بسم الله الرحمن الرحیم

Novel and Multifaceted Approaches to

Screening, Diagnosis, and Monitoring of Osteoporosis

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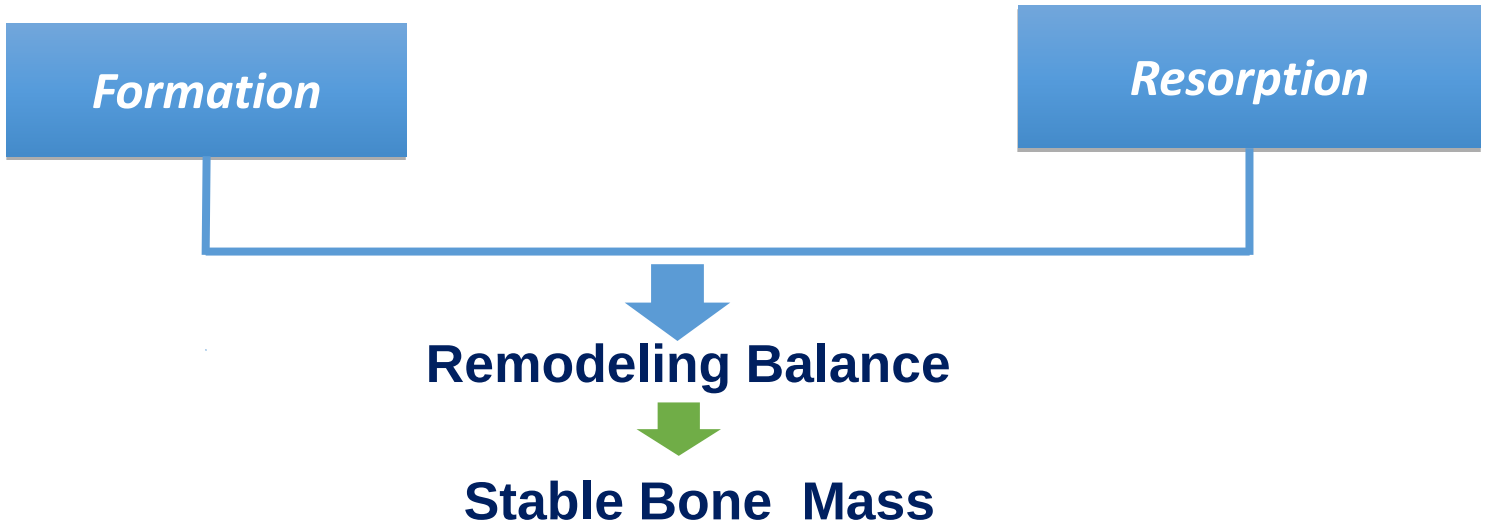
Outline

- Definition of osteoporosis
- Osteoporosis burden and epidemiology
- Importance of screening and early diagnosis
- Currently available diagnosis techniques and their limitations
- Proteomics, genomics, and osteoporosis
- Potentials of Bone Turnover Markers (BTM) in screening, diagnosis, and monitoring of osteoporosis response-to-treatment
- Microfluidic chips for point-of-care screening of osteoporosis
- Comprehensive and multifaceted approach to screening and diagnosis to osteoporosis

