated into two groups, with grade one distinguished from cases of grade two and grade three chondrosarcoma. In addition, the synonym "atypical cartilaginous tumor" was introduced for "grade one chondrosarcoma." These Tumours are locally aggressive but metastasize only extremely rarely; the five year survival rate is 83 percent, with death from disease occurring due to uncontrollable tumor growth, especially in patients with pelvic Tumours. Curettage/simple excision alone is considered adequate treatment. For those Tumours that recur, approximately 10 percent demonstrate an increase in cellularity warranting a change in grade. In contrast, grade two and three chondrosarcomas frequently metastasize and have a five year survival rate of 53 percent; en bloc resection is recommended for this group of patients. Tumours should be graded based on the area of highest histologic grade in cases in which variable histologic grades exist within the same tumor.

Osteogenic Tumours

The only change in the classification of osteogenic Tumours is the incorporation of secondary osteosarcoma into the category of conventional osteosarcoma for descriptive purposes. Conventional osteosarcoma is subclassified based on histologic features (e.g, osteoblastic, chondroblastic), but there remains no relationship between the subtype of conventional osteosarcoma and treatment and prognosis, in contrast to other types of osteosarcoma.

Amplification of mouse double minute two homolog (MDM2) and cyclin-dependent kinase 4 (CDK4) have now been well documented in low-grade central osteosarcoma and parosteal osteosarcoma, and immunohistochemistry for these two markers can be a helpful tool, especially in those cases that have undergone dedifferentiation to a high-grade osteosarcoma and in which recognition of the precursor low-grade lesion is difficult clinically or pathologically.

Fibrogenic Tumours

The definition of "fibrosarcoma of bone" is clarified as an intermediate- to high-grade spindle cell malignant neoplasm that lacks significant pleomorphism and lacks any line of differentiation other than fibroblastic. This clarification addresses several issues. First, a fascicular or "herringbone" pattern of growth may be observed in many different tumor types that can be classified in other specific diagnostic categories. Second, this pattern of growth may also be seen in otherwise unclassified high-grade pleomorphic sarcomas, and if significant pleomorphism is present the tumor is best classified as the latter. Fibrosarcoma of bone is therefore a diagnosis of exclusion, and the diagnosis cannot be made on limited biopsy samples, because thorough sampling is needed to exclude other lines of differentiation. The incidence of fibrosarcoma is likely much less than the five percent documented in the prior WHO classification.

Fibrohistiocytic Tumours

Similar to soft tissue tumor classification, the category of "malignant fibrous histiocytoma" of bone has been removed from the 2013 classification of bone Tumours.

Ewing Sarcoma

The term PNET has been removed as a synonym for Ewing sarcoma. Of round cell sarcomas of bone that do not fulfill criteria for Ewing sarcoma, two genetically distinct groups of Tumours have been recognized that harbor CIC-DUX4 or BCOR-CCNB3 fusion genes.

Osteoclastic Giant Cell-Rich Tumours

In this category, "giant cell tumor of bone" is now separated from "giant cell lesion of the small bones." Giant cell lesion of the small bones is a very rare tumor-like lesion that arises in the small bones of the hands and feet and commonly recurs after initial curettage, but is almost always cured after the second excision. Giant cell tumor of bone is a locally aggressive neoplasm that may very rarely metastasize or undergo malignant transformation into a high-grade sarcoma, either de novo or after radiotherapy.

Notochordal Tumours

Benign notochordal cell tumor was added to this category, which previously only included chordoma.

This benign tumor may represent persistent notochord rather than a true neoplastic proliferation; benign notochordal cell tumor arises at the base of skull, vertebral bodies, and sacrococcygeal bones and is usually an incidental finding.

Vascular Tumours

Hemangioma has been separated from epithelioid hemangioma, a recently characterized neoplasm composed of small vessels lined by epithelioid endothelial cells. Epithelioid hemangioma most often arises in long tubular bones, followed by flat bones and vertebrae. In contrast to conventional hemangioma, epithelioid hemangioma is locally aggressive: recurrence occurs in approximately 10 percent of cases. Treatment consists primarily of curettage, and less often local resection. Epithelioid hemangioma should also be distinguished from epithelioid hemangioendothelioma (EHE), which is classified as a malignant neoplasm that demonstrates endothelial differentiation, and the mainstay of treatment is wide resection. The mortality rate is approximately 20 percent and histologic features do not predict the development of metastases. Recurrent fusion genes have been identified in EHE, namely WWTR1-CAMTA1 and YAP1-TFE3. These fusion genes are not present in angiosarcoma or benign vascular Tumours.

Undifferentiated High-Grade Pleomorphic Sarcoma
High-grade pleomorphic malignant Tumours that lack a specific line of differentiation are classified as “undifferentiated high-grade pleomorphic sarcoma.” This diagnosis is one of exclusion, and thorough sampling is needed to exclude osteoid deposition, which would necessitate a diagnosis of osteosarcoma, as well as other histologic features that may suggest a specific diagnosis.Tumours in this category have a metastatic rate of at least 50 percent. Treatment generally involves neoadjuvant therapy followed by wide excision for potentially resectable lesions. Similar to osteosarcoma, the degree of tumor necrosis after neoadjuvant chemotherapy is an important prognostic factor.

Conclusions

Since the publication of the prior WHO Classification of Tumours of Soft Tissue and Bone 11 years ago, new clinicopathologic and genetic features of many bone Tumours have been characterized, which has led to more reproducible classifications of these Tumours and therefore more effective treatment stratification. This has also allowed obsolete tumor types such as the so called malignant fibrous histiocytoma to be removed from the 2013 WHO classification. In addition, several new entities have been included for the first time in this volume.

Recent Developments in Benign Bone Tumours

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Histopathology

Introduction

Benign tumours of bone frequently pose a diagnostic challenge for general surgical pathologists. Careful clinical and radiological correlation is required for accurate diagnosis. Significant recent advances in some benign bone tumours have occurred at the molecular and cytogenetic level, which have provided a better understanding of the pathophysiology of certain tumours. It has also provided an important aid in the diagnostic workup and differential diagnosis of some bone lesions demonstrating overlapping clinical and pathologic features. Prognostic and therapeutic applications of these findings are the future directions. For the treatment of certain benign bone tumours, newer less invasive therapeutic techniques and medical management have been developed.

Osteoid Osteoma

Osteoid osteoma is a benign bone-forming tumour commonly arises in the cortex of diaphysis or metaphysis of long bones (femur and tibia) or posterior vertebral elements in second and third decades of life. The typical presentation is nocturnal pain relieved by nonsteroidal anti-inflammatory drugs (NSAIDs). The pain is caused by local production of high levels of prostaglandin E2, which is mediated by cyclooxygenases (COX-1 and COX-2).

Radiographically, osteoid osteoma classically presents as an area of cortical thickening and

sclerosis containing a lucent focus "nidus" of less than two cm. Histologically, the nidus consists of an interlacing network of woven bone trabeculae with variable mineralization in a loose fibrovascular stroma (Figure 1). The bone trabeculae are rimmed by osteoblasts, with scattered osteoclastic-type multinucleated giant cells.

