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Biomarkers

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(وَاصْبِرْ لِحُكْمِ رَبِّكَ فَإِنَّكَ بِأَعْيُنِنَا)

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الاهداء

الى كل من ربط الليل بالنهار وترك الدنيا واحب لقاء ربه والنصر لوطنه

الى كل يد في الحشد الشعبي والجيش المقدام

الى كل قطرة دم طاهره روت ارض الوطن لتمسح دنس الأعداء

الى ابي وامي اللذان قدما وضحا من اجلي

الى اساتذتي الذين اناروا لي درب العلم واعانوني على اكمال دراستي

الى كل هؤلاء جميعا اهدي ثمره بحثي المتواضع داعيا من الله ان ينال رضاهم

والله ولي التوفيق

Chapter One

Biomarkers

1.1 Introduction

The use of biomarkers in basic and clinical research as well as in clinical practice has become so commonplace that their presence as primary endpoints in clinical trials is now accepted almost without question. In the case of specific biomarkers that have been well characterized and repeatedly shown to correctly predict relevant clinical outcomes across a variety of treatments and populations, this use is entirely justified and appropriate.[1] In many cases, however, the "validity" of biomarkers is assumed where, in fact, it should continue to be evaluated and reevaluated. This article will consider the current conceptual status of biomarkers as clinical and diagnostic tools and as surrogate endpoints in clinical research with the goal of providing context for interpreting studies that rely heavily on such biological measures.[2]

1.2 What is a Biomarker?

The term "biomarker", "biological marker", refers to a broad subcategory of medical signs – that is, objective indications of medical state observed from outside the patient – which can be measured accurately and reproducibly. Medical signs stand in contrast to medical symptoms, which are limited to those indications of health or illness perceived by patients themselves.[3] There are several more precise definitions of biomarkers in the literature, and they fortunately overlap considerably. In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." [4] A joint venture on chemical safety, the International Programme on Chemical Safety, led by the World Health Organization (WHO) and in coordination with the United Nations and the International Labor Organization, has defined a biomarker as "any substance, structure, or process

that can be measured in the body or its products and influence or predict the incidence of outcome or disease".[3] An even broader definition takes into account not just incidence and outcome of disease, but also the effects of treatments, interventions, and even unintended environmental exposure, such as to chemicals or nutrients. In their report on the validity of biomarkers in environment risk assessment, the WHO has stated that a true definition of biomarkers includes "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction.". Examples of biomarkers include everything from pulse and blood pressure through basic chemistries to more complex laboratory tests of blood and other tissues.[5] Medical signs have a long history of use in clinical practice—as old as medical practice itself—and biomarkers are merely the most objective, quantifiable medical signs modern laboratory science allows us to measure reproducibly. The use of biomarkers, and in particular laboratory-measured biomarkers, in clinical research is somewhat newer, and the best approaches to this practice are still being developed and refined.[6] The key issue at hand is determining the relationship between any given measurable biomarker and relevant clinical endpoints.[7]

1.3 Types of Biomarkers

Biomarkers can be classified based on different parameters. They can be classified based on their characteristics such as imaging biomarkers (CT, PET, MRI) or molecular biomarkers. Molecular biomarkers can be used to refer to nonimaging biomarkers that have biophysical properties, which allow their measurements in biological samples (e.g., plasma, serum, cerebrospinal fluid, bronchoalveolar lavage, biopsy) and include nucleic acids-based biomarkers such as gene mutations or polymorphisms and quantitative gene expression analysis, peptides, proteins, lipids metabolites, and other small molecules.[8] Biomarkers can also be classified based on their application such as diagnostic biomarkers (i.e., cardiac troponin for the diagnosis of myocardial infarction), staging of disease biomarkers (i.e., brain natriuretic peptide for congestive heart failure), disease prognosis biomarkers (cancer biomarkers), and biomarkers for monitoring the clinical response to an intervention (HbAlc for antidiabetic treatment). Another category of biomarkers includes those used in decision making in early drug development. For instance, pharmacodynamic (PD) biomarkers are markers of a certain pharmacological response, which are of special interest in dose optimization studies.[9]

