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# PATHOLOGY MATTERS

Bringing you the latest news in clinical testing

## Bone Turnover Markers (BTM)

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Bone turnover markers (BTMs) released during bone remodeling reflect bone formation and bone resorption. Bone remodeling rate can be estimated by measuring BTMs in blood or urine. Bone formation markers include serum or plasma N & C-terminal propeptides: procollagen 1 amino-terminal extension peptide (P1NP) & procollagen I carboxy-terminal propeptide (P1CP) from type 1 collagen biosynthesis and products of active osteoblasts which are bone-alkaline phosphatase and osteocalcin. Bone resorption markers include degradation products of bone type 1 collagen (plasma C-terminal cross-linking telopeptides CTX and urine N-terminal cross-linking telopeptides NTX) and serum tartrate resistant acid phosphatase 5b (TRACP5b), an osteoclast enzyme that reflects osteoclast number.

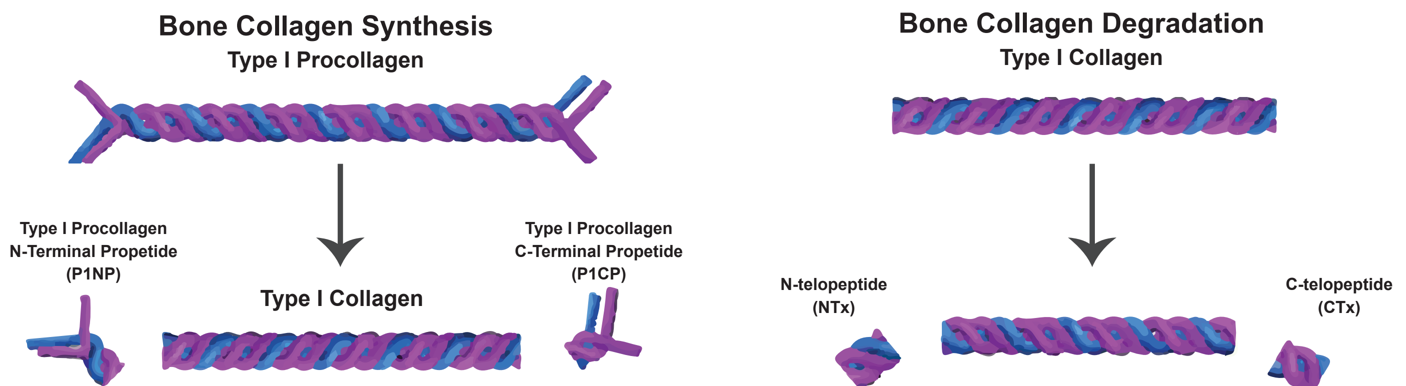


Figure 1: Pathways of bone collagen synthesis and degradation[1].

Advantages of BTMs include easily collected blood and urine samples, relative specificity for bone resorption or bone formation and providing complementary information to bone mineral density (BMD). However, BTMs are not site-specific i.e., reflect total skeletal turnover, and not always specific to bone metabolism, affected by renal function and lack assay standardisation. Hence, serial monitoring requires use of the same laboratory as cut-offs are assay specific. The International Osteoporosis Foundation and International Federation of Clinical Chemistry Working Group on Bone Standards, the National Bone Health Alliance, and the Asia-Pacific group have endorsed serum P1NP and CTX, also known as Beta Crosslaps, markers of bone formation and bone resorption respectively, as reference analytes for BTMs in clinical studies.

CTX/Beta Crosslaps has a large circadian variation, with a peak in the early hours of the morning and nadir in the middle of the day. This is in part mediated by food intake which decreases CTX. Therefore, sample collection for CTX is recommended to be in the fasting state in the morning at a well-defined time to minimise the within-individual variation. Exercise, oral contraceptive use and phase of menstrual cycle among premenopausal women also have an influence on CTX levels. The preferred sample is EDTA plasma. P1NP has little diurnal variation, is not affected by food intake and is stable at room temperature; both serum and plasma are suitable for its

measurement. The inter-individual variation, however, is significant. Hence, the use of individual baseline P1NP values acquired pre-treatment is more relevant than population-based reference intervals when monitoring treatment. Reporting units have been standardised: µg/L for P1NP and pg/ml for CTX in order to enable values to be reported in whole numbers. BTM concentrations are age dependent.

