Markers of Bone Resorption

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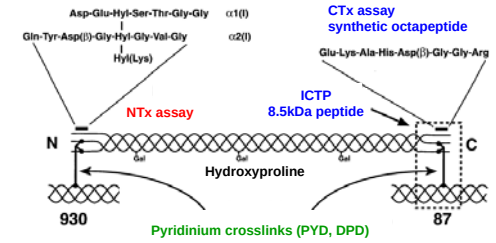
Biochemical Markers of Bone Turnover

Bone Formation	Bone Resorption
Products of active OB:	Degradation products of bone collagen:
▸ Alkaline phosphatase (TAP, BAP)	▸ Hydroxyproline (OHP)
▸ Osteocalcin (OC)	▸ Pyridinium crosslinks (PYD, DPD)
▸ Procollagen type I propeptides (PINP, PICP)	▸ 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



Hydroxypyridinium 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.

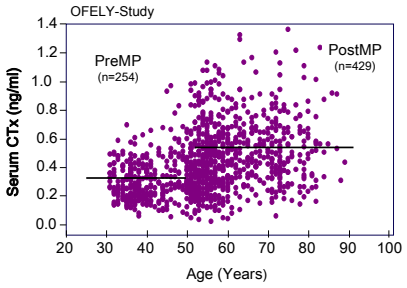
Caveats in the Value of Bone Markers

- 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

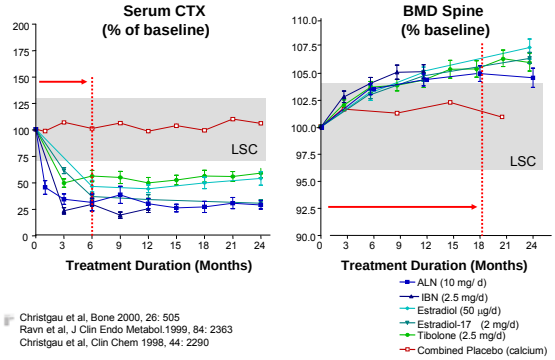
Bone Resorption and Fracture Prediction in Postmenopausal Women

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

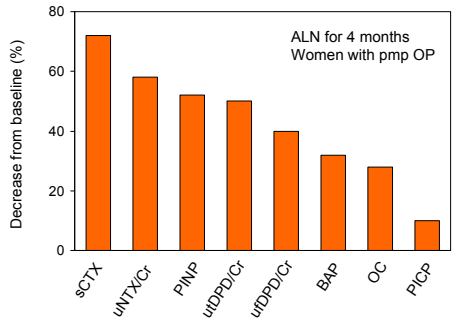
Ross et al, Osteoporos Int 2000, 11: 76
Van Daele et al, BMJ 1996, 312: 482
Gerdhem et al, J Bone Miner Res 2004, 19: 386

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

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



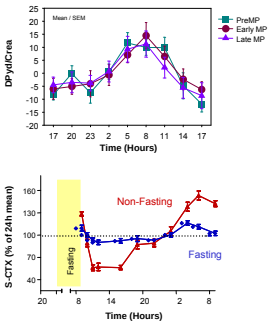
Fink et al, Osteoporos Int 2000, 11: 295

Biochemical Markers of Bone Turnover

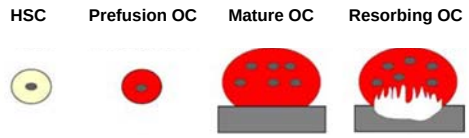
Sources of Preanalytical Variability:

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



TRACP 5b	+	+	+
Cathepsin K		+	+
Collagen-related BRM			+

Henriksen et al, Osteoporos Int 2007, 18: 681

TRACP and Osteoclast Function

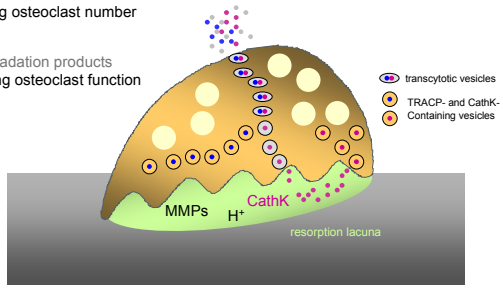
- High amounts of tartrate-resistant acid phosphatase are expressed in OC, alveolar macropaghes and dendritic cells
  Yaziji et al, Am J Clin Pathol 1995; Hayman et al, J Histochem Cytochem 2001
- TRACP has two distinct enzymatic activities. It can function as a phosphatase at acid pH, and as a generator of reactive oxygen species at neutral pH
  Kaija 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

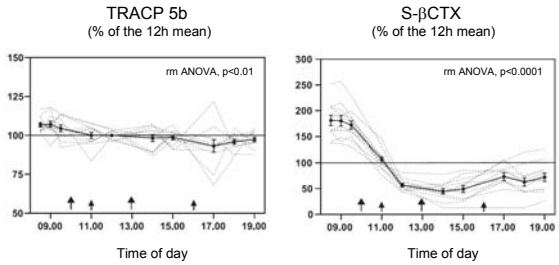
TRACP 5b, cathepsin K
→ proportional to the number of osteoclasts reflecting osteoclast number