1.3.1 Biomarkers can be classified into the following categories [10]

1- Predisposition Biomarkers

A genetic predisposition (also called genetic susceptibility) is an increased likelihood of developing a health disorder based on the presence of a particular biomarker. For example, the presence of the BRCA1 and/or BRCA2 genes indicates that a patient has an increased susceptibility to breast cancer. Genetic testing for disease risk is often considered when someone has a personal or family history that suggests an inherited risk for a particular health condition, and when the results provide information that can help guide a person's future medical care.[11]

2- Diagnostic Biomarkers

Diagnostic biomarkers are used to confirm that a patient has a particular health disorder. For example, the presence of mutations in the CFTR gene indicate that a newborn has cystic fibrosis. A test used to diagnose a disease often measures a type of biomarker called a "surrogate." Diagnostic biomarkers may facilitate earlier detection of a disorder than can be achieved by physical examination of a patient.[12]

3- Prognostic Biomarkers

A prognostic biomarker helps indicate how a disease may develop in an individual when a disorder is already diagnosed. The presence or absence of a prognostic marker can be useful for the selection of patients for treatment but does not directly predict the response to a treatment. For example, Oncotype Dx, a diagnostic test that examines 21 genes, helps determine the likelihood that breast cancer will come back in a patient after initial treatment. This helps the doctor decide whether that patient should continue chemotherapy.[13]

4- Predictive Biomarkers

A predictive biomarker helps determine which patients are most likely to benefit from a specific treatment option. Predictive diagnostics can provide information about how well a treatment is likely to work in a particular patient or about the likelihood of that treatment causing an unwanted side effect.[14]

1.4 Biomarkers versus Clinical Endpoints

Biomarkers are by definition objective, quantifiable characteristics of biological processes. They may but do not necessarily correlate with a patient's experience and sense of wellbeing, and it is easy to imagine measurable biological characteristics that do not correspond to patients' clinical state, or whose variations are undetectable and without effect on health. It is also even easier to imagine measurable biological characteristics whose variance among populations is so great as to render them all but useless as reliable predictors of disease or its absence. In contrast, clinical endpoints are variables that reflect or characterize how a subject in a study or clinical trial “feels, functions, or survives”. They are, in other words, variables that represent a study subject's health and wellbeing from the subject's perspective.[15]

There has long been broad consensus that clinical endpoints are the primary, and to some the only relevant, endpoints of all clinical research, and ultimately of all biomedical research. The goal of clinical practice is to improve morbidity and mortality, not to change quantifiable features of patients' innate biochemistry, for instance, with no outward clinical effect. Similarly, patients seek treatment for their diseases, not for the numerical measures that frequently but not perfectly correlate with their illnesses.[1]

1.5 Biomarkers as Surrogate Endpoints

When used as outcomes in clinical trials, biomarkers are considered to be surrogate endpoints; that is, they act as surrogates or substitutes for clinically meaningful endpoints. But, not all biomarkers are surrogate endpoints, nor are they all intended to be. Surrogate endpoints are a small subset of well-characterized biomarkers with well-evaluated clinical relevance. To be considered a surrogate endpoint, there must be solid scientific evidence (e.g., epidemiological, therapeutic, and/or pathophysiological) that a biomarker consistently and accurately predicts a clinical outcome, either a benefit or harm. In this sense, a surrogate endpoint is a biomarker that can be trusted to serve as a stand-in for, but not as a replacement of, a clinical endpoint.[16][17]

Even biomarkers that are statistically validated to be surrogates for a given clinical endpoint may not actually be part of the pathophysiological pathway that results in that endpoint. In some cases, there may be evidence that the biomarkers measure a process or product of a key pathway stage, but assuming this relationship in all cases risks mistaking correlation for causation. Many possible explanations exist for biomarkers that correlate with clinical endpoints under only limited circumstances. For example, multiple interrelated disease pathways may be involved, or the biomarkers might be indirect signs of a pathway that are not fundamental to the key disease processes.[18][19]

