

Markers of Bone Resorption

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Biochemical Markers of Bone Turnover

Bone Formation

Products of active OB:

- ▶ Alkaline phosphatase (TAP, BAP)
- ▶ Osteocalcin (OC)
- ▶ Procollagen type I propeptides (PINP, PICP)

Bone Resorption

Degradation products of bone collagen:

- ▶ Hydroxyproline (OHP)
- ▶ Pyridinium crosslinks (PYD, DPD)
- ▶ Crosslinked telopeptides of type I collagen (NTX, CTX, ICTP)

Non-collagenous proteins of bone matrix:

- ▶ Bone sialoprotein
- ▶ Osteopontin
- ▶ Osteocalcin fragments (urine)

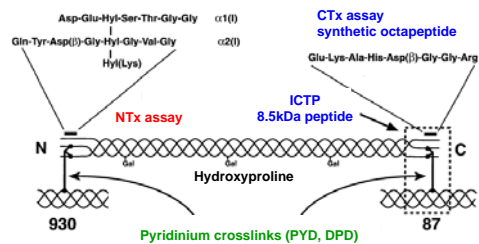
Osteoclast enzymes:

- ▶ Tartrate-resistant acid phosphatase (TRACP 5b)
- ▶ Cathepsin K

Collagen Related Markers of Bone Resorption

| Marker | Tissue of origin | Analytical specimen | Analytical method |
|---|--|-------------------------------|----------------------|
| Hydroxyproline (Hyp) | Bone, cartilage, skin, soft tissue | Urine | Colorimetry HPLC |
| Pyridinoline (PYD) | Bone, cartilage, tendon, blood vessels | Urine Serum | HPLC ELISA |
| Deoxypyridinoline (DPD) | Bone, dentin | Urine Serum | HPLC ELISA |
| Carboxy-terminal crosslinked telopeptide of type I collagen (ICTP, CTX-MMP) | Bone, skin | Serum | RIA |
| Carboxy-terminal crosslinked telopeptide of type I collagen (CTX-I) | All tissues containing type I collagen | Urine (α/β) Serum (β only) | ELISA RIA |
| Amino-terminal crosslinked telopeptide of type I collagen (NTX-I) | All tissues containing type I collagen | Urine Serum | ELISA CLIA RIA |

Molecular Origin of Markers of Collagen Degradation



Hydroxyproline: HPLC, EIA
 Hydroxyproline crosslinks (PYD, DPD): HPLC, EIA
 Crosslinked telopeptides: ICTP (CTX-MMP, carboxyterminal type I collagen telopeptide; RIA)
 CTX (Linear octapeptide derived from carboxyterminal type I collagen telopeptide; ELISA)
 NTx (Aminoterminal crosslinked type I collagen telopeptide; ELISA)

Response in Collagen-related Markers in Different Clinical Conditions

- ▶ Serum and urine CTX or NTX levels are markedly increased in postmenopausal osteoporosis, and their values decrease rapidly with antiresorptive treatment (in contrast to ICTP)
- ▶ Serum ICTP is a sensitive marker in other pathological conditions (metastatic bone disease, myeloma)
- ▶ Differences in marker responses may result from differences in the enzymatic pathways leading to the release of CTX/NTX and ICTP from collagen type I.

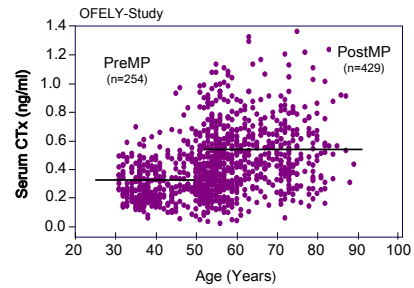
Caveats in the Value of Bone Markers

- ▶ Collagen-related markers are based primarily on type I collagen, which is not bone specific and is widely distributed in several tissues
- ▶ Changes in bone markers are not disease specific, but reflect alterations in skeletal metabolism independent of the underlying cause
- ▶ Systemic levels of biochemical markers reflect global skeletal turnover, i.e. no distinct information on the remodeling of trabecular and cortical bone
- ▶ Some markers are characterized by significant intra-individual variability

Biochemical Markers in the Assessment and Monitoring of Osteoporosis

- ▶ Evaluation of bone turnover and bone loss
- ▶ Fracture risk assessment
- ▶ Short-term evaluation of treatment effect

