What is the laboratory basis of diagnosing H1N1.

According to the CDC protocol a typical H1N1 test has four targets: The Influeneza A primer and probe set is designed for universal detection of type A influenza viruses. The swine Influenza A primer and probe set is designed to specifically detect all swine influenza A viruses. The swine H1 primer and probe set is designed to specifically detect swine H1 influenza. The fourth target is RNase P which is present in all human beings. This is used to determine the adequate collection and processing of the sample and should come out to be positive in all cases. In case RNase P is negative, a repeat sample should be analysed.

What is the diagnosis if Influenza A and RNase P is positive.

The diagnosis is "Positive for Influenza A"

What is the diagnosis if only RNase P is positive, all other targets are negative.

RNase P is a target which is used as a control to check the adequacy of sample collection and processing; it should come out to be positive for sample which has been processed adequately and has no inhibitors of PCR reaction. If all the other targets are negative, the sample is negative for H1N1 and Influenza A.

What is the diagnosis if only 3 out of 4 targets are detected.

A specimen is considered presumptive positive for influenza A virus if the InfA reaction is positive. If the reaction for influenza A is positive, it may also be positive for Univ Swine Influenza and/or Swine H1. A specimen is considered presumptive positive for swine influenza A/H1 if BOTH the InfA and the respective subtype (swInfA or swH1) reaction are positive.. If a specimen is positive for InfA and only one of the subtype a repeat sample is recommended.

Should I wear a face mask or respirator?

Short answer: Maybe. Face masks and respirators may very well offer extra protection, but should not be your first line of defense against either pandemic or seasonal flu. Every day, newspapers carry pictures of people wearing face masks to prevent swine flu transmission. But very little is known about whether face masks actually protect against the flu. There's a difference between a face mask and a respirator. A face mask does not seal tightly to the face. Face masks include masks labeled as surgical, dental, medical procedure, isolation, or laser masks. Respirators are N95- or higher-rated filtering face pieces that fit snugly to the face. Respirators filter out virus particles when correctly adjusted -- which is not as simple as it sounds. But it's hard to breathe through them for extended periods, and they cannot be worn by children or by people with facial hair. People who have flu-like symptoms should carry disposable tissues to cover their coughs and sneezes. When going out in public, or when sharing common spaces around the home with family members, they should put on a face mask -- if one is available and tolerable

How long does the flu virus survive on surfaces?

Flu bugs can survive for hours on surfaces. One study showed that flu viruses can live for up to 48 hours on hard, nonporous surfaces such as stainless steel and for up to 12 hours on cloth and tissues. The virus seems to survive for only minutes on your hands -- but that's plenty of time for you to transfer it to your mouth, nose, or eyes.

Can I still eat pork?

Yes. You can't get swine flu by eating pork, bacon, ham, or other foods that come from pigs. You can only get the 2009 H1N1 swine flu from another person.

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FROM THE EDITOR'S DESK

BIOCHEMICAL MARKERS of bone turnover are commonly used but underrated as tests in the management of bone disorders. Such tests do not establish the diagnosis of a disease, but rather they reflect the activity of the skeleton.

The utility of these markers in general practice is not well appreciated. In part this is because the results can vary if the tests are not appropriately done, causing frustration for some clinicians, who erroneously conclude that these markers lack utility.

In addition, there are opinions that the tests are not diagnostic, that they do not predict risk, and that they are not useful in patient management.

Wrong on all counts!

First of all, these markers were never meant to diagnose a specific bone disease. They reflect high bone activity or turnover and potential bone loss, and high levels indicate that further assessment is needed. (In much the same way, an elevated prostate-specific antigen level may or may not mean the patient has prostate cancer, but it does mean further assessment is needed.)

Second, these tests do address fracture risk, either when used alone or when combined with bone densitometry measurements. A high level of a turnover marker indicates a risk of fracture similar to that of a T score lower than −2.5, with an odds ratio in the range of 2.4 to 2.8. Moreover, if a patient has a low T score and a high marker level, his or her risk is even higher, with an odds ratio of 4.1.

Third, the argument about the tests' lack of ability to help in patient management is completely untrue, as these tests can indicate whether bone physiology is responding to antiresorptive and anabolic drug therapy: marker activity should decline with antiresorptive drugs and increase with anabolic agents.

And this occurs months to years before bone densitometry even reflects a change!

The failure of test values to respond appropriately should prompt physicians to find out why. Is the patient not taking the medicine appropriately? Or more worrisome, is he or she not taking it at all?

Bone markers may be a solution to this dilemma. Changes in a bone marker help clinicians know that the patient is properly using therapy. Moreover, these changes tell the patient that treatment is working. In my experience, relaying this type of information to the patient encourages adherence. Studies have indicated that markers do indeed help patients stay adherent to therapy and avoid fractures. Hence, these markers can indicate the risk of fracture and are useful in managing patients and promoting compliance.