1.6 Characteristics of the ideal biomarker

An ideal biomarker may fill one of many different roles. A biomarker may be suitable for the early diagnosis of a disease, either as part of a routine screening exam or at the first sign of a questionable symptom. A biomarker may also appear or disappear over the course of disease progression and thus be useful in determining the prognosis of a disease within an individual. Another biomarker may change as a drug therapy is started, adjusted or discontinued, ultimately aiding in the monitoring of the patient's response to that particular therapy.[20]

In all uses of biomarkers – research and development of new therapies, and diagnosis, prognosis and monitoring progression of a disease or response to treatment – the ideal biomarker should be easily obtained with minimum discomfort or risk to the patient. For example, a blood or urine sample is preferred over an invasive and risky biopsy of an internal organ.[21]

In addition, the rapid return of results for early initiation of treatment and monitoring effectiveness is highly desirable. This could be a test performed during a patient's office visit with an immediate result. Finally, a reliable biomarker will have a detection method that is both sensitive and specific and is highly reproducible among clinical laboratories.[20]

1.7 Challenges of biomarker researches

Biomarkers isolated from patient specimens may be proteins, polypeptides, lipids, hormones, metabolic intermediates, or nucleic acids and their derivatives. Each of these compounds require different collection, processing and storage procedures and conditions. Furthermore, the time between collection of the specimen and processing for long-term storage must be minimized to limit time and temperature-dependent loss of potential biomarkers. For example, temperature changes of only a few degrees can induce degradation of biomarker proteins, so most investigators and large-scale studies act conservatively by choosing ultra-low freezers (-80oC) for storing biofluids like serum, plasma, and urine. However, this option is probably the most expensive in terms of operating costs and maintenance, and replacement costs for freezers can also be high. While maintaining sample temperature is a top priority, this requires considerable investment to ensure that measures are in place to avoid transient warming cycles and to cope with potential mechanical failure. An alternative is to store samples in the vapor phase of liquid nitrogen at -150oC. Capital costs are higher, but operating costs and temperature stability can be much improved.[22][20]

1.8 Disease-related biomarkers and drug-related biomarkers

It is necessary to distinguish between disease-related and drug-related biomarkers. Disease-related biomarkers give an indication of the probable effect of treatment on patient (risk indicator or predictive biomarkers), if a disease already exists (diagnostic biomarker), or how such a disease may develop in an individual case regardless of the type of treatment (prognostic biomarker).[23] Predictive biomarkers help to assess the most likely response to a particular treatment type, while prognostic markers shows the progression of disease with or without treatment. In contrast, drug-related biomarkers indicate whether a drug will be effective in a specific patient and how the patient's body will process it.[24]

In addition to long-known parameters, such as those included and objectively measured in a blood count, there are numerous novel biomarkers used in the various medical specialties. Currently, intensive work is taking place on the discovery and development of innovative and more effective biomarkers. These "new" biomarkers have become the basis for preventive medicine, meaning medicine that recognises diseases or the risk of disease early, and takes specific countermeasures to prevent the development of disease. [25] Biomarkers are also seen as the key to personalised medicine, treatments individually tailored to specific patients for highly efficient intervention in disease processes. Often, such biomarkers indicate changes in metabolic processes.[26]

The "classic" biomarker in medicine is a laboratory parameter that the doctor can use to help make decisions in making a diagnosis and selecting a course of treatment. For example, the detection of certain autoantibodies in patient blood is a reliable biomarker for autoimmune disease, and the detection of rheumatoid factors has been an important diagnostic marker for rheumatoid arthritis (RA) for over 50 years. For the diagnosis of this autoimmune disease the antibodies against the bodies own citrullinated proteins are of particular value.