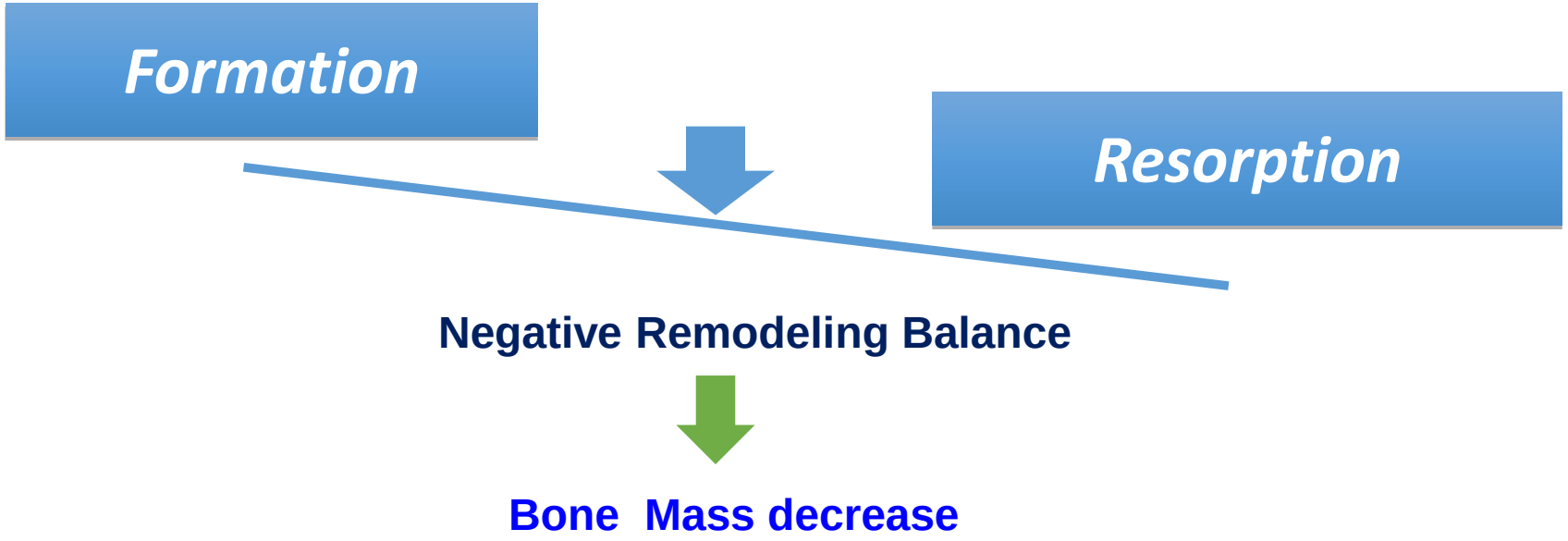
Physiological bone turnover

- Bone turnover is regulated by two mechanisms:
  - Bone resorption
  - Bone formation



Khosla, Sundeep, and Lorenz C. Hofbauer. "Osteoporosis treatment: recent developments and ongoing challenges." ***The lancet Diabetes & endocrinology (2017).***

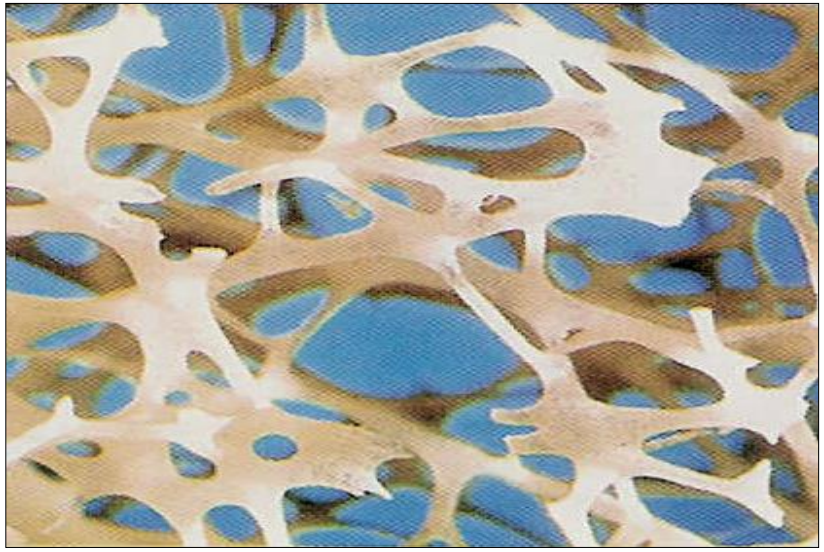
Definition of Osteoporosis



Osteoporosis develops when **bone resorption exceeds bone formation**.

Khosla, Sundeep, and Lorenz C. Hofbauer. "Osteoporosis treatment: recent developments and ongoing challenges." ***The lancet Diabetes & endocrinology (2017).***

Bone damage in osteoporosis



Healthy bone

osteoporosis

Osteoporosis is **the most common metabolic bone disease** and leads to damage to bone structure

Canalis, Ernesto. "MANAGEMENT OF ENDOCRINE DISEASE: Novel anabolic treatments for osteoporosis."***European journal of endocrinology* 178.2 (2018):** R33-R44.