In this issue, Dr Nimmi Kansal details the utility of these tests and emphasizes the need to recognize that there is a valid reason for using these tests.

Also addressed in this issue are some frequently asked questions related to swine flu testing.

Your appreciative feedback is very encouraging and we look forward to suggestions to help us improve in our endeavor to provide you the best quality information in the field of diagnostics. We look forward to your participation and continued support.

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Dr Lal PathLabs

INSIGHT

BIOCHEMICAL MARKERS OF BONE TURNOVER

With the ageing population in most countries, disorders of bone and mineral metabolism are becoming increasingly relevant to every day clinical practice. Consequently, the interest in, and the need for effective measures to be used in the screening, diagnosis and follow up of such pathologies have markedly grown. Together with clinical and imaging techniques, biochemical tests play an important role in the assessment and differential diagnosis of metabolic bone diseases. These biochemical markers are non invasive, comparatively inexpensive and when applied correctly, helpful tools in the diagnostic and therapeutic assessment of metabolic bone diseases.

Bone is constantly undergoing a metabolic process called remodeling. This includes a degradation process, bone resorption, mediated by the action of osteoclasts, and a building process, bone formation, mediated by the action of osteoclasts. Remodeling is required for the maintenance and overall health of bone and is tightly coupled; that is resorption and formation is in balance. In abnormal state of bone metabolism, this process becomes uncoupled and when resorption, exceeds formation, this results in a net loss of bone.

Osteoporosis is a metabolic bone disease characterized by abnormal bone remodeling. It is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue with a consequent increase in susceptibility of fractures. The most common type of osteoporosis occurs in postmenopausal women as a result of estrogen deficiency produced by the cessation of ovarian function. Restoration of premenopausal estrogen levels by replacement therapy prevents bone loss and osteoporosis. Estrogens and a class of compound known as bisphosphonates are antiresorptive therapies that can be used to prevent bone loss or treat osteoporosis. Osteoporosis can also result from an inadequate peak bone mass during the growing years, an age related imbalance of bone remodeling with not excess of resorption, and a number of clinical conditions and therapies that induce bone loss or bone remodeling imbalances. These include endocrine diseases such as hypogonadism, hyperthyroidism, hyperparathyroidism, and hypercorticolism; gastrointestinal diseases related to nutrition and mineral metabolism; connective tissue disease; multiple myeloma; chronic immobilization, alcoholism, and chronic therapy with heparin or corticosteroids. Other diseases characterized by bone remodeling include Paget's disease and metastasis to bone.

Bone turnover markers, are indicators of bone metabolism derived from bone matrix or bone cells. They are important diagnostic aid in the evaluation and management of osteoporosis. Bone markers allow for efficient therapy monitoring and compliance testing of osteoporotic patients. They can also help in fracture risk prediction. The international osteoporosis foundation (IOF) supports the use of bone markers with published guidelines.

OF recommendations for the use of bone markers in postmenopausal osteoporosis

1. **Therapeutic monitoring**

Which marker to choose preferentially and when to measure?

a. Type of marker :
   - Bone resorption markers: Urinary N-telopeptide (U-NTX) or Urinary C-telopeptide (U-CTX) or Serum C-telopeptide (S-CTX) for monitoring biphosphonate therapy, the same markers or Deoxypyridinoline (U-DPD) for monitoring hormone response therapy (HRT).
   - Bone formation markers: Bone alkaline phosphatase (bone ALP), osteocalcin (OC), aminoterminal propeptide (P1NP).
   - Use one marker or one formation and bone resorption marker.

b. Timing of sample :
   - Serum: morning sample (before 9.00 AM) after overnight fast.
   - Urine: Either first or second morning void with creatinine correction, after overnight fast.

c. Interval of measurement :

- Resorption markers: before starting treatment, and 3 to 6 months after treatment has been initiated.
- Formation markers: before starting treatment, and 6 months after treatment has been initiated.
- More than one measurement before starting treatment will reduce the variability of measurement.

Which cut-off to use?

- Ideally cut-off values should be based on fracture probability, but data are not yet available. Currently cut offs are based on BMD changes during treatment with HRT and alendronate. These cutoffs are consistent with least significant changes of bone marker.
- For a given marker, the decrease is more pronounced with alendronate treatment than with HRT. Thus the lower value of ranges of following cut-off applies to alendronate, upper values to HRT.
- For a 90% specificity to predict a positive bone mineral densitometry (BMD) response, cut-offs expressed as percentage decrease from baseline, are:
  - -45% to –65% for U-NTX and U-CTX
  - -35% to –55% for S-CTX
  - -20% to –30% for total or free U-DPD
  - -20% to –40% for OC & bone ALP
- For a 90% sensitivity, cut –offs are higher by approximately 20%, i.e., -25% to –45% for U-NTX and U-CTX In the case of equivocal change in bone markers, a third measurement should be performed 3 months later.