These ACPAs, (ACPA stands for Anti-citrullinated protein/peptide antibody) can be detected in the blood before the first symptoms of RA appear. They are thus highly valuable biomarkers for the early diagnosis of this autoimmune disease. In addition, they indicate if the disease threatens to be severe with serious damage to the bones and joints, which is an important tool for the doctor when providing a diagnosis and developing a treatment plan.[27][28][29]

There are also more and more indications that ACPAs can be very useful in monitoring the success of treatment for RA. This would make possible the accurate use of modern treatments with biologicals. Physicians hope to soon be able to individually tailor rheumatoid arthritis treatments for each patient.[30]

1.9 Biomarkers in Drug Development

In evaluating potential drug therapies, a biomarker may be used as a surrogate for a natural endpoint such as survival or irreversible morbidity. If a treatment alters the biomarker, which has a direct connection to improved health, the biomarker serves as a surrogate endpoint for evaluating clinical benefit. Some of the main areas in which molecular biomarkers are used in the drug development process are: early drug development studies, safety studies, proof of concept studies, and molecular profiling.[31]

Molecular biomarkers are often used in early drug development studies. Safety molecular biomarkers have been used for decades both in preclinical and clinical research. Since these tests have become mainstream tests, they have been fully automated for both animal and human testing. Among the most common safety tests are those of liver function (e.g., transaminases, bilirubin, alkaline phosphatase) and kidney function (e.g., serum creatinine, creatinine clearance, cystatin C). Others include markers of skeletal muscle(e.g., myoglobin) or cardiac muscle injury (e.g., CK-MB, troponin I or T), as well as bone biomarkers (e.g., bone-specific alkaline phosphatase).[32][8]

1.10 Potential disadvantages

Not all biomarkers should be used as surrogate endpoints to assess clinical outcomes. Biomarkers can be difficult to validate and require different levels of validation depending on their intended use. If a biomarker is to be used to measure the success of a therapeutic intervention, the biomarker should reflect a direct effect of that medicine.[1]

Chapter Two

Application of biomarkers

Bone Biomarker

2.1 Bone Markers

The field of bone turnover markers has developed considerably in the past decade. Biochemical monitoring of bone metabolism depends upon measurement of enzymes and proteins released during bone formation and of degradation products produced during bone resorption. Various biochemical markers are now available that allow a specific and sensitive assessment of the rate of bone formation and bone resorption of the skeleton. Although these markers are not recommended for use in diagnosis of osteoporosis yet, they appear to be useful for the individual monitoring of osteoporotic patients treated with antiresorptive agents[33]

The renewal of bone is responsible for bone strength throughout our life. Old bone is removed (resorption) and new bone is created (formation). During childhood and the beginning of adulthood, bone becomes larger, heavier and denser, bone formation is then more important than bone resorption. [34]

2.2 Bone biology

Bone is an active connective tissue composed of different types of bone cells. Osteoblasts are involved in the creation and mineralisation of bone; osteocytes and osteoclasts are involved in the reabsorption of bone tissue. The mineralised matrix of bone tissue has an organic component mainly of collagen and an inorganic component of bone mineral made up of various salts.[34]

2.2.1 OSTEOBLASTS

Osteoblasts are responsible for bone matrix synthesis. They secrete a collagen rich ground substance essential for later mineralization of hydroxyapatite and other crystals. The collagen actually strands to form osteoids: spiral fibers of bone matrix. Osteoblasts cause calcium salts and phosphorus to precipitate from the blood, these minerals bond with the newly formed osteoid to mineralize the bone tissue. Alkaline phosphatase is contained in osteoblasts and secreted during osteoblastic activity [35]

2.2.2 OSTEOCYTES

Osteoblasts that have been trapped in the osteoids produced by other surroundings osteoblasts are called Osteocytes. Osteocytes maintain bones, they play a role in controlling the extracellular concentration of calcium and phosphate, and are directly stimulated by calcitonin and inhibited by PTH (Parathyroid hormone). Their exact role is actually still to be defined. [36][37]

2.2.3 OSTEOCLASTS

These cells derive from bone marrow mononuclear cells. Their characteristic feature is a ruffled edge where active resorption takes place. The osteoclasts secrete bone-reabsorbing enzymes, which digest bone matrix. The mode of differentiation, recruitment and inhibition is controlled by numerous hormonal and growth factors. [38]

2.2.4 Bone matrix

Is comprised primarily of collagen, which takes the shape of chains in various sizes and dimensions. Bone matrix is created from various collagens, which are the most abundant proteins in the body. Collagen is abundant, strong and stable, which makes it ideal for creating bone as well as cartilage, skin, tendons and tissues throughout the body. [39]