Currently, two assays for CTX measurement by ELISA method and automated immunoassay are available: Beta-CrossLaps Roche Elecsys (ECLIA, Roche Diagnostics, Mannheim, Germany) and CTX-1 (CrossLaps) IDS-iSYS (CLIA, Immunodiagnostic Systems, Tyne and Wear, UK). The reference intervals for the two automated methods for CTX cannot be used interchangeably as there is a significant proportional bias between the two methods. Currently available P1NP automated immunoassays include the total P1NP (trimeric molecule and monomer) and the intact P1NP (trimeric form only) assays measured on Elecsys (Roche Diagnostics) and IDS-iSYS (Immunodiagnostic Systems), respectively. A radioimmunoassay is also available for intact P1NP (UniQ P1NP RIA Orion Diagnostica, Epsoo, Finland). The two automated immunoassays give similar results for serum P1NP in subjects with normal renal function and hence reference intervals are interchangeable. Monomeric fragments accumulate in renal failure, affecting the measured P1NP concentration of total (Roche) but not intact P1NP (IDS-iSYS).

Osteocalcin is the most abundant non-collagenous bone protein secreted by osteoblasts and measurable in blood. It is specific to bone; hence its concentration reflects bone formation rate. It has circadian variation thus time of sample collection should be standardised. It is not affected by food intake. Osteocalcin accumulates in renal failure as it is excreted by the kidneys. There are 5 immunoassays available for osteocalcin and 4 of these are automated. Not all methods have the same reference intervals due to a variable detection of osteocalcin; therefore, the reference intervals cannot be used interchangeably.

## Clinical Use of Bone Turnover Markers

The use of BTMs for the diagnosis and monitoring of Paget's disease of bone is well established and shows promise for malignant bone disease. Serum PINP and plasma CTX are useful for monitoring therapy in osteoporosis. After the initiation of osteoporosis treatment, the change in BTMs is large and occurs by 3–6 months, compared to BMD where the change is small and slow, approximately after 18–24 months. The direction, magnitude and time course of the response vary by treatment and by BTM. Significant reductions in CTX are seen after 1 month of bisphosphonate treatment, indicating inhibition of osteoclastic activity, and reach a plateau from 3 months onwards. Reductions are seen earlier with intravenous compared to oral therapy. The decrease in P1NP is delayed by about 4 weeks compared to CTX, reaching a plateau after 3–6 months of treatment. In contrast, with teriparatide, an anabolic agent, there is an initial increase in P1NP reflecting direct stimulation of bone formation followed by a later increase in CTX. In addition to detecting a significant change in BTMs following initiation of treatment, optimum treatment effect is reflected by the BTMs attaining treatment targets. The use of BTMs in management of osteoporosis in patients with chronic kidney disease (CKD) may be adversely impacted. CTX and total P1NP (Roche) are both increased in CKD whilst intact P1NP (IDS-iSYS) is not as it recognises only the trimeric form. The Malaysian Clinical Practice Guidelines on Management of Osteoporosis advocates the use of P1NP and CTX to evaluate treatment efficacy and compliance.

## Innoquest Pathology offers:

| Panel Code | Tests | Specimen Requirements |
|---|---|---|
| OCL | Osteocalcin | 8ml Plain (Gel-YELLOW) |
| BXL | Beta Cross Laps | 8ml Plain (Gel-YELLOW) - freeze serum (Test runs every Thurs) |
| P1P | P1NP | 8ml Plain (Gel-YELLOW) - freeze serum (Test runs every Tues & Thurs) |
| =BONE1 | Bone Profile 1 | 8ml Plain (Gel-YELLOW) (Profile runs every Thurs) |
| =BONE2 | Bone Profile 2 | 8ml Plain (Gel-YELLOW) & 4ml EDTA (PURPLE) (Profile runs every Thurs) |


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# Detection of DNA Methylation Promotes Early Detection of Colorectal Cancer

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DNA methylation is a common type of epigenetic modification that occurs naturally in cells, involving addition of a methyl group (-CH3) to the cytosine base of cytosine-guanine dinucleotide (CpG)[1]. One of the crucial roles of DNA methylation is to regulate the gene expression, where the methylation on gene promoter controls the gene transcription activity without modifying underlying DNA sequences[2].