Epidemiology

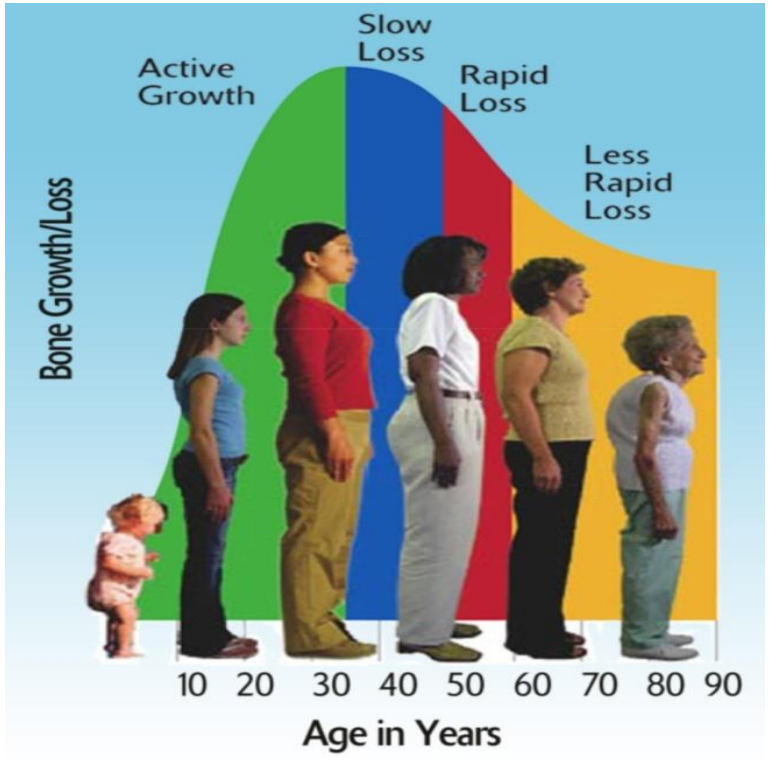
- Osteoporosis is a **multifactorial disease** affecting large number of people of **both genders** worldwide.

One-Third of women

More prevalent than Breast Cancer

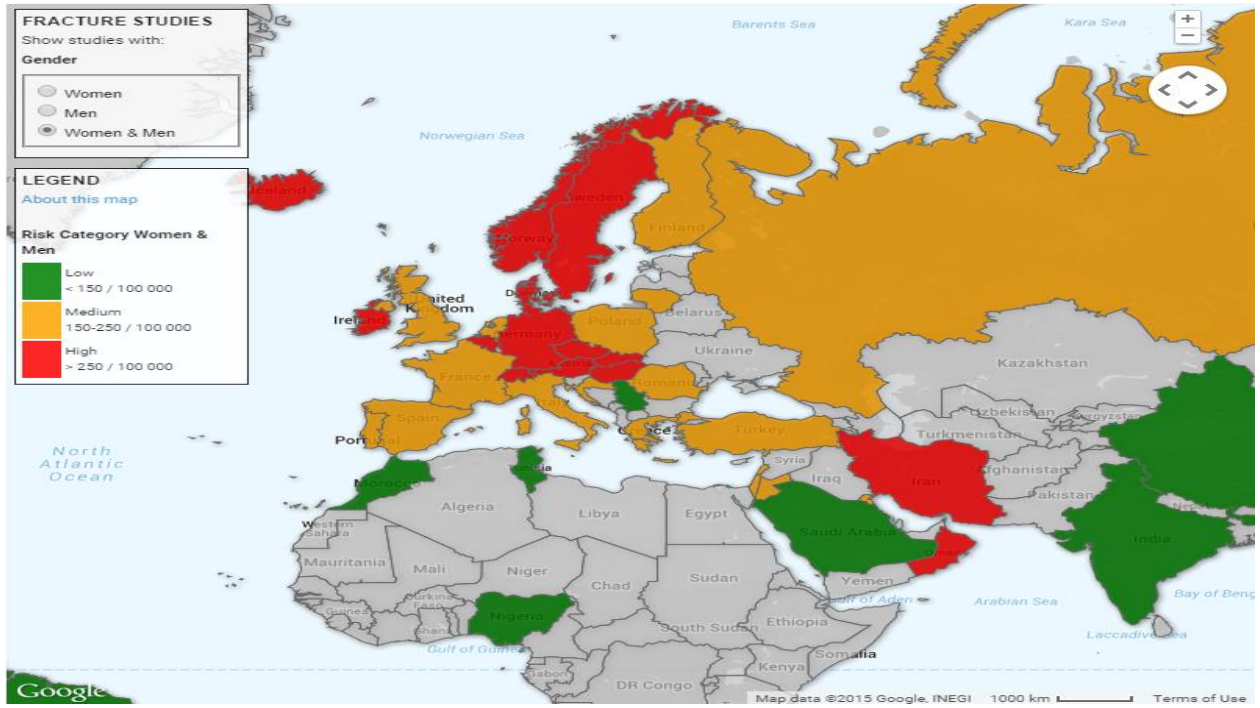
One-Fifth of men

More prevalent than Prostate Cancer



*Tabatabaei-Malazy, O., Salari, P., Khashayar, P., & **Larijani, B. (2017)**. New horizons in treatment of osteoporosis. DARU Journal of Pharmaceutical Sciences, 25(1), 2.*

Global burden of osteoporosis



Every 30 seconds someone in the EU experiences a fracture as a result of osteoporosis

Burden of osteoporosis in the Middle East

- Because of **increasing life expectancy**, osteoporosis is becoming a major **global health issue**