2.3 The bone remodeling cycle.

The bone remodelling cycle lasts 150–200 days and is primarily mediated by osteoblastic signals which promote the differentiation and maturation of osteoclast precursors. Activated osteoclasts create resorption pits with low pH to dissolve the inorganic matrix and lysomal enzymes, such as TRAP and cathepsin K, effectively digest the exposed type-1 collagen releasing specific degradation products. Osteoblasts are attracted to this eroded surface and begin to form new osteoid. Type-1 collagen, abundant in osteoblasts, is secreted as a procollagen precursor molecule into the extracellular space where it is cleaved at the amino- and carboxy-terminals releasing pro-peptides into the blood. Initially hydroxyapatite crystals are deposited in the osteoid then a slower mineralisation process continues over several months, followed by a period of quiescence. RANKL, an essential osteoclastogenic cytokine, is expressed on the surface of osteoblasts, it binds to its cellular receptor RANK on pre-osteoclasts and promotes their differentiation and activation. OPG a decoy receptor for RANKL, is secreted by osteoblasts and other stromal derived cells and reduces bone resorption by binding to RANK and preventing osteoclastic activity.[40][41]

2.3.1 bone mineral density (BMD)

Is the amount of bone mineral in bone tissue. The concept is of mass of mineral per volume of bone (relating to density in the physics sense), although clinically it is measured by proxy according to optical density per square centimeter of bone surface upon imaging. Bone density measurement is used in clinical medicine as an indirect indicator of osteoporosis and fracture risk. It is measured by a procedure called densitometry, often performed in the radiology or nuclear medicine departments of hospitals or clinics. The measurement is painless and non-invasive and involves low radiation exposure. Measurements are most commonly made over the lumbar spine and over the upper part of the hip. The forearm may be scanned if the hip and lumbar spine are not accessible.[42][43]

2.4 Bone markers types

❖ **Formation markers [44]**

- Bone Alkaline Phosphatase (BAP)
- Osteocalcin (OC)
- Procollagen type 1 Carboxy-terminal Propeptide (P1CP)
- *Procollagen type 1 amiNo-terminal Propeptide (P1NP)
- Resorption markers Collagen derived [45]
- *Carboxy-Terminal cross-linked telopeptides of type 1 collagen (CTX)
- Carboxy-Terminal cross-linked telopeptides of type 1 collagen (ICTP or CTX-MMP)
- amiNo-Terminal cross-linked telopeptides of type 1 collagen (NTX)
- Type 1 collagen alpha 1 helicoidal peptide (HELP)
- Deoxypyridinoline (DPD)
- Pyridinoline (PYD)

❖ **Resorption markers Osteoclastic Enzymes [33]**

- Tartrate Resistant Acid Phosphatase –isoform 5b (TRAP5b)
- Cathepsin K

❖ **Osteocyte activity markers[44]**

- Receptor Activator of Nuclear factor Kappa B Ligand (RANKL)
- Osteoprotegerin (OPG)
- Dickkopf-related protein 1 (DKK1)
- Sclerostin (SCL)

*P1NP and CTX (highlighted in bold) are the markers of choice, recommended by the IOF, IFCC (2011) and NBHA (2012).

2.4.1 Preptin

Preptin, a peptide that corresponds to Asp69–Leu102 of pro- insulin-like growth factor-2 (pro-IGF-2), was identified as an additional molecule stored in secretory vesicles of pancreatic beta cells, and co-secreted with insulin and amylin. Preptin increases glucose-mediated insulin secretion.. Similar to the other betapancreatic hormones, preptin has anabolic activity in bone. In vitro studies demonstrated that preptin stimulates the proliferation of primary rat osteoblasts and osteoblast-like cell lines and reduces osteoblast apoptosis induced by serum deprivation. Preptin-induced phosphorylation of p42/p44 MAP kinases in osteoblastic cells and its proliferative effects were blocked by MAP kinase inhibitors. In human primary osteoblasts, preptin promoted proliferation and alkaline phosphatase activity through induction of p42/p44MAP kinase and CTGF .Preptin did not affect bone resorption in mouse bone marrow cultures. In contrast,a recent study that compared the effect of IGFI,IGF-II, insulin, and preptin on human bone cells ,suggested that preptin induces differentiation and activity of both osteoblasts and osteoclasts. In vivo, local administration of preptin increased bone formation and bone area in adult male mice. Indications for the anabolic effect of preptin on bone in humans arise from a number of clinical observations. A study measured preptin levels and bone metabolic markers in serum samples from male patients with osteoporosis, osteopenia, and normal bone mass. Serum preptin levels were lowest in the osteoporosis group, and were positively correlated with bone formation markers, whereas no correlation was observed with markers of bone resorption.[46][47]