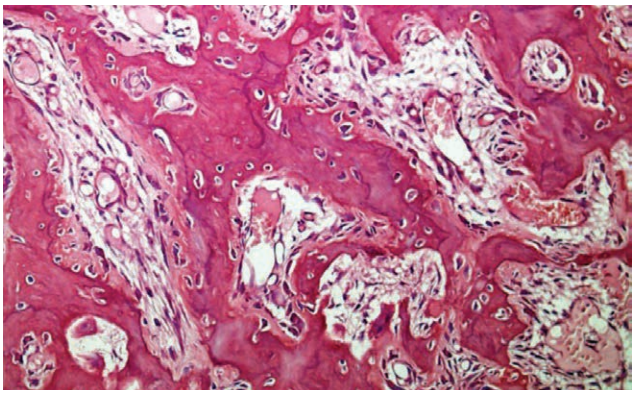

Figure 1. Osteoid osteoma exhibiting interconnecting bone trabeculae with osteoblastic rimming and loose fibrovascular stroma

Recent development in osteoid osteoma is in its treatment, to control pain. Minimally invasive techniques have been developed include CT-guided core drill excision, percutaneous radiofrequency ablation (RFA), cryoablation, or laser photocoagulation. Radiofrequency ablation has gained great popularity because of its advantages over traditional surgery. It is minimally invasive, safe, and highly effective (pain relief within 24 hour to one week after treatment). It is useful in the treatment of lesions in atypical or technically challenging locations (intra-articular, spinal, or short bone lesions). Tissue necrosis is induced by thermal coagulation through insertion of an electrode into the lesion. The main advantages of the procedure include minimal bone loss and structural weakening, less morbidity (infections), shorter anesthesia, minimal restriction of physical activity, short hospitalization and recovery time (usually a few hours), and lower cost.

Osteoblastoma

Osteoblastoma is a rare, benign bone neoplasm which is histologically and clinically similar to osteoid osteoma but has a potential for progressive growth and local recurrence. It is characterized by larger size (>2 cm) and dull pain that may not be relieved by NSAIDs. The peak incidence is in the second decade of life and it is more common in males. Osteoblastoma has predilection for the axial skeleton (posterior vertebral elements) but also occurs in the appendicular long bones and the mandible. Most cases are radiographically round to oval, well-demarcated, lytic lesions surrounded by a rim of reactive bone. Histologically, osteoblastoma is similar or identical to the nidus of an osteoid osteoma. It is composed of inter anastomosing trabeculae of woven bone in a loose fibrovascular stroma with conspicuous osteoblastic rimming and abundant osteoclasts (Figure 2). The lesion is sharply demarcated from the adjacent bone and is often surrounded by a rim of reactive bone. Osteoblastoma does not permeate cortical and host bone or invade into soft tissue.

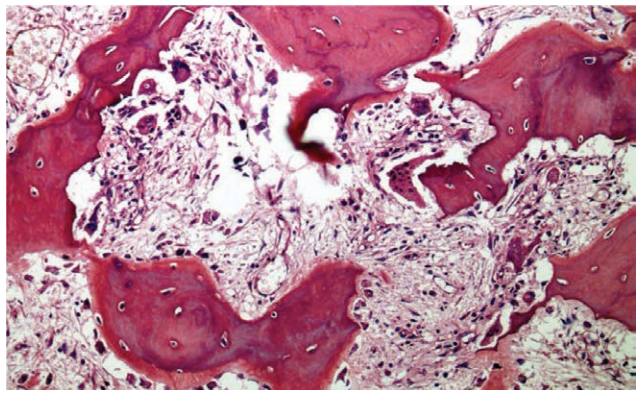

Figure 2. Osteoblastoma showing interconnecting bone trabeculae with osteoblastic rimming and loose fibrovascular stroma and few osteoclasts

A unique three-way translocation involving chromosomes 1, 2, and 14 [t(1;2;14)(q42;q13;q24)] and rearrangement of 1q42 have been reported in osteoblastoma.
An epithelioid variant of osteoblastoma has been described with a tendency to local recurrence but no metastatic potential has been described. Conventional osteoblastoma has a good prognosis with a local recurrence rate of 16 percent – 20 percent. The tumors are treated with surgical removal either by curettage or by en bloc resection.

Fibrous Dysplasia

Fibrous dysplasia (FD) is a dysplastic anomaly of bone forming mesenchymal tissue. There is focal or multifocal inability to produce lamellar bone, with an arrest at the level of woven bone. This leads to the formation of a mass of immature isolated woven bone trabeculae enmeshed in dysplastic fibrous tissue which never complete the remodeling process. Consequently, there is substantial loss of mechanical strength, pain, deformity, and pathologic fractures.

Most lesions are diagnosed before the age of 30 years. It may be monostotic or polyostotic. The monostotic form represents 60 percent or more of all patients with the disease and it is usually asymptomatic. Fibrous dysplasia may develop in

practically any bone (craniofacial bones, femur, tibia, and ribs are preferred sites). Radiologically the lesions are lytic and expansile, with thinning of the adjacent cortices and internal "ground glass" pattern of opacification caused by mineralization of the woven bone trabeculae.

Histologically, FD is composed of cytologically bland spindle cells with interspersed slender trabeculae of woven bone devoid of prominent rimming osteoblasts or osteoclasts (Figure 3).

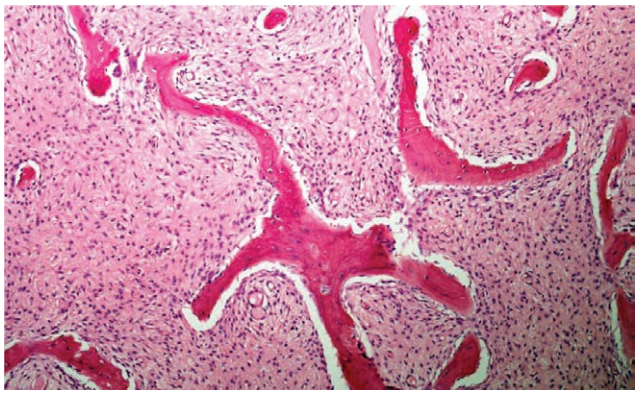

Figure 3. Fibrous dysplasia composed of irregular woven bone trabeculae with interspersed fibrous stroma

Recent developments in FD involve new insights into its pathophysiology with the description of a gene mutation and biological pathways involved. It is now regarded as a genetic, non-inheritable disease caused by missense mutations occurring post zygotically in the gene coding for the alpha subunit of the stimulatory G protein Gs, in the GNAS complex locus in chromosome 20q13 (gsp mutation). The resulting proteins display reduced GTPase activity, with increased adenylyl cyclase activation. The mutated cells constitutively generate high levels of cAMP and have a high proliferation rate. Activation of the Gs alpha/PKA/CREB pathway induces c-fos overexpression in mesenchymal precursor cells, which interferes with normal osteoblast differentiation. It has also been suggested that increased cAMP may down regulate the osteoblastic transcription factor Runx two, contributing to abnormal osteoblastic differentiation. This is the basis for the histologic hallmark of extensive proliferation of fibrous tissue produced by the abnormally differentiated preosteoblastic cells. Another downstream effect of increased cAMP is increased levels of interleukin six, which in turn may be responsible for osteoclast recruitment and activation and consequently bone resorption. It is now possible to test for the genetic mutation in peripheral blood samples by genetic amplification techniques such as PCR, which may confirm the diagnosis in difficult cases. FGF-23 can be elevated in patients with FD, leading to renal phosphate wasting and hypophosphatemia. The presence of structural alterations involving chromosomes 3, 8, 10, 12, and 15 suggests that FD may be a neoplastic condition with a predisposition to somatic mutations of bone-forming mesenchymal tissue.

Curettage with bone grafting is the traditional treatment for FD. However, there have been changes over the past decades with the introduction of medical management with bisphosphonates (mainly pamidronate), which are potent inhibitors of bone resorption and have a lasting effect on bone turnover. Radiographic improvement of the lesions, decrease in bone pain, and biochemical markers of bone remodeling have been observed with this treatment. Surgery is indicated for correction of deformities and prevention of impending fractures.

Osteochondroma

Osteochondroma is a relatively common benign bone tumour. It is more common in males in the first and second decades of life and occurs in bones formed by endochondral ossification. The metaphyses of long bones are the preferred site (distal femur, proximal tibia, and proximal humerus) but it may arise in flat bones like the ilium and scapula. Most lesions are solitary but approximately 15 percent of patients have multiple lesions. Multiple osteochondromatosis or hereditary multiple osteochondromas (HMO) is an autosomal dominant inherited condition where multiple osteochondromas develop in several bones, leading to significant deformity.