2. Prediction of fragility fractures :

- High levels of bone resorption makers are associated with approximately two fold increased risk of osteoporotic fractures.
- Resorption markers can be used in the assessment of fracture risk in selected patients in whom BMD and clinical risk factors are not sufficient to take a treatment decision.
- In a patient with osteoporosis, very high level of bone turnover markers (T> 3) is suggestive of other metabolic bone disease, including malignancy.
- Normal values are reference values established in healthy premenopausal women of 30-45 yrs of age.

[Delmas et.al (2000). The use of biochemical makers of bone turnover in osteoporosis, osteoporos Int, suppl 6:2-17]

Markers of bone formation :

Bone formation markers are products of osteoblast expressed during different phase of osteoblast development. They are considered to reflect different aspects of osteoblast function and of bone formation. All markers of bone formation are measured in serum or plasma.

1. Bone alkaline phosphotase

The activity of bone ALP is elevated with increased oseoblast activity such as in Paget's disease, high turnover osteoporosis, hyperparathyroidism, osteomalacia and rickets etc. Advantages of using the marker

- Long half life of 1 to 2 days in serum.
- Unaffected by diurnal variation
- Not affected by declining GFR, thus more useful than osteocalcin in impaired renal function.
- Highly sensitive & specific marker for diagnosis of Paget's disease.

Bone ALP is not a reliable marker in liver disease and in patients with abnormal level of 1, 25 dihydroxy vitamin D.

2. Osteocalcin

Osteocalcin is synthesized in the skeleton by osteoblasts the cells responsible for bone formation. Its synthesis is stimulated by 1,25 dihydroxyvitamin D and it is rapidly cleared by kidney, thus has short half life. It is highly sensitive & specific maker of bone formation. Its levels are increased in hyperparathyroidism, Pagets' disease, high turnover osteoporosis hyperthyroidism, renal osteodystrophy, fracture, acromegaly and are decreased in hyperparathyroidism, Growth hormone deficiency, during estrogen replacement therapy, during treatment with glucocorticoid, bisphosphonates and calcitonin. Osteocalcin may be misleading in certain situation as it may be elevated in patients of renal failure and in patients on treatment with Vitamin D.

3. Procollagen peptides

Another test for assessing bone formation or osteoblastic collagen synthesis is determination of the propeptides of type I collagen. Type I is collagen is the most abundant collagen found in bone. It is also found in other tissues such as skin, dentin, cornea, vessels etc. and tendons in bone; collagen is synthesized as pre-procollagen containing both N and C terminal extensions. These extensions or propeptides are cleared from type I collagen during fiber formation and circulate in blood, Assays are available both for the amino (N-) terminal propeptide (P1NP) and the carboxy (C-) terminal propeptide (P1CP). But clinical relevance of P1CP is still in evaluation and serum P1NP appears to be of greater diagnostic validity. From practical point of view thermostability of P1NP is an advantage in that extended transport and storage times are well tolerated without significant loss of activity. Measurement of PINP is helpful for assessing bone formation in patients treated with Vitamin D or in patients with abnormal level of these hormones where osteocalcin and bone alkaline phosphatase may be misleading.

Markers of bone resorption

Makers of bone resorption include collagen cross links such as pyridinolines and N- and C- telopeptides, acid phosphatase, urinary galactosyl hydroxylysine and urinary hydroxyproline.

1. Pyridinolines (Deoxypyridinoline (DPD) and pyridinoline (PYD)

PYD and DPD are formed during the extracellular maturation of fibrillar collagens. They bridge several collagen peptides and mechanically stabilize the collagen molecule. During bone resorption, crosslinked collagens are proteolytically broken down and these crosslinked components are released into circulation and urine. DPD is found in significant amount in bone, ligaments and aorta. DPD appears to be specific and sensitive marker of bone resorption for several reasons:

- It is formed during collagen maturation and not during biosynthesis. Thus, originates only as degeneration product of the mature matrix.
- It does not appear to be metabolized prior to excretion in urine.
- Bone is the major source of DPD.
- DPD does not appear to be absorbed from the diet.

Thus, pyridinium cross links are currently viewed as the best indices for assessing bone resorption.

2. Cross linked telopeptides of Type I collagen

The crosslinked telopeptides of type I collagen are derived from specific region of the collagen type I molecule, namely aminoterminal telopeptide (NTP) and carboxy terminal (CTP) telopeptide.