2.4.2 Terminal Telopeptide

In bone physiology, the C-terminal telopeptide (or more formally, carboxyterminal collagen crosslinks, and known by the acronym CTX), is a telopeptide that can be used as a biomarker in the serum to measure the rate of bone turnover. It can be useful in assisting clinicians to determine a patient's nonsurgical treatment response as well as evaluate a patient's risk of developing complications during healing following surgical intervention. [47]

The bone resorption markers include; serum C-telopeptide cross-link of type I collagen (CTXI) is a highly sensitive indicator of bone resorption. Serum CTX-I is released from cathepsin K-mediated proteolytic degradation of α1-chain Ctelopeptide of type I collagen during osteoclastic resorption and is assessed by enzyme-linked immunosorbent assay (ELISA).[44][47]

2.4.3 The enzyme-linked immunosorbent assay (ELISA)

Is a test that uses antibodies and color change to identify a substance. ELISA is a popular format of "wet-lab" type analytic biochemistry assay that uses a solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample.[47]

2.4.4 Interpretation

C-terminal telopeptide levels may indicate the following [45][48]:

- Elevated levels of C-terminal telopeptide indicate increased bone turnover.
- Elevated levels are found in osteoporosis patients with elevated bone turnover who are at increased risk for rapid disease progression.
- Increased levels are also associated with osteopenia, Paget disease of the bone, hyperthyroidism, and hypothyroidism.
- This test can be used to monitor and assess how effective antiresorptive therapy has been in patients treated for disorders such as osteopenia, osteoporosis, and Paget disease.

This test can also serve as an adjunct means of monitoring patient response to other treatments for diseases with increased bone turnover, such as rickets, osteomalacia, and hyperthyroidism.

Using pretreatment beta-CTX levels as a baseline, adequate therapeutic response is indicated by a decrease of 25% or more 3-6 months after the initiation of therapy.

Reference ranges for males are as follows[49]:

- < 18 years - Not established
- 18-30 years - 155-873 pg/mL
- 31-50 years - 93-630 pg/mL
- 51-70 years - 35-836 pg/mL
- >70 years - Not established

The reference ranges for females are as follows:

- < 18 years - Not established
- Premenopausal - 25-573 pg/mL
- Postmenopausal - 104-1008 pg/mL

2.5 Variability in markers of bone turnover

An understanding of the source and magnitude of the absolute inter and intra-person variability, including biological, pre-analytic and analytical variation, of each marker is necessary to interpret serial measurements and individualise treatment.[50]

2.5.1 Biological variability

2.5.1.1 Intra-individual variation

Bone turnover shows a circadian rhythm, this is more obvious in the serum and urinary markers of bone resorption. βCTX for example is highest between 01:30 and 04:30 hours and may be more than twice that at the nadir between 11:00 and 15:00 hours , this may be attenuated by several factors such as; age, gender, ethnicity, menopausal status, osteoporotic stage and anti-resorptive agents or calcium supplementation , but the disparity is diminished with fasting. Osteocalcin and P1CP follow the same diurnal pattern but show only twenty percent difference and BSAP has two peaks at 14:00 and 23:30 hours with a nadir thirty percent reduced at 06:30 . Therefore timing of the sample collection and fasting status should be tightly controlled.[44]