Histologically, the cap resembles an epiphyseal plate, particularly at its base. All osteochondromas usually stop growing when the parent growth plates close.

The most significant recent advances are related to the identification of the gene mutations that characterize HMO and their link to its pathophysiology. Mutations of the EXT1 (8q24.1), EXT2 (11p11-12), and the recently described EXT3 (19p) tumor suppressor genes are associated with this condition. Loss of genetic material from the long arm of chromosome eight is the distinctive cytogenetic alteration of osteochondroma. It is hypothesized that EXT mutations affect the signaling pathways of the growth plate chondrocytes. Indian hedgehog (IHH) and parathyroid hormone-like hormone (PTHLH) function in a negative feedback loop to inhibit the differentiation of proliferating chondrocytes in the growth plate. Prehypertrophic chondrocytes express

IHH, which stimulates perichondrial cells to produce PTHLH. PTHLH in turn inhibits both growth plate chondrocyte differentiation and the expression of IHH. PTHLH binds to proliferating chondrocytes, inhibiting differentiation and apoptosis through induction of Bcl-2 production.EXT1 and EXT2 are transmembrane glycoproteins involved in the synthesis of heparin sulfate proteoglycans (HSPGs). HSPGs bind to IHH ligands in the extracellular environment, which controls their diffusion. Mutations involving EXT genes lead to abnormal processing of HSPGs and their accumulation in the cytoplasm of chondrocytes. Defective HSPG production associated with EXT mutations causes a disruption in this signaling pathway, which in turn causes aberrant maturation and bone growth within the proliferating cartilage.
Treatment is reserved for symptomatic cases. Complete excision of the osteochondroma including the perichondrium is usually curative.

Malignant transformation to chondrosarcoma has been reported in 1 percent of solitary osteochondromas and 1 percent – 3 percent of multiple osteochondromas. Most cases are low-grade chondrosarcomas.

Enchondroma

Enchondroma is a benign intramedullary mass of hyaline cartilage. About 41 percent of the cases occur in the small bones of the hands and feet. It may also arise in long tubular bones (proximal humerus, proximal and distal femur), the ribs, and the spine. The metaphysis is a preferred location and the lesion is usually asymptomatic. Radiographically, enchondroma is well demarcated and radiolucent with stippled calcification. Expansion and thinning of the surrounding cortex with endosteal scalloping may be seen in the small bones.

Histologically, enchondroma is composed of lobules of hypocellular hyaline cartilage without mitotic activity or nuclear pleomorphism (Figure 4). It lacks evidence of permeation of marrow spaces or entrapment of preexisting bony trabeculae. The differential diagnosis between enchondroma and low-grade chondrosarcoma is challenging and requires careful clinical, radiological, and histopathological correlation. The treatment of enchondroma is curettage and bone grafting. Local recurrence is uncommon. Most enchondromas have a normal karyotype. Structural abnormalities of chromosomes 6 and 12 have been described. In enchondromas, PTHLH signaling is active,

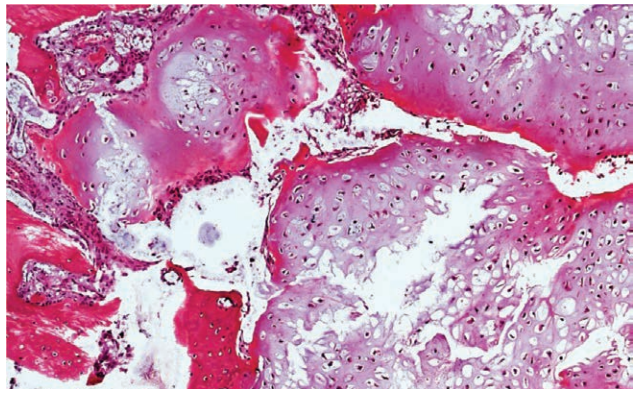

Figure 4. Enchonroma composed of lobules of hyaline cartilage encased by thin rim of reactive bone.

but independent of Indian hedgehog (IHH), irrespective of the presence or absence of absence of enchondromatosis. Heterozygous somatic mutations have been detected in at least 50 percent of solitary enchondromas and approximately 90 percent of enchondromas from patients with enchondromatosis.

Chondroblastoma

Chondroblastoma is a benign bone neoplasm with predilection for the epiphyses of the long bones in skeletally immature individuals. Most cases occur during the second decade of life. The proximal and distal femur, proximal tibia, and proximal humerus are most commonly affected. Radiographically, eccentric, well-circumscribed, lytic lesion centered in the epiphysis with partially sclerotic margins is seen. Histologically, chondroblastomas are composed of round to polygonal cells with well-defined cytoplasmic borders. The cytoplasm is clear to slightly eosinophilic and ovoid nuclei with longitudinal grooves. Randomly distributed osteoclast-type giant cells are invariably present. Linear pericellular calcification (“chicken-wire”) is characteristic (Figure 5).

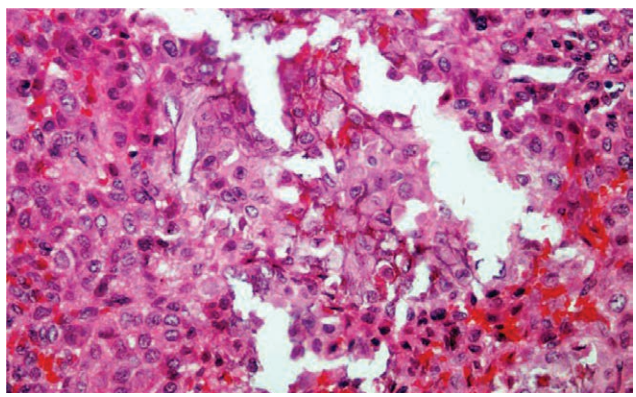

Figure 5. Chondroblastoma composed of sheets of polygonal cells with focal chicken wire calcification.

Chondroblastomas generally express S-100 protein, CK8, CK18, and CK19.
Strong expression of the cartilage lineage regulator Sox9 has been recently demonstrated.
Structural abnormalities of chromosomes 5 and 8 seem to occur frequently in chondroblastoma.

Chondromyxoid Fibroma

Chondromyxoid fibroma (CMF) is one of the least common bone tumours. The metadiaphysis of the proximal tibia and the ilium are commonly affected sites. Radiographically, it appears as an eccentric, sharply marginated lytic lesion with scalloped margins and a sclerotic bony rim. Histologically, the tumor is composed of lobules with loose myxoid stroma containing spindle- to stellate-shape cells. The lobules are separated by fibrous septa containing multinucleated osteoclast- type giant cells (Figure 6). CMF has a high expression of

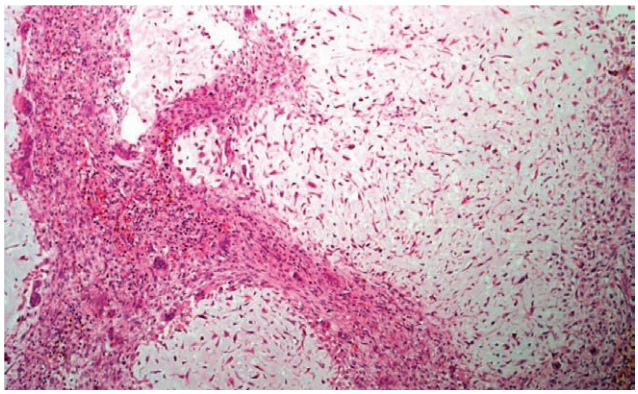

Figure 6. Chondromyxoid fibroma exhibting a lobulated architecture composed of spindle cells with peripheral osteoclast type giant cells.

certain cell cycle progression molecules and cell-to-cell adhesion molecules such as p16, cyclin D1, and ALCAM (CD166). These can be assessed by immunohistochemistry and have been suggested as an aid in distinguishing CMF from chondrosarcoma.