Changes in Bone Resorption with Menopause



Garnero et al. Clin Chem 2001;47:694

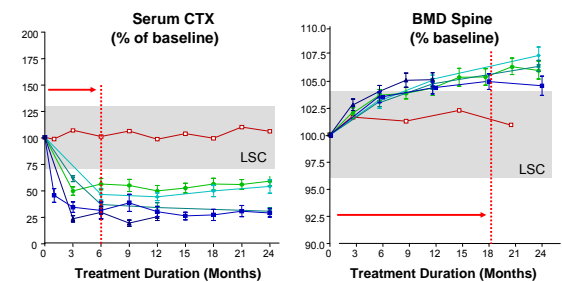
Bone Resorption and Fracture Prediction in Postmenopausal Women

| Prospective Studies | Age (yrs) | Study follow-up (yrs) | Fx type | Marker | RR (95%CI) for levels >+2SD premp |
|---------------------|-----------|-----------------------|---------|-----------------|-----------------------------------|
| EPIDOS | >75 | 1.8 | hip | u-CTX u-FDPD | 2.2 (1.3, 3.6) 1.9 (1.1, 3.2) |
| | >75 | 3.3 | hip | s-CTX | 1.9 (1.0, 3.8) |
| OFELY | 50-89 | 5.0 | all | u-CTX s-CTX | 2.3 (1.3, 4.1) 1.9 (1.0, 3.6) |
| HOS | 43-80 | 2.7 | all | u-CTX | 1.5 (1.2, 2.0) |
| Rotterdam | >55 | 4.0 | all | u-DPD | 1.9 (1.2, 3.8) |
| Malmö | 75 | 4.6 | spine | TRAP5b | 2.3 (1.3, 4.1) |

Garnero et al. J Bone Miner Res 1996, 11: 1531
Chapurlat et al. Bone 2000, 27: 283
Garnero et al. J Bone Miner Res 2000, 15: 1526

Ross et al. Osteoporos Int 2000, 11: 76
Van Daele et al. BMJ 1996, 312: 482
Gerdhem et al. J Bone Miner Res 2004, 19: 386

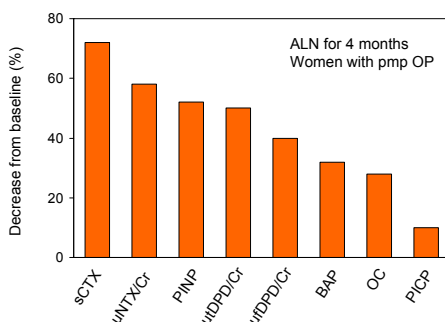
Change of Bone Turnover and BMD on Treatment



Christgau et al. Bone 2000, 26: 505
Ravn et al. J Clin Endo Metabol 1999, 84: 2363
Christgau et al. Clin Chem 1998, 44: 2290

ALN (10 mg/d)
IBN (2.5 mg/d)
Estradiol (50 µg/d)
Estradiol-17 (2 mg/d)
Tibolone (2.5 mg/d)
Combined Placebo (calcium)

Suppression of Bone Markers during Antiresorptive Therapy depends on selected Bone Marker



Fink et al. Osteoporos Int 2000, 11: 295

Biochemical Markers of Bone Turnover

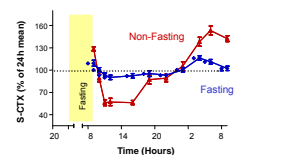
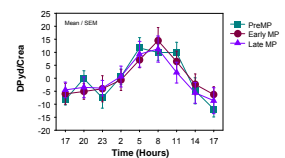
Sources of

Preanalytical Variability:

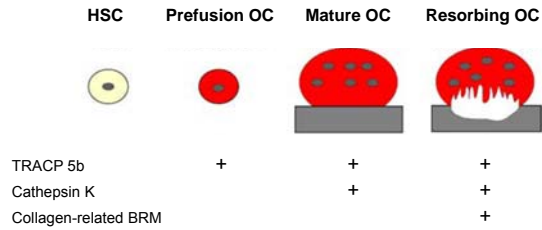
- Controllable factors:
- Sample storage
 - Diurnal variability
 - Diet
 - Exercise
 - Seasonal rhythms

Uncontrollable factors:

- Age
- Gender
- Recent fractures
- Renal function
- Immobility
- Non-skeletal diseases



Release of Osteoclast Enzymes and Resorption Markers during Osteoclast Differentiation



Henriksen et al, Osteoporos Int 2007, 18: 681

TRACP and Osteoclast Function

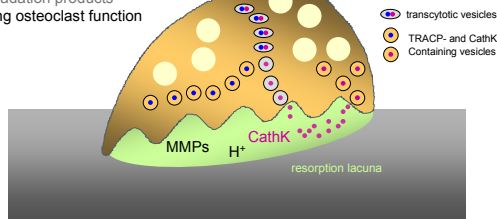
- ▶ High amounts of tartrate-resistant acid phosphatase are expressed in OC, alveolar macrophages and dendritic cells
Yaziji et al, Am J Clin Pathol 1995; Hayman et al, J Histochem Cytochem 2001
- ▶ TRACP has two distinct enzymatic activities. It can function as a phosphatase at acid pH, and as a generator of reactive oxygen species at neutral pH
Kajja et al, BBRC 2002
- ▶ Two isoforms of type 5 TRACP found in human serum:
 - TRACP 5b: only secreted by osteoclasts (pH 5.8)
 - TRACP 5a: secreted by macrophages, dendritic cells (containing sialic acid residues, pH 5.2)