Cancer is a complex disease arisen from accumulation of genetic and epigenetics alterations, and DNA methylation is one of the earliest genomic changes during a carcinogenesis process[3,4]. In many cancers, the genes which regulate cell proliferation, such as oncogenes and tumour suppressor genes, are frequently observed with altered DNA methylation patterns, either as hypomethylation or hypermethylation. Such aberrations disrupt the normal gene function by dysregulating gene expression, lead to carcinogenesis with uncontrolled cell growth[5]. As abnormal DNA methylation events are observed in early phase of carcinogenesis before prominent cancer symptoms, detection of the DNA methylation become a promising approach for early detection of many cancer types[6].

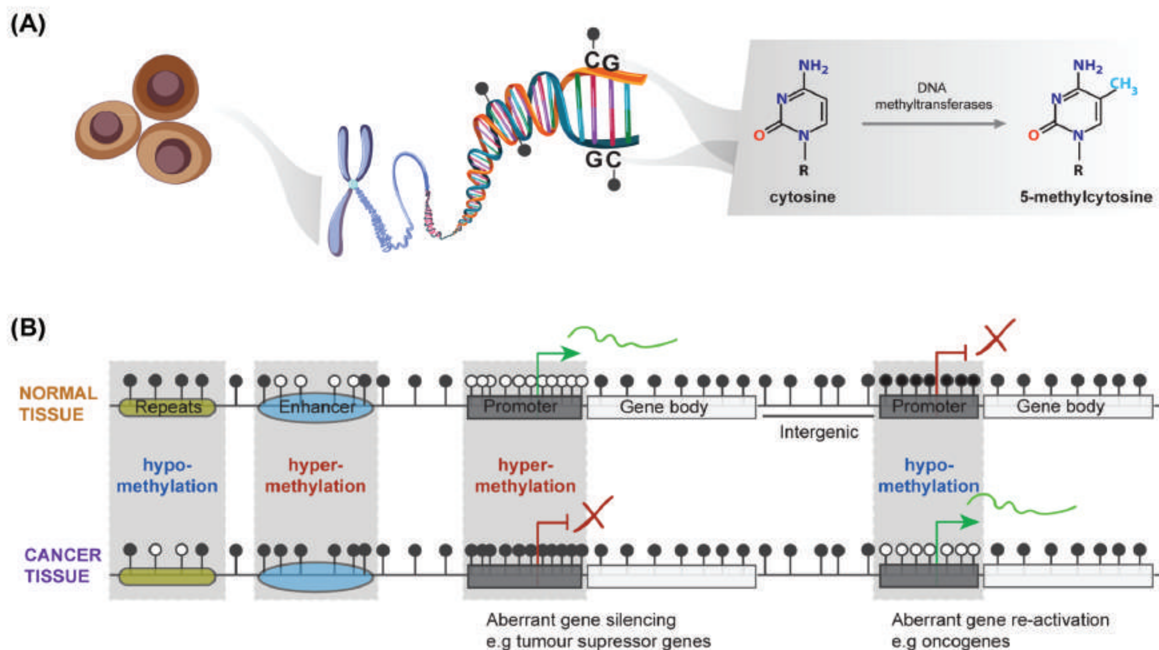


Figure 2(A) Principles of DNA methylation in cell. (B) Distinct DNA methylation of cancer cell compared to normal cell, leading to suppression of tumor suppressor gene and oncogenes expression, which drive tumorigenesis[5].