CMF demonstrates strong expression of the cartilage lineage regulator S0x9. Chromosome 6 aberrations is frequent but heterogenous; regions commonly involved include 6p23-25, 6q12- 15, and 6q23-27. It has been suggested that these may be helpful in distinguishing CMF from chondrosarcoma where rearrangements involving these sites of breakpoint clustering on chromosome six are uncommon. The treatment for CMF is curettage and bone grafting. Recurrence occurs in about 25 percent of cases.

Giant Cell Tumour of Bone

GCT is a benign, locally aggressive neoplasm of skeletally mature individuals (peak incidence is between ages 20 and 45 years), commonly arises in the epiphysis of long bones, most commonly the distal femur, proximal tibia, distal radius, and proximal humerus. Radiographically, GCT appears as an epiphyseal, eccentric, and expansile lytic lesion without significant marginal bony sclerosis, extending into the adjacent metaphysis. Histologically, the tumour is composed of uniformly distributed osteoclast-type giant cells in a background of mononuclear, round, polygonal, and spindle cells lacking cytologic atypia (Figure 7).

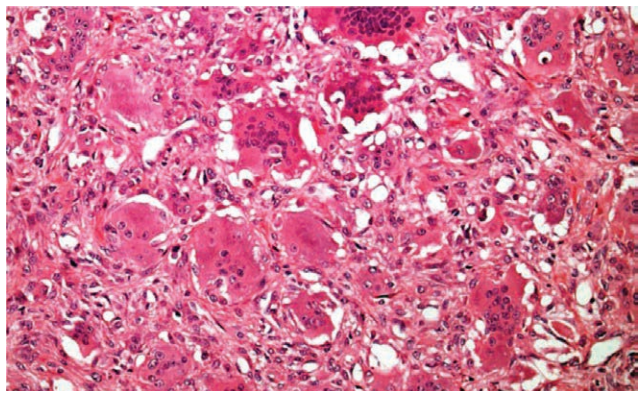

Figure 7. Giant cell tumor composed of evenly dispersed osteoclast type giant cells among mononuclear cells.

Mitoses are variably seen in the mononuclear cells and atypical forms are absent. The mononuclear cells constitute the neoplastic cell population, whereas the multinucleated giant cells are reactive. Vascular invasion may be identified in GCT and this finding does not correlate with the rate of pulmonary metastases. The tumor frequently invades and destroys the subchondral bone plate supporting the articular cartilage. The behavior of a GCT cannot be predicted from its histopathological features. Local recurrence occurs in about 20 percent of cases after curettage. Lung metastases may occur in two percent of cases and most are clinically indolent. Malignant transformation may occur at the site of a previously excised GCT or following irradiation.

Recent studies suggest that the neoplastic mononuclear cells of GCT are derived from primitive pluripotential mesenchymal stem cells and exhibit a preosteoblastic phenotype. They express collagen type I, alkaline phosphatase, osteocalcin, matrix metalloproteinases, macrophage colony-stimulating factor, osteoprotegerin, and receptor activator for nuclear factor kappa B ligand (RANK-L). The latter is a cytokine belonging to the tumor necrosis factor family (TNF-alpha) that is a potent stimulator of osteoclast formation, differentiation, and maturation. The osteoclast-like giant cells express RANK receptor and are most

likely derived from recruited monocytes under the provision of the neoplastic mononuclear cells. RANK-L expression by the neoplastic mononuclear cells of GCT is responsible for giant cell formation and bone resorption. Matrix metalloproteinases (MMP-2 and MMP-9) are also produced by the mononuclear stromal cells and may play a role in osteolysis. GCT has been reported to exhibit strong expression of TP73L gene and p63 protein in 69 percent – 100 percent of cases.P63 has been proposed as a useful marker for the diagnosis of GCT. The characteristic chromosomal aberrations seen in GCT are telomeric associations. Their significance is also unclear. Recurrent numeric or unbalanced structural abnormalities are uncommon.
Treatment of GCT is surgery and curettage with bone grafting or cementation are most commonly used. Bisphosphonates have been used as adjuvant treatment for problematic primary, recurrent, and metastatic GCT. Bisphosphonates aid in controlling the osteolysis associated with tumour growth by reducing osteoclast numbers and inhibiting osteoclastic resorption. Recent advances in the understanding of molecular cell signaling in bone remodeling and osteoclast differentiation and activation have led to new therapeutic targets, mainly anti RANK-L therapy. Denosumab is a human monoclonal antibody against RANK-L that inhibits osteoclast-mediated bone destruction. Clinical trials are underway with promising results as an adjuvant treatment to surgery or as sole treatment in inoperable cases, particularly those with pulmonary metastases.

Conclusions

The most important advances at the molecular and cytogenetic level have shed some light on the pathogenesis of certain tumours such as Gs alpha mutation in fibrous dysplasia and EXT gene mutations in osteochondroma. Clonal aberrations found in these tumours have been proposed as evidence suggestive of a neoplastic nature. In other instances, mutational analysis demonstrates specific gene rearrangements that may be useful in the differential diagnosis of lesions with clinical and pathologic similarities such as fibrous dysplasia and low-grade osteosarcoma, chondromyxoid fibroma, and chondrosarcoma or GCT and aneurysmal bone cyst. One of the most significant recent advances is the identification of the RANK/RANK-L system of cell signaling, which is the main regulatory mechanism of osteoclast formation, activation, and survival. Its role in normal bone remodeling, metabolic bone disease and tumour osteolysis has unveiled an important therapeutic target for the treatment of such conditions with antibodies directed to RANK-L. It has also helped us partially understand the molecular and cellular biology of GCT of bone. RFA and bisphosphonates are therapeutic modalities that are gaining great popularity and widespread use by reducing the invasiveness and morbidity associated with traditional surgical management and providing treatment alternatives to inoperable or advanced stage tumours.

Rheumatoid Arthritis: Anti CCP Antibodies and Rheumatoid Factor

Dr Hafsa Majid
Chemical Pathology

Rheumatoid Arthritis (RA) is the commonest inflammatory joint disease, affecting nearly one percent of the adult population worldwide and 0.9-1.98 percent of Pakistani population. Although the precise etiology of RA remains unknown, there is strong evidence for autoimmunity since several autoantibodies are associated with the disease.

It is characterized by multiple deformities and is associated with considerable morbidity and mortality. Patients with RA follow a variable disease course with regard to outcome measures; functional status or radiological assessment of joint damage. Early identification of patients with RA and, in particular, those likely to assume a more rapidly destructive form of disease, is important because of the possible benefit from early, aggressive intervention with disease modifying agents. However there is no single clinical, radiologic, or serologic test that enables a diagnosis of RA to be made with certainty. As with other autoimmune diseases, the diagnosis depends upon the aggregation of characteristic symptoms, signs, laboratory data, and radiologic findings. The main clinically useful biologic markers in patients

with RA include rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

1. Rheumatoid Factor

Rheumatoid factor is the autoantibody directed against the Fc portion of Immunoglobulin G (IgG). The RF is a well-established diagnostic and prognostic test in Rheumatoid Arthritis. High titer RF is relatively specific for the diagnosis of RA in the context of a chronic polyarthritis, and often appear many years before the onset of clinical RA. It was for decades the sole serologic criterion widely used as the diagnostic and prognostic markers of RA.
The RF is considered an early marker since its presence is linked with an increased risk of developing RA in people with mild arthritic symptoms. Rheumatoid factor may have some prognostic value with regard to disease manifestations and activity, and the severity of joint erosions. A seropositive RA (ie, RA associated with a positive rheumatoid factor test) is often associated with more aggressive joint disease, and is more commonly complicated by extra-articular manifestations than seronegative RA. RF also occurs in other diseases, including a variety of rheumatic disorders, many of which share similar features, such as symmetric polyarthritis and Non-rheumatic disorders characterized by chronic antigenic stimulation, shown in Table 1. Rheumatoid factors have been found in up to four percent of young, healthy individuals, higher incidence in elderly.