Elevated levels of pyridinolines and telopeptides have been reported in osteoporosis, Paget's disease, metastatic bone disease, primary and secondary hyperthyroidism, and other disease with increased bone resorption. When postmenopausal women are compared with premenopausal controls, telopeptides are usually increased more than other markers of resorption and formation. Response of antiresorptive therapy is generally greatest with telopeptides, intermediate with total pyridinolines and lowest with free pyridinolines.

CLINICAL UTILITY OF BONE MARKERS

Therapy monitoring

The most persuasive evidence to date for the use of bone markers is in the area of monitoring of osteoporosis treatment. The aim of the treatment is to prevent fractures. Biphosphonates, estrogens and raloxifene decrease bone resorption and bone formation markers; strontium ravelate treatment causes a mild reduction in bone resorption makers and a mild increase in bone formation markers; teriparatide (recombinant PTH) increases both bone formation and bone resorption markers. The advantage of using bone markers instead of BMD is the significant changes in bone markers can be observed at three to six months after initiation of therapy whereas changes in BMD become significant only after 18-24 months; a long time to detect treatment failure. The reduction in fracture risk following anti resorptive treatment was seen early, similar to change in bone markers, whereas the changes in BMD are gradual and later. No surprisingly the change in bone markers explains a much greater proportion of reduction in fracture risk than any change in BMD. Taken together, these data suggest bone markers are arguably better tools than BMD for monitoring antiresorptive treatment.

Fractures risk assessment

The diagnosis of osteoporosis is based on bone density scanning by virtue of WHO definition of osteoporosis and patients with a low bone density have increased risk of fracture. However, a considerable body of data indicates that bone markers predict bone loss independent of bone density; individuals with increased bone turnover marker loose bone at a faster rate than subjects with normal or low bone turnover markers. Bone turnover markers, in combination with other risk factors for osteoporotic fracture, may be used to define fracture risk and intervention thresholds. The relative fracture risk, as defined by either low BMD or an increased bone turnover marker, is similar and increased fracture is accentuated when both are present. Thus, in clinical practice, increased bone turnover makers in the presence of a low BMD would favor initiation of treatment for that patient.

To identify noncompliant patients

Bone markers react very sensitively and quickly (within days) to irregular drug taking and therefore rapidly reveal inconsistent intake of medications. Positive feedback to the patient has been shown to improve the patients' adherence to the prescribed medication. Studies have shows that up to 50% of patients stop treatment too early and medication is taken irregularly. Adherent patients were shown to suffer fewer fractures and to incur lower hospital costs.

To identify non responders to therapy

Most therapies are highly efficient as proven in many studies and true treatment failures are rare. However, there are some patients who do not respond to therapy for the following reasons:

- Malabsorption
- Alcholism
- Immobilization
- Glucocorticoid treatment
- Hyperthyroidism

Bone markers are able to identify these patients at a very early point in time to allow for appropriate measures to prevent future fractures.

Bone markers can help you to

- Monitor therapy effectiveness are early as three months after therapy initiation.
- Identify non-compliant and non persistent patients under therapy.
- Identify non responders to therapy.
- Predict fracture risk reduction and BMD changes under therapy.
- Identify people at risk of losing bone mass.

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SWINE FLU

2009 H1N1 (sometimes called "swine flu") is a new influenza virus causing illness in people. This new virus was first detected in people in the United States in April 2009. This virus is spreading from person-to-person worldwide, probably in much the same way that regular seasonal influenza viruses spread. This virus was originally referred to as "swine flu" because laboratory testing showed that many of the genes in this new virus were very similar to influenza viruses that normally occur in pigs (swine) in North America. But further study has shown that this new virus is very different from what normally circulates in North American pigs. It has two genes from flu viruses that normally circulate in pigs in Europe and Asia and bird (avian) genes and human genes. Scientists call this a "quadruple reassortant" virus.

The frequently asked questions about H1N1 are

Why is the swine flu virus called H1N1:

The type A viruses are the most virulent human pathogens among the three influenza types (A,B and C) and cause the most severe disease. The influenza A virus can be subdivided into different serotypes based on the antibody response to these viruses. Hemagglutinin (HA) and neuraminidase (NA) are the two large proteins (glycoproteins) on the outside of the influenza A viral particles. HA protein mediates binding of the virus to target cells and entry of the virus into the target cell while NA is involved in the release of progeny virus from infected cells. Influenza A viruses are classified into subtypes based on antibody responses to HA and NA. These different types of HA and NA form the basis of the H and N distinctions , for example, H5N1 or H1N1. There are 16 H and 9 N subtypes known, but only H 1, 2 and 3, and N 1 and 2 are commonly found in humans.

Dr Lal PathLabs