The existence of intra-individual low-frequency biological rhythms, imply that biomarkers can also vary between consecutive days, this is more noticeable in the urinary resorption markers. There is a degree of controversy regarding seasonal variation with some researchers suggesting that overall seasonal changes are insignificant, whilst others have found a substantial wintertime increase, which may be due in part to reduced levels of vitamin D. Physical activity is also significant, TRAP and to a lesser extent BSAP and CTX are reduced immediately after plyometrics, but return to pre concentrations within two hours. Interestingly similar changes were found in PTH. Details of exercise in the previous twenty-four hours should therefore be recorded.[51]

Bone turnover varies with the menstrual cycle, research suggests that osteoblastic activity is higher during the luteal period and bone resorption is increased during the follicular phase. Pregnancy affects all BTMs due in part to the calcium requirements of the foetus, but also to changes in maternal glomerular filtration rate (GFR) affecting renal clearance. However the time change is contentious, one study following ten women at regular intervals reported an increase in urinary resorption markers throughout pregnancy with a significant increase in bone formation in the third trimester. A more recent study measured serum OPG, RANKL, osteocalcin and CTX in twenty six different women at each trimester. The study found increased bone formation in the first trimester and increased resorption in the second which surprisingly decreased again in the third trimester. Postpartum, levels gradually start to decrease but may still be higher than pre-pregnancy levels for up to a year.[52][53]

A comprehensive drug history should also be taken into account when interpreting bone marker results. Anti-resorptive drugs such as bisphosphonates and hormone replacement therapy (HRT) have a major effect on markers of bone resorption and long-term corticosteroid therapy is known to suppress bone formation.[54]

Inflammatory conditions are major precipitators for bone loss, especially rheumatoid arthritis (RA) which is further aggravated by decreased functional activity and the use of glucocorticoids. In a prior study, we found that B-cell depletion increases bone formation and decreases bone resorption in RA patients. This may be a direct effect on osteoblasts and osteoclasts respectively and be at least partially explained by the decreased inflammation and disease activity.[55] In diabetes serum osteocalcin is negatively correlated with glucose levels and advanced glycation end products (AGEs) are known to have a negative impact on bone. Thyroid disorders such as thyrotoxicosis are well known to affect bone turnover. Thyroid stimulating hormone (TSH) receptors are present in both osteoblasts and osteoclasts and the low TSH levels observed in thyroidectomised patients on L-thyroxine are associated with an increase in OPG and decrease in RANKL and are significantly correlated with vertebral fractures. Bone markers are cleared through the liver or kidneys and are also influenced by diseases affecting these systems, decreased GFR for example will decrease the urinary excretion of CTX and therefore increase serum levels. They are also affected by any disease states leading to increased periods of bed rest and immobility.[56][57]

2.5.1.2 Inter-individual variation

Between person variability is much harder to control, e.g. age, gender, and menopausal status, but is equally important to validate results. Bone metabolism rates are higher in infants up to three years of age, they are relatively stable throughout adolescence but sex-specific increases in bone marker levels are evident during the pubertal growth spurt and are reportedly influenced by pubertal stage rather than age. BTMs are higher in men between twenty and thirty years of age then reach their lowest levels during their fifties, whereas in females there is a substantial increase in bone turnover corresponding to oestrogen deficiency during the menopause.[44] Given the large observed differences observed between genders, different ages and developmental stages means that care must be taken when comparing populations and in the design of research studies.[58]

2.5.2 Analytical variability

2.5.2.1 Technical variation

Over the last decade many of the traditional BTM immunoassays have been automated, improving technical performance and increasing their availability. Nevertheless, analytical aspects such as within and between batch precision, accuracy and standardisation, remain problematic. Inter-laboratory variation is also crucial; a European study in 2001, measuring pooled samples of serum and urine in seventy-three laboratories concluded that even with identical assays results for the majority of the markers were significantly different. Similarly an American study in 2010 comparing six commercial laboratories over an eight month period concluded that reproducibility varied substantially for urine NTX and serum BSAP. Moreover there is an extensive list of bone markers being offered making it very difficult to compare research evidence. Consequently, the International Osteoporosis Foundation (IOF), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the National Bone Health Alliance (NBHA), have recommended that a marker of bone formation and resorption, namely P1NP and CTX, are used as reference analytes in clinical studies. They go on to stipulate that these markers should be measured by standardised assays to minimise immunochemical heterogeneity and recommend that manufacturers adopt international reference standards and minimise batch to batch variability.[44]