- Osteoporosis is more prevalent in **developing countries**, particularly in the **Middle East** region

- Osteoporosis imposes **great financial burdens** on health systems of different countries

*Maalouf, G., Gannagé-Yared, M. H., Ezzedine, J., **Larijani, B.**, Badawi, S., Rached, A., ... & Nammari, R. (2007). Middle East and North Africa consensus on osteoporosis. Journal of Musculoskeletal and Neuronal Interactions, 7(2), 131.*

Need for early diagnosis and management

- In most cases, restoration of diminished bone volume is not possible
- Besides health consequences, osteoporotic fracture imposes great financial and social burdens to health systems
- Therefore, identification of individuals at high-risk of disabling fractures and early interventions can be of great benefit to both individuals and society as a whole

Ferrari, Serge Livio. "Prevention of fractures in patients with osteoporosis." The ***Lancet 391.10117 (2018):*** *184-186.*

The role of screening in reducing morbidity

- Screening and early diagnosis can prevent from many osteoporosis complications such as hip fracture

Outcome	Control (n=6250)	Screening (n=6233)	Hazard Ratio (95% CI)	P-value
Osteoporosis related fracture	852 (13.6%)	805 (12.9%)	0.94 (0.85-1.03)	0.178
Hip fracture	218 (3.5%)	164 (2.6%)	0.72 (0.59-0.89)	0.002
Any clinical fracture	1002 (16.0%)	951 (15.3%)	0.94 (0.86-1.03)	0.183
Mortality	525 (8.4%)	550 (8.8%)	1.05 (0.93-1.19)	0.436

Screening in the community to reduce fractures in older women (SCOOP): a randomised controlled trial

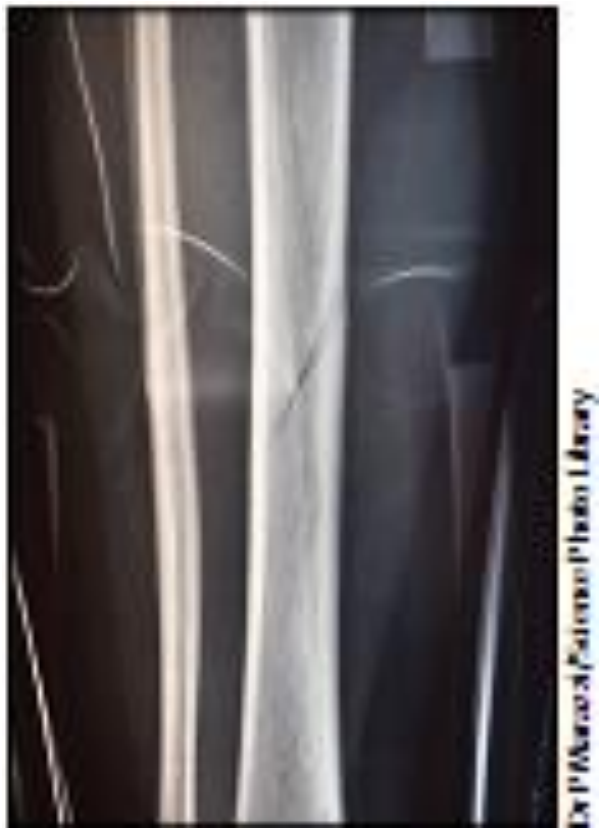
*Shepstone, Lee, et al. Screening in the community to reduce fractures in older women (SCOOP): a randomised controlled trial." **The** "**Lancet (2017**).*

Falls and their Burden on Health Systems

- Falls are a great cause of **morbidity and mortality** in old persons

- **A third of community-living people** aged 65 years or older fall at least once per year, with half of this number having **multiple falls** in this period

- Falls impose major **social and economic burdens** for individuals, their families, health services, and the economy.