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_a, 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

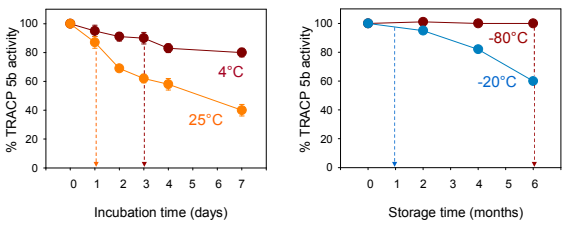
Effect of Feeding

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

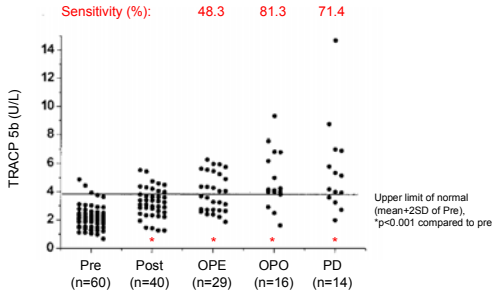
Healthy premenopausal women, n=20; *p<0.05, **p<0.01, ***p<0.0001

Stability of Serum TRACP 5b



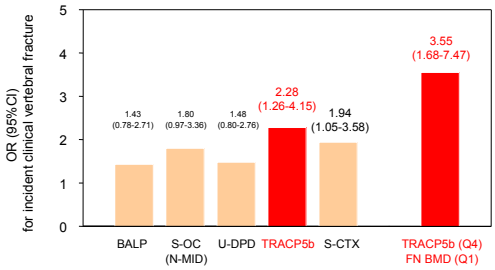
Halleen et al, J Bone Miner Res 2000, 15: 1337

TRACP 5b in Metabolic Bone Diseases



Halleen et al, Clin Chem 2001, 47: 597

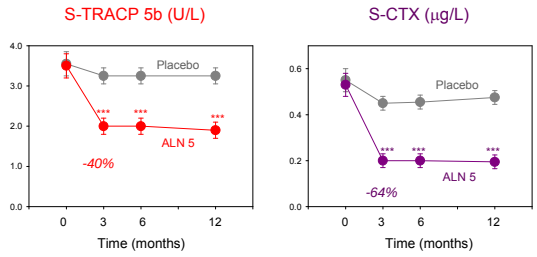
TRACP 5b and Fracture Prediction



Gerdhem et al, J Bone Miner Res 2004, 19: 386

Malmö OPRA Study
n=1040 elderly women (>75 yrs); n=49 VFx
Mean follow-up 4.6 yrs (3-6.5); non-fasting samples

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

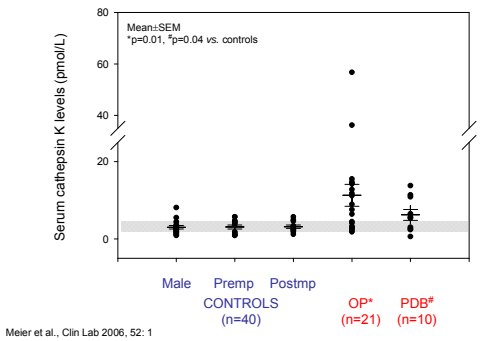
- Serum TRACP 5b is a reliable osteoclast-specific and sensitive marker of bone resorption
- TRACP 5b is proportional to the number of osteoclasts, may be used as a marker of osteoclast number (may be of interest in novel treatments inhibiting bone resorption without affecting OC number, i.e. CIC-7 inhibitors)
- 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)

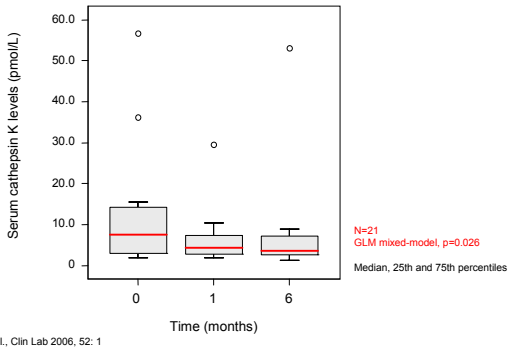
- Pycnodysostosis, autosomal recessive bone sclerosing disorder, is caused by a deficiency in cathepsin K activity characterised by decreased bone turnover and an accumulation of undigested collagen fibrils in OC (osteopetrosis and short-stature)
  Gelb et al, Science 1996
  Fratzl-Zelman et al, J Clin Endocinol Metab 2004
- Cathepsin K null mouse manifest osteopetrosis, characterized by dysfunctional matrix digestion
  Saftig 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



Meier et al., Clin Lab 2006, 52: 1

Effect of Bisphosphonate Treatment on Serum Cath K in postmp OP



Meier et al., Clin Lab 2006, 52: 1

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.