Lam et al, Clin Chem 1978; Lam et al, Clin Biochem 1981; Halleen et al, JBMR 2000

TRACP and Cathepsin K in Resorbing Osteoclast

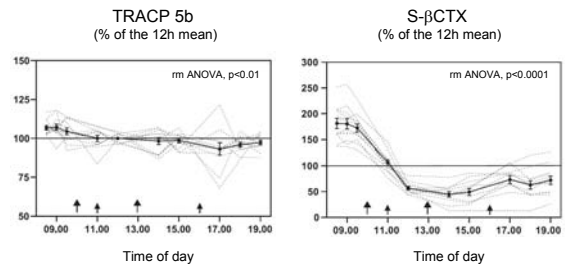
TRACP 5b, cathepsin K
→ proportional to the number of osteoclasts reflecting osteoclast number

Bone degradation products
→ reflecting osteoclast function



adapted from Halleen et al, J Bone Miner Res 2003

Low Diurnal Variability of TRACP 5b



Hannon et al, Bone 2004, 34: 187

Healthy postmenopausal women, n=20; mean values ± SEM

Clinical Performance of Immunoreactive TRACP 5b

Analytical Performance

| Bone marker | Analytical variability (CV _w , fasting) | Individual variability (CV _i , fasting) |
|-------------|--|--|
| TRACP 5b | 3.2 | 6.6 |
| S-βCTX | 1.1 | 19.1 |
| S-NTX | 9.7 | 12.2 |
| U-CTX | 7.5 | 24.6 |
| U-NTX | 6.9 | 43.7 |

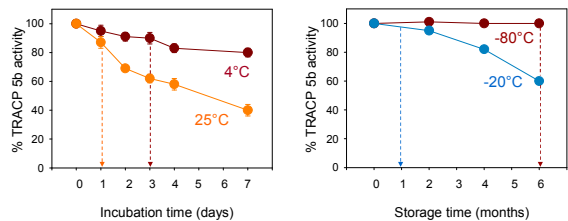
Effect of Feeding

| Bone marker | % Difference (fed-fasting) ± SE |
|-------------|---------------------------------|
| TRACP 5b | -2.4 ± 0.79 * |
| S-βCTX | -17.8 ± 2.6 *** |
| S-NTX | -8.5 ± 1.7 *** |
| S-CTX | -7.0 ± 2.6 ** |
| U-NTX | -7.9 ± 3.7 * |

Hannon et al, Bone 2004, 34: 187

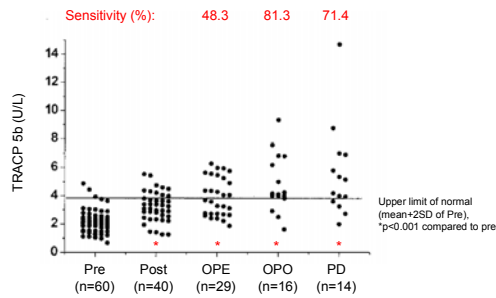
Healthy premenopausal women, n=20; *p<0.05, **p<0.01, ***p<0.0001

Stability of Serum TRACP 5b



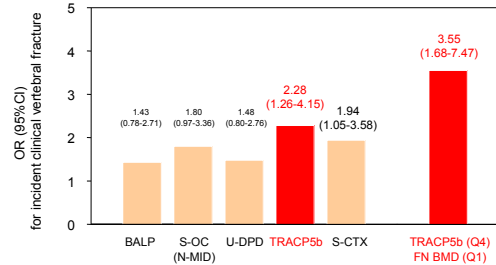
Halleen et al, J Bone Miner Res 2000, 15: 1337

TRACP 5b in Metabolic Bone Diseases



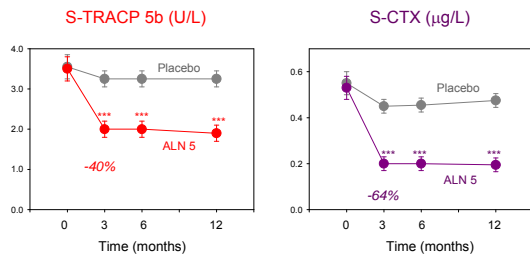
Halleen et al, Clin Chem 2001, 47: 597

TRACP 5b and Fracture Prediction



Malmö OPRA Study
n=1040 elderly women (>75 yrs); n=49 VFX
Mean follow-up 4.6 yrs (3-6.5), non-fasting samples
Gerdem et al, J Bone Miner Res 2004, 19: 386