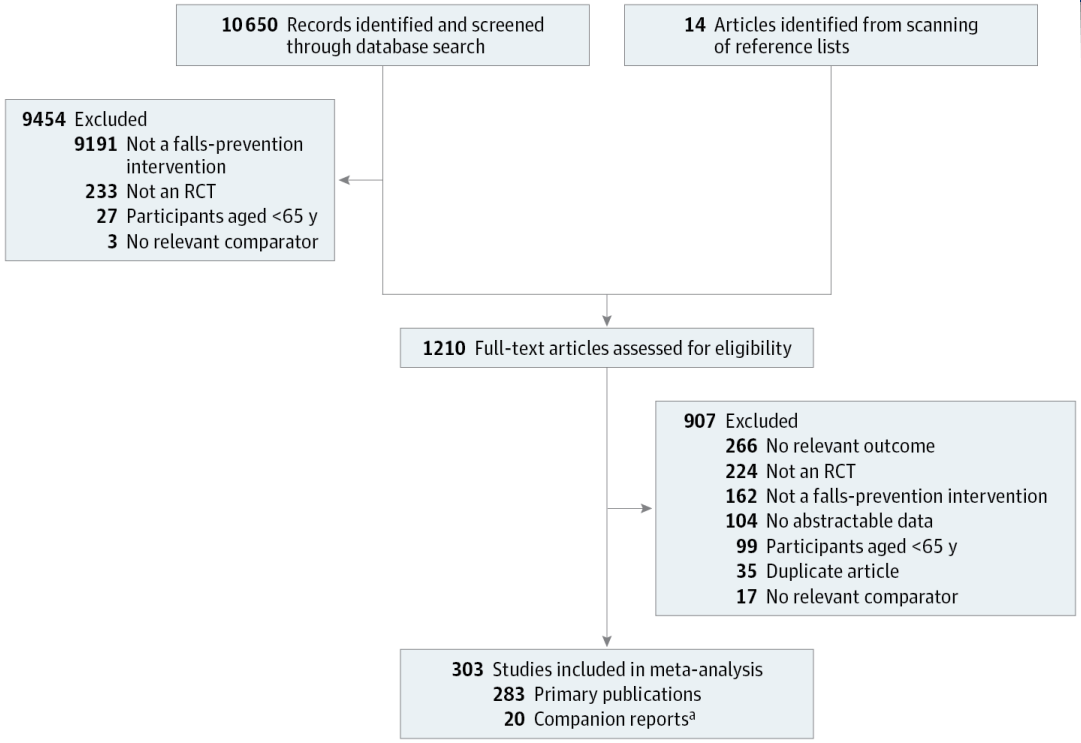


*Bouillon, Roger. "Can vitamin D prevent falls and fractures?."**The Lancet Diabetes & Endocrinology 5.6 (2017):** 407-409.*

Early diagnosis of osteoporosis and falls

- Result of a systematic review by Tricco et al in 2017 demonstrated that **early diagnosis** and timely intervention can prevent from many incidents of **falls**

- Following early diagnosis, various combinations of **interventions** can be used for prevention form **fall-caused injuries**



Comparisons of Interventions for Preventing Falls in Older Adults
A Systematic Review and Meta-analysis

*Tricco, Andrea C., et al. "Comparisons of interventions for preventing falls in older adults: a systematic review and meta-analysis." **Jama 318.17 (2017):** 1687-1699.*