2.5.2.2 Sample stability

Appropriate control of sample collection and preparation is vital for successful BTM measurement. Several BTMs, especially osteocalcin and TRAP5b, are sensitive to thermo degradation and levels can be significantly reduced after only a few hours at room temperature. TRAP5b activity is also reduced during storage, samples must be kept at −70°C or lower and multiple freeze-thaw cycles should be avoided. No significant decrease has been detected in CTX stored at −20°C or lower for up to three years, nevertheless it rapidly decreases in serum at both 4°C and 37°C. The molecular mechanism is unknown but this decrease is minimised by ethylenediaminetetraacetic acid (EDTA). CTX is reportedly stable in EDTA blood tubes before separation for up to forty-eight hours, likewise osteocalcin becomes stable for up to eight hours at room temperature. Consequently blood should be collected into EDTA tubes and separated as soon as possible, if samples are not analysed immediately they should be stored at −20°C or lower. Both P1NP and BSAP were found to be stable in any sample type. Notably current TRAP5b assays are not affected by haemolysis, but erythrocytes are known to contain proteases which degrade osteocalcin. Grossly haemolysed samples in general should always be avoided.59

2.5.2.3 Clinical usefulness of bone turnover markers in osteoporosis

BTMs are frequently used in clinical trials and provide valuable information on the efficacy of osteoporotic treatments, but their predictive value is limited by their large biological variation and diagnostically they are less often used for individualized patient care. Other routine laboratory investigations are frequently used to identify or exclude secondary causes of osteoporosis such as hyperparathyroidism, vitamin D status, thyrotoxicosis and hypogonadism.[60]

2.6 Treatment selection and monitoring

BMD and BTMs are independent predictors of fracture risk, recent evidence does not support the use of BTMs to select the optimal treatment, but BTMs can be used to monitor treatment efficacy before BMD changes can be evaluated. Additionally early changes in BTMs can be used to measure the clinical efficacy of an anti-resorptive treatment and to reinforce patient compliance. The effectiveness of osteoporotic therapy can be assessed by serial BMD measurements usually by DXA, but quantifiable changes in bone mass are small and are only apparent after twelve to twenty-four months, furthermore they only measure net balance in a very small portion of the skeleton. DXA reproducibility is also affected by machine and operator error plus patient variability (weight or degenerative changes)[61]

Meta-regression analysis has found no evidence of a relationship between BMD changes and reduction in risk of fractures among patients receiving calcium with or without vitamin D supplementation. Calcium and/or Vitamin D may reduce fracture rates through a mechanism independent of bone density. Osteoporosis treatments such as bisphosphonates, strontium ranelate, denosumab, hormone replacement therapy (HRT) and selective estrogen receptor moderators (SERMs) act by reducing BTM levels by forty to sixty percent within three to six months. Thus one use of BTMs is to give an early indication of the success of the treatment. Baseline measurements can be repeated at the next follow up appointment say three to six months later. In the meantime the change in BTM supplies reassurance to the clinician and can be used to encourage the patient. Unfortunately, as BTMs are highly variable this is at best only an indication.[62]

There has been considerable discussion about how long to treat with bisphosphonates, because these drugs accumulate in the skeleton, leading to a reservoir that continues to be released for months or years after treatment has stopped. These medications also result in a low bone turnover state over time with both resorption and formation reduced.. The BTMs should be used in conjunction with the clinical circumstances and with repeated BMD after appropriate time intervals.[63]

More recently anabolic agents such as PTH, e.g. teriparatide, have become available which stimulate osteoblastic activity. Markers of bone formation increase early after the initiation of teriparatide therapy with a delayed, but significant, increase in resorption markers. It has been proposed clinically to measure P1NP at baseline and three months post treatment a positive response is defined as a change of greater than 10 μg/L.[64]

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