TRACP 5b for Monitoring ALN Treatment



Nenonen et al, J Bone Miner Res 2005, 20: 1804

RCT, healthy postmenopausal women, n=148
***p<0.001

TRACP 5b for Monitoring ALN Treatment

| Bone marker | Signal-to-noise ratio | |
|-------------|--|---|
| | Hannon et al. Bone 2004 ALN+Ca, n=23, evaluation at 24 wks | Nenonen et al. JBMR 2005 ALN+Ca/VD (RCT), n=148, evaluation at 12 wks |
| S-TRACP 5b | 5.3 | 3.2 |
| S-CTX | 3.9 | 2.8 |
| S-PINP | | 2.9 |
| S-BALP | | 2.8 |
| S-OC | | 1.8 |
| U-CTX | 1.9 | |
| U-NTX | 1.6 | |
| U-DPD | | 2.3 |

TRACP 5b

- ▶ Serum TRACP 5b is a reliable osteoclast-specific and sensitive marker of bone resorption
- ▶ TRACP 5b is proportional to the number of osteoclasts, may be used as a marker of osteoclast number (may be of interest in novel treatments inhibiting bone resorption without affecting OC number, i.e. CIC-7 inhibitors)
- ▶ Serum TRACP 5b activity has low technical and biological variability, does not accumulate in renal and hepatic failure, but has low storage stability above -70°C
- ▶ Serum TRACP 5b has a favorable signal-to-noise ratio, hence may be a useful marker in monitoring antiresorptive therapy

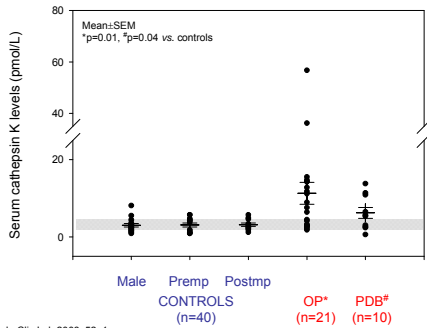
Pycnodysostosis (Toulouse-Lautrec Disease)



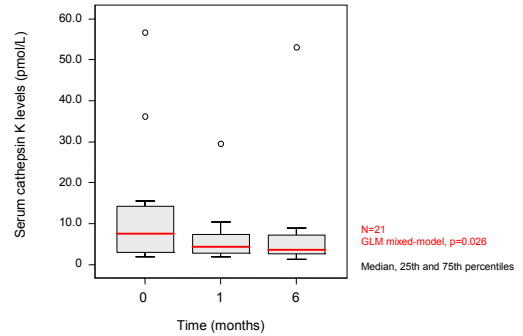
Henri Toulouse-Lautrec (1864-1901)

- ▶ Pycnodysostosis, autosomal recessive bone sclerosing disorder, is caused by a **deficiency in cathepsin K activity** characterised by decreased bone turnover and an accumulation of undigested collagen fibrils in OC (osteopetrosis and short-stature)
Gelb et al, Science 1996
Fratzl-Zelman et al, J Clin Endocrinol Metab 2004
- ▶ **Cathepsin K null mouse** manifest osteopetrosis, characterized by dysfunctional matrix digestion
Saitig et al, Proc Natl Acad Sci USA 1998
Gowen et al, J Bone Miner Res 1999

Serum Cath K Levels in postmp OP, PDB and Controls



Effect of Bisphosphonate Treatment on Serum Cath K in postmp OP



Clinical Studies Measuring Serum Cathepsin K

- ▶ Cathepsin K decrease with age in women and men
(Kershan-Schindl et al, *Experimental Gerontology* 2005)
- ▶ Cathepsin K correlates with BMD and fracture history
(Holzer et al, *J Lab Clin Med* 2005)
- ▶ Cathepsin K also expressed in synovial fibroblasts and macrophages.
Serum cathepsin K levels are increased patients with rheumatoid arthritis and correlates with radiological destruction in longstanding disease
(Skoumal et al, *Arthritis Res Ther* 2005; Skoumal et al, *Rheumatol Int* 2008)

Cathepsin K: Conclusion

- ▶ Serum concentrations of cathepsin K do not appear to reflect the activity of osteoclasts as compared to biochemical markers of bone resorption.
- ▶ Cathepsin K may be a better surrogate for osteoclast number than for osteoclast function.
- ▶ The clinical utility of cathepsin K measurement as a marker of bone resorption seems to be limited.