Table 4. Subgroup Analyses of Network Meta-analysis for Injurious Falls Outcome

Comparison by Subgroup	Studies, No.	Patients, No.	Proportion With Event (95% CI)		Odds Ratio (95% CI)	Absolute Mean Difference in Proportions (95% CI)[a]
			Intervention	Control		
Exercise vs Usual Care						
Overall analysis					0.51 (0.33 to 0.79)	
Participants <75% women	37	20 354	0.36 (0.16 to 0.59)	0.41 (0.29 to 0.53)	0.49 (0.31 to 0.78)	−0.71 (−1.17 to −0.25)
Study duration ≤12 mo	44	32 890	0.30 (0.13 to 0.52)	0.33 (0.22 to 0.44)	0.48 (0.29 to 0.80)	−0.72 (−1.23 to −0.22)
Age <80 y of age	32	24 869	0.25 (0.08 to 0.48)	0.35 (0.19 to 0.53)	0.44 (0.26 to 0.75)	−0.81 (−1.35 to −0.28)
Mixed history of falling[b]	40	37 010	0.36 (0.16 to 0.59)	0.37 (0.25 to 0.49)	0.49 (0.30 to 0.82)	−0.71 (−1.22 to −0.20)
History of falling only[c]	11	3830	0.16 (0.07 to 0.27)	0.24 (0.07 to 0.47)	0.90 (0.24 to 3.30)	−0.11 (−1.41 to 1.19)
Low risk of contamination bias	24	26 969	0.40 (0.00 to 0.96)	0.26 (0.15 to 0.37)	0.59 (0.29 to 1.18)	−0.53 (−1.23 to 0.17)
Combined Exercise and Vision Assessment and Treatment vs Usual Care						
Overall analysis					0.17 (0.07 to 0.38)	
Participants <75% women	37	20 354	0.51 (0.42 to 0.59)	0.41 (0.29 to 0.53)	0.16 (0.07 to 0.39)	−1.82 (−2.68 to −0.95)
Study duration ≤12 mo	44	32 890	NA	0.33 (0.22 to 0.44)	NA	
Age <80 y of age	32	24 869	0.51 (0.42 to 0.59)	0.35 (0.19 to 0.53)	0.17 (0.07 to 0.43)	−1.76 (−2.66 to −0.85)
Mixed history of falling[b]	40	37 010	0.51 (0.42 to 0.59)	0.37 (0.25 to 0.49)	0.16 (0.06 to 0.42)	−1.82 (−2.77 to −0.86)
History of falling only[c]	11	3830	NA	0.24 (0.07 to 0.47)	NA	
Low risk of contamination bias	24	26 969	NA	0.26 (0.15 to 0.37)	NA	
Combined Exercise, Vision Assessment and Treatment, and Environmental Assessment and Modification vs Usual Care						
Overall analysis					0.30 (0.13 to 0.70)	
Participants <75% women	37	20 354	0.65 (0.57 to 0.73)	0.41 (0.29 to 0.53)	0.30 (0.12 to 0.71)	−0.22 (−2.09 to −0.35)
Study duration ≤12 mo	44	32 890	NA	0.33 (0.22 to 0.44)	NA	
Age <80 y of age	32	24 869	0.65 (0.57 to 0.73)	0.35 (0.19 to 0.53)	0.31 (0.13 to 0.78)	−1.16 (−2.07 to −0.24)
Mixed history of falling[b]	40	37 010	0.65 (0.57 to 0.73)	0.37 (0.25 to 0.49)	0.30 (0.11 to 0.78)	−1.22 (−2.18 to −0.25)
History of falling only[c]	11	3830	NA	0.24 (0.07 to 0.47)	NA	
Low risk of contamination bias	24	26 969	NA	0.26 (0.15 to 0.37)	NA	
Combined Clinic-Level Quality Improvement Strategies, Multifactorial Assessment and Treatment, Calcium Supplementation, and Vitamin D Supplementation vs Usual Care						
Overall analysis					0.12 (0.03 to 0.55)	
Participants <75% women	37	20 354	0.03 (0.00 to 0.07)	0.41 (0.29 to 0.53)	0.12 (0.03 to 0.56)	−2.08 (−3.58 to −0.58)
Study duration ≤12 mo	44	32 890	0.03 (0.00 to 0.07)	0.33 (0.22 to 0.44)	0.12 (0.03 to 0.54)	−2.08 (−3.56 to −0.61)
Age <80 y of age	32	24 869	NA	0.349 (0.191 to 0.527)	NA	
Mixed history of falling[b]	40	37 010	NA	0.37 (0.25 to 0.49)	NA	
History of falling only[c]	11	3830	0.03 (0.00 to 0.07)	0.24 (0.07 to 0.47)	0.12 (0.04 to 0.44)	−2.08 (−3.34 to −0.83)
Low risk of contamination bias	24	26 969	NA	0.26 (0.15 to 0.37)	NA	

Abbreviations: NA, not applicable.

[a] Odds ratios derived from each network meta-analysis were transformed to risk differences using established methods.[34]

[b] Studies that included participants regardless of whether they had fallen in the past or not.

[c] Studies that only included participants who had fallen in the past.

Diagnosis of osteoporosis

- The **first line** of medical testing for diagnosis of osteoporosis is measurement of **Bone Mineral Density**, most commonly using dual-energy X-ray absorptiometry **(DXA)**