Table 1: Rheumatoid and non-Rheumatic Diseases Associated with raised RF.

Rheumatic Diseases	Non-Rheumatic Diseases
Rheumatoid arthritis — 26 to 90 %	Indolent or chronic infection e.g., Hepatitis B virus infection
Sjögren's syndrome — 75 to 95 %	Inflammatory or fibrosing pulmonary disorders, such as sarcoidosis.
Mixed connective tissue disease — 50 to 60%	Hepatitis C especially when accompanied by cryoglobulinemia, - 54 to 76% of cases
Mixed cryoglobulinemia — 40-100%	Malignancy
Systemic lupus erythematosus — 15-35%	Primary biliary cirrhosis
Polymyositis/dermatomyositis — 5-10%	-

2. Anti-CCP Antibodies

The aAti-CCP testing is a clinically useful tool in diagnosis or exclusion of RA in patients with polyarthritis. Patients with an established diagnosis of RA who have a positive test for RF, anti-CCP antibodies, or both are at a higher risk of developing erosive joint damage and functional impairment. As a result, such patients should receive anti-rheumatic therapy that suppresses disease activity early in the course of their disease. The anti-CCP testing offers certain advantages including:

- The sensitivity of anti-CCP antibodies is similar to that of RF, but specificity is high (90 to 96 percent).
- Anti-CCP antibodies predict erosive disease more effectively in RA patients than RF.
- It may also be valuable in identifying those patients with early RA who are at increased risk of progressive joint damage.
- Anti-CCP testing is a clinically useful tool in diagnosis or exclusion of RA in patients with polyarthritis. Anti-CCP may be useful in the differential diagnosis of early stage RA, particularly in the ability to distinguish RA from primary Sjögren's syndrome or SLE.
- Among patients with early oligo- or polyarthritis, anti-CCP testing appears to be of predictive value in the RF negative subgroup.
- In contrast to RF, anti-CCP antibodies are rarely present in the serum of patients with HCV infections.

3. Combination of RF and Anti-CCP Antibodies

Testing for both anti-CCP antibodies and RF may be better for excluding the diagnosis of RA than testing for either antibody alone. Those with early arthritis who are RF or anti-CCP antibody positive are at an increased risk of developing RA and erosive joint disease, while those with neither of these markers are less likely to develop joint damage. Thus, earlier intervention with disease modifying antirheumatic drug therapy may be warranted in those with positive markers, while symptomatic treatment may be appropriate for those lacking both RF and anti-CCP antibodies. Diagnoses other than RA should be considered in patients who are both RF and anti-CCP antibody negative. Unless it is demonstrated that there is an intervention that effectively and safely reduces the risk of developing RA, there is no role for screening asymptomatic individuals for either RF or anti-CCP antibodies.

Clinical Utility of Urinary Calcium

Dr Sibtain Ahmed
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Calcium (Ca) is a fundamental element necessary to form electrical gradients across membranes, an essential cofactor for many enzymes, and the main constituent in bone. Under normal physiologic conditions, the concentration of calcium in serum and in cells is tightly controlled. Calcium exists in three states in the body; bound to protein, bound to small anions, and in the free (ionized) state. The concentration of serum calcium in the ionized state is regulated by parathyroid hormone (PTH) and 1, 25 dihydroxy vitamin D.

Excretion of Calcium

Circulating calcium is excreted by glomerular filtration and reabsorbed in the proximal tubules. Calcium reabsorption in the proximal tubule is affected by tubular sodium concentration, whereas PTH induces calcium uptake in the distal tubule and the collecting duct. Excess calcium is excreted in the urine and the feces. Urine calcium levels also reflect dietary intake. In average adult urine sample collected over 24 hours, 100–300 mg of calcium excretion is considered normal. It is also important to note that calcium excretion is heavily influenced by sodium excretion. Low-sodium diets tend to decrease calcium excretion and vice versa.

Laboratory Indices for Evaluation of Calcium Excretion

Although a 24-hour collection is best, random urine calcium measurement can also be performed to calculate calcium/creatinine ratio or fractional excretion of calcium (FECa) on urine specimens.

- 24 hour Ca excretion: The reference range for urine calcium is 100-300 mg/24-hour. Hypercalciuria is >350 mg/24-hour specimen.
- Ca: Cr ratio: A normal reference interval for the urine calcium (mg/dL): urine creatinine (mg/dL) ratio is <0.14. Values exceeding 0.20 (for men) or 0.57 (for women) are found in patients with hypercalciuria.
- FECa: Simultaneously 3 - 5 cc of serum is also required for analysis of Ca and Cr.

The formula for FECa is as follows: (urine Ca × serum Cr) / (serum Ca × urine Cr) × 100

In Familial hypocalciuric hypercalcemia (FHH) the FECa results are usually ≤0.01.

Clinical Utility of Measuring Ca Excretion

The primary clinical value of urine calcium measurement is to aid in the differential diagnoses of patients and direct optimal treatment options for patients with abnormal serum calcium. Various conditions associated with abnormal urinary Calcium levels are enumerated in Table 1.

Table 1: Conditions associated with abnormal Urinary Calcium levels

Hypercalciuria Associated with Hypercalcemia
Primary Hyperparathyroidism Hypervitaminosis D Sarcoidosis Bone metastases Multiple Myeloma Corticosteroids Prolonged immobilization Paget's disease
Hypercalciuria with Normocalcemia
Increased Calcium intake Idiopathic Hypercalciuria Renal tubule acidosis X-Linked Hypercalciuria (Dent's Disease)
Hypercalciuria Associated with Hypocalcemia
Calcium-sensing receptor activating mutation
Hypocalciuria
Hypoparathyroidism Pseudo-Hypoparathyroidism Vitamin D Deficiency Low calcium diet Familial Hypocalciuric Hypercalcemia Renal Osteodystrophy
Medications Causing Hypercalcemia
Thiazide Diuretics Oral Contraceptives

HLA B-27 at a Glance

Sheeba Parveen
Molecular Pathology

HLA (Human leukocyte antigen) B-27 is class I surface antigen which is encoded by genes present on short arm of chromosome 6 (Fig.

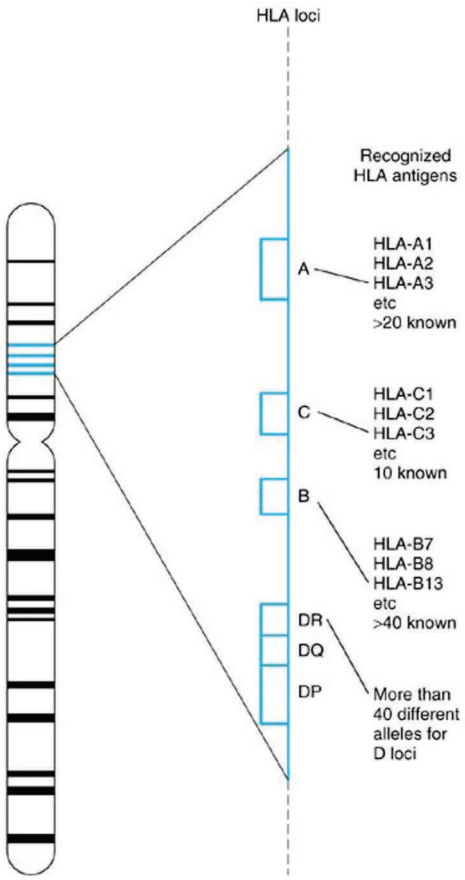

Fig.1.A. The Organization of HLA Genes on Chromosome 6

1A). The HLA genes are the human versions of MHC genes that are found in most vertebrates. The proteins encoded by certain genes are also known as antigens, as a result of their historic discovery as a factor in organ transplantation. The major HLA antigens are essential elements for immune function. Different classes have different functions.