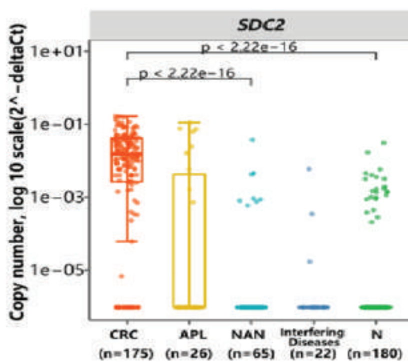


Figure 3:
Performance of methylated *SDC2, ADHFE1 and PPP2R5C* in differentiating colorectal cancer cases from healthy cases[16]

The significance of epigenetic alterations in colorectal cancer (CRC) pathogenesis has been reported widely[7,8]. According to comprehensive integrated methylation profiles of Cancer Genome Atlas (TCGA) database, a number of methylated genes found to be closely associated with CRC , including:

1. *SDC2*, gene encodes intracellular membrane protein with biological functions in cell division and migration[9]. Studies discover that *SDC2* plays a role in early stages of CRC development, with significant differences in *SDC2* methylation between tissue adjacent to carcinoma tissues and an obvious decreasing detection rate in the cancer tissue, adenoma tissue, polyp tissues, and normal tissues[10,11].

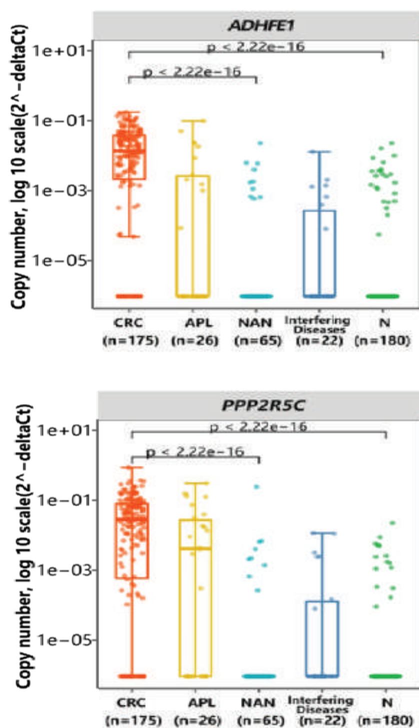


Figure 3 (con't):
Performance of methylated *SDC2, ADHFE1 and PPP2R5C* in differentiating colorectal cancer cases from healthy cases[16]

2. *ADHFE1*, gene encodes hydroxyacid-oxoacid transhydrogenase involving in cell proliferation and differentiation. *ADHFE1* hypermethylation found to accelerate the G1-S transition in cell cycle progression of CRC cells[12]. It exhibits similarly high predictive capability over FDA-approved methylation-based plasma biomarker SEPT9 in identifying CRC[13].
3. *PPP2R5C*, gene encodes regulatory subunit of PP2A phosphatase acting as negative regulator of cell growth and proliferation[14]. It regulates dephosphorylation of p53 protein in response to DNA damage, thus inhibiting the growth of CRC cells[15].

National Comprehensive Cancer Network (NCCN) guideline recommends the multi-targeted stool DNA-based test as regular CRC screening test[17]. Test principle behind multi-targeted stool DNA-based test is to analyze target methylated marker changes in cancerous cells that shed from colon lining into the stool[16]. Stool DNA-based testing offers many advantages, including:

1. Analysis on methylation status of three biomarkers in single sample address higher reliability in detecting CRC
2. Test do not rely on presence of prominent CRC symptom (such as faecal occult blood)
3. Non-invasive and safe test
4. Convenient test with self-collection design
5. Preparation or sedation before test are not required
6. Screening can be repeated every 3 years instead of annually

CRC remains as a major contributor to global cancer incidence and mortality and frequent related concern on late case diagnosis[1]. Multi-targeted stool DNA-based test becoming a promising approach to improve early detection of CRC, mitigate the delay in timely diagnosis and treatment. It demonstrates its potential as a valuable tool for the early detection of colorectal cancer, but also its cost-effectiveness in cancer management.

**Innoquest Pathology offers:**

| Panel Code | Tests | Specimen Requirements |
|---|---|---|
| CTS | Colotect™ Colorectal Cancer Screening Test | Stool (Collection kit will be provided) |

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