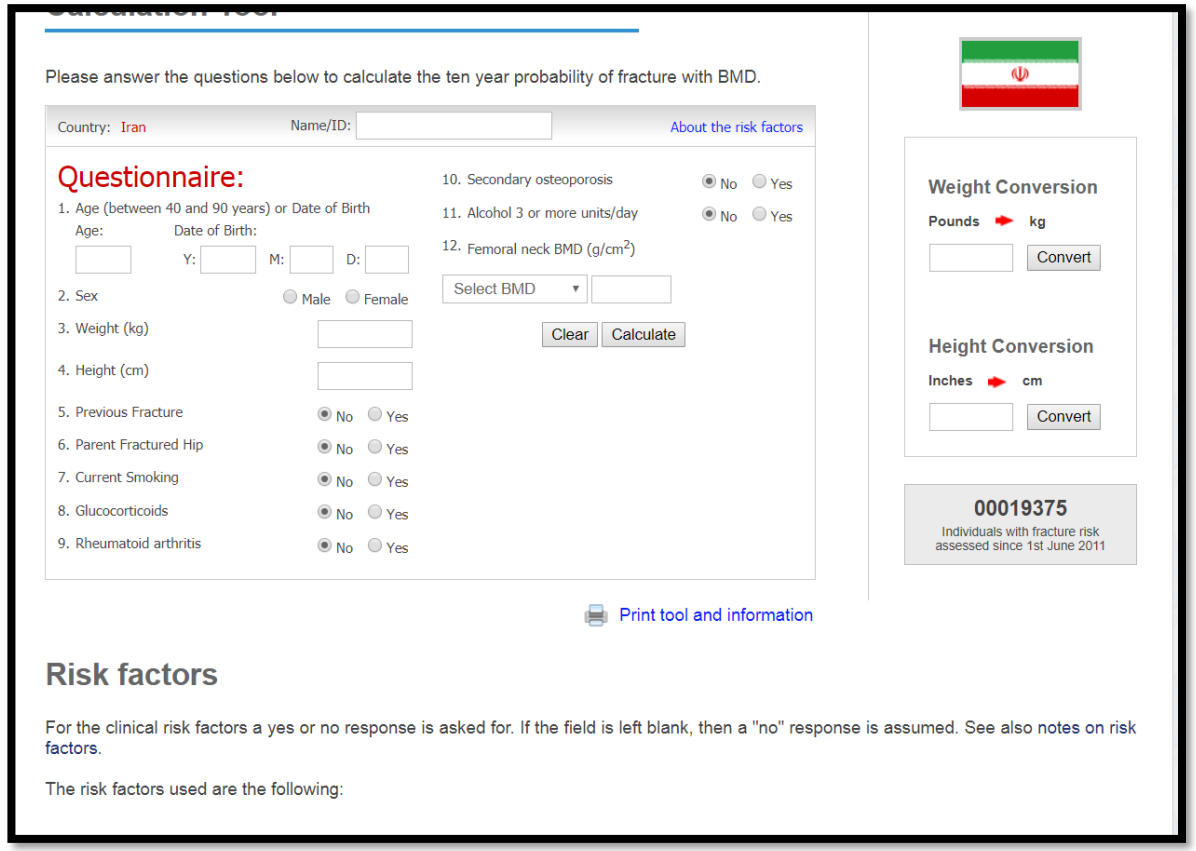
- **Algorithms** such as **FRAX®** combine **BMD** data with **clinical information** to form a diagnostic tool for estimation of fracture risk based on BMD and other clinical features

*Cotts, Kamala Gullapalli, and Adam S. Cifu. "Treatment of Osteoporosis." **Jama 319.10 (2018):** 1040-1041.*

FRAX: a Country-specific tool for evaluation of fracture risk

- The FRAX® tool has been developed to **evaluate fracture risk** of patients

- It is based on **individual patient models** that integrate the risks associated with **clinical** risk factors as well as bone mineral density **(BMD)** at the femoral neck



Osteoporosis risk assessment, diagnosis and management

Recommendations restricted to postmenopausal women and men aged >50 years

Royal Australian College *of* General Practitioners | osteoporosis australia

Minimal trauma hip or vertebral fracture

DXA to establish baseline BMD – recommended but not essential

Initiate treatment with anti-osteoporosis medication
- Bisphosphonates (Grade A)
- Denosumab (Grade A women, Grade B men)
- Oestrogen replacement therapy (Grade A)
- Strontium ranelate – second line only (Grade A)

Where appropriate
- Implement falls reduction strategies (Grade A)
- Encourage exercise participation (Grade A)
- Modify diet, smoking and alcohol intake (Grade C)
- Provide education and psychosocial support (Grade D)

Minimal trauma fracture at any other site*

DXA of spine and proximal femur (Grade A)

T-score ≤–1.5

T-score >–1.5

Refer for specialist review

No history of minimal trauma fracture

Aged ≥70 years‡ (Grade D Consensus)

Assess risk factor profile (Grade B) Major risk factors that qualify for MBS reimbursement of DXA†

Non-modifiable
- Parental history of fracture

Modifiable and lifestyle
- Premature menopause†
- Hypogonadism†
- Multiple falls
- Low physical activity or immobility
- Low body weight
- Low muscle mass and strength
- Poor balance
- Protein or calcium undernutrition
- Smoking
- Alcohol >2 standard drinks/day
- Vitamin D insufficiency

Diseases or conditions§
- Rheumatoid arthritis†
- Hyperthyroidism†
- Hyperparathyroidism†
- Chronic kidney disease†
- Chronic liver disease†
- Coeliac disease or malabsorption†
- Diabetes mellitus
- Myeloma or MGUS
- Organ transplant
- Bone marrow transplant
- HIV infection
- Depression

Medications (large effect)
- Glucocorticoids†‖ (>3 months≥7.5 mg/day)
- Excess thyroid hormone replacement
- Aromatase inhibitors
- Anti-androgen therapy†

Medications (modest effect)
- SSRIs
- Anti-psychotics
- Thiazolidenediones
- Anti-epileptic medications
- PPIs

DXA of spine and proximal femur (Grade A)

T-score ≤–2.5? → No → **Estimate absolute fracture risk** Garvan Fracture Risk Calculator or FRAX (Grade D Consensus)