The role of HLA B-27 molecules is presentation of peptides to T-cells. The immune system uses HLAs to distinguish the body its own proteins 'self' from the proteins made by foreign invaders such as viruses and bacteria 'non-self'. HLA B-27 is strongly associated with the condition ankylosing spondylitis, which is named due to the terms 'Ankylosing' or fusing together and 'spondylitis' or inflammation of bones of spine. This and other associated inflammatory diseases are collectively referred to as 'spondyloarthritis'. It is uncertain how HLA B-27 causes increase risk of ankylosing spondylitis.

Researchers speculates that HLA B-27 may abnormally display to immune system peptides that trigger arthritis other researchers suggests that joint inflammation characteristic of this disorder may result from improper folding of HLA B-27 protein or the presence of abnormal forms of the protein on the cell surface.

Ankylosing spondylitis is two to three times more common in males than in females, and it affects all age groups including children. The most common age of onset of symptoms is the second and third decade of life. The effect of the disease is depicted in Fig. 1B Symptoms of ankylosing spondylitis include back pain and stiffness (worst at night and

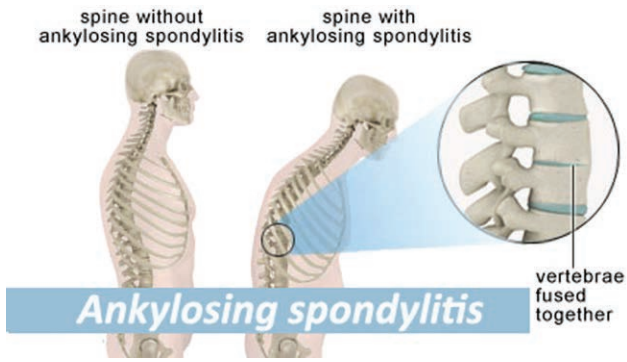

Fig.1.B. Diagrammatic Representation of the effect of Ankylosing Spondylitis on the Spine.

in morning), weight loss, feeling unwell, tiredness, pain and swelling of hips, knee or joints, plantar fasciitis (pain under heel of foot), aching in chest, around ribs. It may be associated with psoriatic, colitic and reactive arthiritis. The complications of the disease are uveitis (inflammation of eye) and issues related to heart and lungs.

For molecular diagnosis of ankylosing spondylitis a venous blood sample is required for the test. DNA is extracted from blood using the sodium dodecyl sulphate (SDS)-proteinase K lyses method and then amplified by conventional polymerase chain reaction (PCR). Using Sequence specific primer (HLA-A and HLA–B) primers. In addition, PCR for the growth hormones (GH 1 and GH 2) genes is used as an internal control. Amplified product is than analysed by agarose gel electrophoresis (Fig. 2).

A positive band of 144 kb obtained in the PCR reaction indicates the presence of the HLA-B27 allele, and based on this the result of the test is reported as a 'presence' or an 'absence' of the HLA B27 allele. An internal control is included in each PCR reaction to monitor the presence of PCR inhibitors in the specimen.

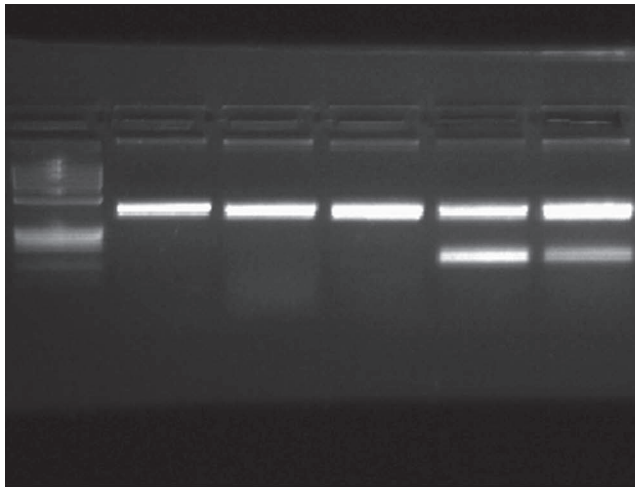

Fig. 2. Detection of the HLA B-27 all ele. The picture depicts the presence of DNA on an agarose gel stained with ethi dium bromide. Fluorescent bands indicate the presence of DNA in each lane

Clinical Utility of Serum 1,25OHD Testing

Dr Shabnam Khawaja
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Introduction

1, 25 dihydroxyvitamin D (1,25 $(OH)_2D$) is the most potent and active vitamin D metabolite. It is produced in kidney by enzymatic hydroxylation of 25-hydroxyvitamin D (25(OH) D). Plasma 1,25-dihydroxyvitamin D ($1,25(OH)_2$ D) is tightly controlled by plasma parathyroid hormone (PTH), serum calcium, serum phosphate, and fibroblast-like growth factor 23 (FGF-23)
$1, 25(OH)_2D$ alone provides essentially no information about the nutritional status of vitamin D. Consequently, 25OHD is the preferred initial test for assessing vitamin D status. Compared to 25(OH) D, $1,25(OH)_2$ D circulates in the human body at a very low concentration, making its serum levels challenging to assess. 1,25 $(OH)_2D$ is a steroid like hormone. In target cells, such as classic steroid hormones, it binds to a specific cytoplasmic vitamin D receptor (VDR); the vitamin D bound to VDR then translocates to the nucleus, where its effects are initiated at a transcriptional level. The main established actions of $1,25(OH)_2$ D collectively increase calcium in the body and modulate the skeleton. It increases the intestinal absorption of calcium and phosphate, decreases renal excretion of calcium and phosphate, suppresses PTH production, and regulates osteoblast function and bone resorption.

Clinical utility of1, 25 $(OH)_2D$ Testing

Measuring serum levels of $1,25(OH)_2$ D should be considered upon suspicion of deficiency or excess of $1,25(OH)_2$ D (Table 1).

It is a preferred test for individuals with hypercalcemia or renal failure in addition to 25(OH) D testing. In patients with hypercalcemia, both 25(OH) D and $1, 25(OH)_2D$ levels are done to rule out vitamin D intoxication with disorders that can increase $1,25(OH)_2$ D synthesis, such as lymphoma, sarcoidosis, and other granulomatous diseases.

It may also be used as a second-order test in the assessment of vitamin D status, especially in patients with renal disease and in investigation of vitamin D deficiency when suspecting vitamin D-dependent rickets due to hereditary deficiency of renal 1-alpha hydroxylase or end-organ resistance to 1,25 $(OH)_2D$.

$1,25(OH)_2$ D may be helpful in diagnosing parathyroid function disorders. A high serum level of $1,25(OH)_2$ D, for example, may suggest of primary hyperparathyroidism, whereas a normal or low serum level is more likely found in secondary hyperparathyroidism.

Serial $1,25(OH)_2$ D levels can be used in monitoring the efficacy of treatment in patients receiving $1,25(OH)_2$ D supplementation.

NOTE:

The Section of Chemical Pathology, Department of Pathology and Laboratory Medicine has initiated the testing of 1,25 $(OH)_2$D. The reference interval of1,25 $(OH)_2$D used in our laboratory is 19.9-79.3 pg/mL.

Table 1: Conditions causing high or low 1, 25-Dihydroxyvitamin D Levels

Decreased 1, 25-Dihydroxyvitamin D Levels	Increased 1,25-Dihydroxyvitamin D Levels
Chronic kidney disease	Lymphoproliferative disorders
Tumor-induced osteomalacia,	Sarcoidosis,
Use of HIV protease inhibitors	Tuberculosis
Severe 25OHD deficiency	Inflammatory bowel disease
Vitamin D–dependent rickets type 1 (inactivating mutation in the 1-hydroxylase gene)	Hereditary vitamin D-resistant rickets (mutations of vitamin D receptor coding genes)
Autosomal-dominant hypophosphatemic rickets (mutation of the gene coding for FGF-23, which prevents its breakdown)	Primary hyperparathyroidism
X-linked hypophosphatemic rickets (mutations that elevate levels of FGF-23)	
Hypoparathyroidism	

Answer to LABRAD Quiz

1.1: Hypoparathyroidism is the most common cause of hypocalcemia, which is causing symptoms in this patient. Her presentation soon after birth suggests presence of congenital hypoparathyroidism.

1.2: Serum magnesium levels: Magnesium is required for optimal activity of parathyroid gland. Levels are usually low in hypoparathyroidism.

1.3: The goal of treatment is to normalise the levels of calcium, magnesium and phosphorus. A treatment regimen typically includes replacing calcium by giving oral calcium carbonate or gluconate along with 1 alpha vitamin D.

2.1: Symptoms of bone pain and weakness can mean that patient have a coexistent vitamin D deficiency along with senile osteoporosis. For many people, the symptoms are subtle. Yet even without bone symptoms, inadequate vitamin D levels can pose other health risks

2.2: Biochemical tests to measure calcium, phosphorus, magnesium, vitamin D and parathyroid hormone and bone turnover markers can be performed. Depending upon the symptomatology, tests to screen for secondary osteoporosis can also be considered.

2.3: Bone tumor markers (BTM) are helpful in monitoring bisphosphonate therapy. Early changes in BTM measure the clinical efficacy of an anti-resorptive treatment and to reinforce patient compliance. At AKUH Clinical Laboratory, N-telopeptide of Type I collagen is performed.

Role of Clinical and Radiological Correlation in Orthopaedic Pathology

Dr Nasir Ud Din
Histopathology

Clinical and radiological information required for the pathological diagnosis of bone tumours. Histopathological assessment of a bone tumour needs to take into account the clinical background and features of the lesion, its radiological appearances and the results of relevant laboratory investigations. Diagnostic evaluation and treatment should optimally be carried out at a centre which specialises in the diagnosis and treatment of bone tumours.

Relevant clinical information should be provided on the pathology request form and its content should be recorded in the final pathology report. The age of the patient is crucial for bone tumour diagnosis as a number of bone tumours, both benign and malignant, tend to develop most commonly within a given age range (Table 1).

Table 1: Peak Age Predilection of Bone Tumors

Age (years)	Benign	Malignant
Less than 20	Nonossifying fibroma, simple bone cyst,aneurysmal bone cyst, chondroblastoma, Langerhans cell histiocytosis, osteoblastoma, osteoid osteoma, osteofibrous dysplasia, chondromyxoid fibroma, fibrous dysplasia, enchonroma	Ewing Sarcoma, osteosarcoma (conventional, periosteal, telangiectatic), Leukemia,neuroblastoma,
20-40	Enchondroma, giant cell tumor, osteoblastoma, osteoid osteoma, chondromyxoid fibroma, fibrous dysplasia	Osteosarcoma (parosteal), adamantinoma
More than 40	Fibrous dysplasia, Paget disease	Metastatic disease (most common), Chondrosarcoma,myeloma, Non-hodgkin lymphoma, Osteosarcoma (secondary)

Some tumours and tumour-like lesions have a predilection to arise in certain bones,e.g simple bone cyst occurs most often in the proximal humerus of a child or adolescent (Table 2). Most bone tumours present with bone pain and swelling. Bone pain is dull, aching and characteristically worse at night characteristically seen in osteoid osteoma. Rapid growth is characteristic of some malignant tumours but is also seen in some benign tumours and tumour-like lesions, such as aneurysmal bone cyst, eosinophilic granuloma and osteomyelitis. A history of trauma may be notable in cases where a post-traumatic lesion (eg haematoma) is a possible diagnosis. Local and systemic signs of infection need to be distinguished from those associated with the growth of a bone tumour, such as Ewing sarcoma. Information regarding a pre-existing skeletal condition should be provided, including developmental conditions where there are multiple skeletal lesions (e.g fibrous dysplasia, osteochondromatosis). It is also important to receive information on any relevant extraskeletal disease (e.g history of carcinoma) elsewhere in the body. The results of laboratory investigations which may help in evaluating a bone lesion should be communicated to the reporting pathologist. Details of the white blood cell count and erythrocyte sedimentation rate should be noted if there is a possibility that the lesion is a bone infection, eosinophilic granuloma, leukaemia or other haematological malignancy. Ewing sarcoma and 'toxic' osteoclasts may present with clinical and laboratory features that resemble osteomyelitis. If myeloma is suspected, protein electrophoresis for the identification of monoclonal immunoglobulin components in the serum or urine should be undertaken. Laboratory tests reflecting bone turnover, such as the serum calcium, phosphate and alkaline phosphatase should also be known, particularly if there is a need to exclude a metabolic cause for the development of a bone tumour, such as a "brown tumour" of hyperparathyroidism or Paget's disease. The alkaline phosphatase may also be elevated in osteosarcoma, 'blastic' metastases, fracture, polyostotic fibrous dysplasia and other conditions. The acid phosphatase may be elevated in prostate carcinoma.

Radiological information is essential for bone tumour diagnosis and it is strongly recommended that, wherever possible, the pathologist should

Table 2. Typical Locations of Bone Lesions

Location	Benign	Malignant
Epiphyseal	Chondroblastoma, Giant cell tumor,Intraosseous ganglion/ geode(associated with arthritis)	Clear cell chondrosarcoma (very rare tumor)
Metaphyseal		
Medullary	Simple bone cyst,Aneurysmal bone cyst, Enchondroma, Fibrous dysplasia,Osteomy elitis,Localized Langerhans cell histiocy-tosis, chondromyxoid fibroma	Conventional osteosarcoma,chondrosarcoma, metastasis, myeloma, lymphoma,
Cortical	Fibrous cortical defect, osteoid osteoma	Metastasis (especially lung)
Juxtacortical	Juxtacortical chondroma	Periosteal osteosarcoma, parosteal osteo-sarcoma, juxtacortical chondrosarcoma
Diaphysis		
Medullary	Fibrous dysplasia, Localized Langerhans cell histiocytosis	Ewing sarcoma, lymphoma, myeloma, Metastatic disease
Cortical	Osteofibrous dysplasia	Adamantinoma, Metastasis (especially lung)

personally view the radiological images of a bone tumour before issuing a diagnostic report. Where this is not possible, it should be recorded in the pathology report.

The precise anatomical location of a lesion in bone is important because tumours have a tendency not only to develop more commonly in certain bones but also more frequently to involve the particular anatomical region of an affected bone (Table 3). It should also be evident from the radiology whether a lesion has originated in bone or extended into it from surrounding soft tissues.

Table 3: Specific Sites of Selected Tumours

Tumor	Location
Adamantinoma	Anterior cortex of tibia
Osteofibrous dysplasia	Anterior cortex of tibia
Parosteal osteosarcoma	Posterior cortex of distal femur
Periosteal desmoid	Posterior cortex of distal femur
Osteoblastoma	Posterior elements of spine
Aneursmal bone cyst	Posterior elements of spine
Haemangioma	Vertebral bodies
Chordoma	Clivus, vertebral bodies, sacrum
Epidermal inclusion cyst	Terminal tuft of phalanx
Glomus tumour	Terminal tuft of phalanx
Simple bone cyst	Calcaneous

The matrix composition of the lesion may point to specific diagnostic possibilities (e.g calcification within cartilage tumour or ossification within a bone-forming tumour). The interface between the lesion and surrounding bone, particularly whether the lesion is well or poorly defined, should be noted as this may favour a particular benign or malignant diagnosis. A sclerotic rim is commonly present around slow growing lesions and usually points to a benign diagnosis. A non-sclerotic margin is usually found around a more rapidly growing bone lesion; malignant lesions are commonly poorly defined and have a broad zone of transition.

The pattern of bone destruction should be identified as it indicates the rate of growth of a bone lesion. A geographic pattern of bone destruction is characterized by the presence of well-circumscribed lytic areas (maximum dimension more than 1 cm) with a well-defined margin; this reflects the slow growth rate of these lesions, which are usually benign tumours (e.g non-ossifying fibroma) or locally aggressive/low-grade malignant tumours (e.g giant cell tumour of bone, low-grade chondrosarcoma). A rim of sclerosis between normal host bone and the lytic area may or may not be present. A moth-eaten pattern of bone destruction is characterized by the presence of multiple small lytic areas (usually 2-5 mm) separated by identifiable bone; this indicates an aggressive pattern of growth and is most often seen in malignant neoplasms, although it can be seen in some forms of osteomyelitis and langerhans cell histiocytosis. A permeative pattern of bone destruction is characterized by diffuse marrow involvement in which there are multiple tiny lytic areas (< 1 mm maximum dimension). This is usually accompanied by a broad zone of transition and reflects rapid growth of a bone lesion. A permeative pattern occurs in malignant tumours such as Ewing sarcoma and osteosarcoma, but can also be seen in some benign entities such as osteomyelitis and langerhans cell histiocytosis.

Radiological evidence of extension of the tumour through the bone cortex and involvement of

surrounding soft tissue should be noted as this provides evidence of a locally aggressive or malignant tumour. The nature of the periosteal reaction associated with a bone lesion oftens reflects the growth rate of the tumour. When the tumour grows slowly, the periosteum forms a thick layer of bone. Multiple layers of periosteal new bone are formed when there is a succession of fast and slow growth phases associated with the enlargement of the underlying lesion. The presence of tumour on both sides of the cortex (which is not yet destroyed) often indicates a very aggressive lesion.

The presence of multiple lesions within bone should be determined as this may suggest particular conditions such as multiple cartilage tumours (e.g enchondromatosis, multiple osteochondromas) or langerhans cell histocytosis, brown tumor, fibrous dysplasia, multiple myeloma. This feature is also useful in assessing whether a malignant tumour is more likely to be primary or secondary. With regard to primary malignant bone tumours, it may also point to a diagnosis of multifocal osteosarcoma or metastatic Ewing sarcoma.

Stop at One. Make your first break your last!

CME Seminar on 'World Osteoporosis Day'

Dr Lena Jafri
Chemical Pathology

The Metabolic Bone Disease Forum of AKU and the Department of Pathology & Laboratory Medicine, Aga Khan University in collaboration with Pakistan Society of Chemical Pathology, Pakistan Orthopedics Association and Pakistan Society of Rheumatology, organized a CME Seminar on 'World Osteoporosis Day' on Tuesday, October 20, 2015, at the Medical College, Aga Khan University, Karachi Pakistan. Renowned experts from Aga Khan University delivered talks and complicated cases related to osteoporosis were discussed by invited experts. Dr Imran Siddiqui, Associate Professor and Interim Chair, Department of Pathology & Laboratory Medicine who welcomed the participants and discussed the importance of such academic activities. He appreciated the efforts of Metabolic Bone Disease Forum in creating awareness of bone health on 'World Osteoporosis Day'.

The links between vitamin D and osteoporosis were highlighted by Dr Aysha Habib Khan, Associate Professor and Section Head of Chemical Pathology, Department of Pathology & Laboratory Medicine. She described the work of Metabolic Bone Research Group and Metabolic Bone Disease Forum which have a strong record of activity over the past years. She gave an overview of the burden of osteoporosis in Pakistan and shared findings of various studies from Aga Khan University Hospital. She shared the reality that most hospitals and clinics fail to capture the first fracture – leaving patients open

Dr Aysha Habib Khan delivering a talk on Burden of Osteoporosis in Pakistan

to a future of suffering and debility. Over 80% of fracture patients are never offered screening and/or treatment for osteoporosis, despite the fact that there are effective medications that can reduce fracture risk by as much as 30–70 %. She also emphasized on the importance of screening with appropriate laboratory investigation in the assessment of metabolic bone diseases.

Dr Maseeh uz Zaman, Associate Professor and Section Head Nuclear Medicine, Department of Radiology gave a comprehensive talk on interpretation and limitations of Dual Energy X-ray Absorptiometry (DXA) Scan. He discussed the sensitivity of DXA reporting with real life cases. He further explained how DXA findings can assist in managing osteoporosis. Additionally he talked about

FRAX, the fracture risk assessment tool a free web-based clinical scale assessing the 10-year fracture risk and need for lifestyle advice, DXA scanning or preventive treatment.

Ms. Muzamila Mughal, a Nutritional Expert, who highlighted the importance of diet, exercise and lifestyle for good musculoskeletal health, including its role in maintaining future mobility and quality of life. In line with World Osteoporosis theme 2015 'Serve up bone strength throughout life', she stressed that adequate dietary intake of calcium and vitamin D is essential for good bone health.

Management of primary osteoporosis was discussed by Dr. Masood Umar, Associate Professor and Orthopedic Surgeon. The key aspect of his discussion was a comprehensive treatment strategy for patients with osteoporosis. He identified the pros and cons of various pharmacological therapies available for osteoporosis.

Dr Lena Jafri Assistant Professor and Member Executive Council Pakistan Society of Chemical Pathology emphasized on the need to identify secondary osteoporosis in clinical set-up. She presented an outline of the approach to secondary osteoporosis. She gave an insightful talk on clinical utility of available biochemical and radiological investigations for identifying secondary osteoporosis and provided the audience with a diagnostic approach. Additionally she emphasized the importance of the need for a Pakistani fracture risk assessment tool and discussed the impediments in creating one, like lack of fracture registries and paucity of data on post- fracture mortality rates. Dr Mehmood Riaz, Assistant Professor, Department of Medicine, gave an enlightening talk on steroid induced osteoporosis. He stated that glucocorticoid-induced osteoporosis is under recognized and

One of the presenters with the Dean Medical College, Dr Farhat Abbas and Acting Chair Dept. of Pathology and Laboratory Medicine, Dr Imran Siddiqui

undertreated in an estimated one million patients who receive glucocorticoid therapy for a wide variety of medical disorders, emphasizing that glucocorticoids are the most common cause of secondary osteoporosis and non-traumatic osteonecrosis.

Real life challenging cases of osteoporosis were presented by Dr Hafsa Majid, Resident Chemical Pathology and panel of experts pitched in with their expertise on diagnosis and management. Panel of experts comprised of renowned endocrinologist. Saeed Maher, rheumatologist Dr. Saleha Ishaq, orthopaedic surgeon Dr. Amin Chinoy and Director Kiran Hospital Dr. Akhter. Many questions were elicited which made for great discussion among all. The closing remarks were delivered by Dean Medical College, Dr Farhat Abbas who appreciated the role of faculty in holding a seminar on World Osteoporosis Day and in creating awareness of an ignored disease affecting a large part of our population. The CME seminar was successful in bringing together delegates from the different disciplines. The osteoporosis seminar exposed the risk factors, highlighted the preventive tools, diagnostic caveats and management strategies of osteoporosis. The attendees were remarkably diverse and showed high level of enthusiasm. The goal of providing a forum of information exchange among all the professionals was successfully achieved.

Group picture of presenters and members of Pakistan Society of Chemical Pathology at the CME Seminar on World Osteoporosis Day at AKUH, Karachi Pakistan

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