Yes → **High 10-year risk of fracture** Hip fracture >3%, any fracture >20% OR T-score ≤–2.5

Low risk of fracture

Initiate treatment with anti-osteoporosis medication
- Bisphosphonates (Grade A)
- Denosumab (Grade A women, Grade B men)
- Oestrogen replacement therapy (Grade A)
- Strontium ranelate – second line only (Grade A)

Treatment not recommended

Where appropriate
- Implement falls reduction strategies (Grade A)
- Encourage exercise participation (Grade A)
- Modify diet, smoking and alcohol intake (Grade C)
- Provide education and psychosocial support (Grade D)

BMD	Bone mineral density
DXA	Dual energy X-ray absorptiometry
HIV	Human immunodeficiency virus
MBS	Medicare Benefits Schedule
MGUS	Monoclonal gammopathy of undetermined significance
PPIs	Proton pump inhibitors
SSRIs	Selective serotonin reuptake inhibitor

* Excluding fingers and toes
† Qualifies for MBS reimbursement of BMD testing
‡ Consensus recommendation. The MBS reimburses costs for measurement of BMD testing in any person aged ≥70 years
‖ See other guidelines specific to glucocorticoid treatment for more information and recommendations regarding glucocorticoid use and risk of osteoporosis and fracture
§ Treatment of an underlying condition may improve bone strength

4428

DXA: the Gold Standard in Diagnosis of Osteoporosis

- The diagnosis of osteoporosis is based on the assessment of bone mineral density
- The recommended technique is dual energy X-ray absorptiometry (DXA) at the site proximal femur

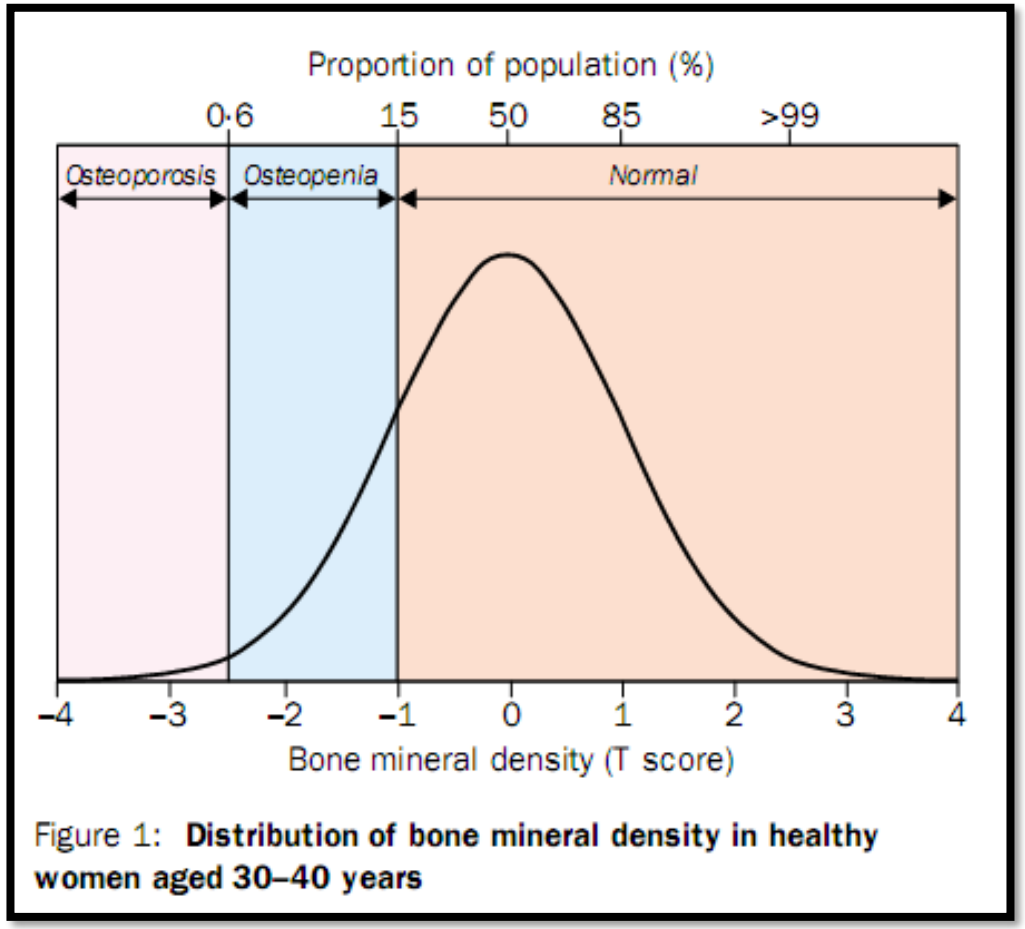
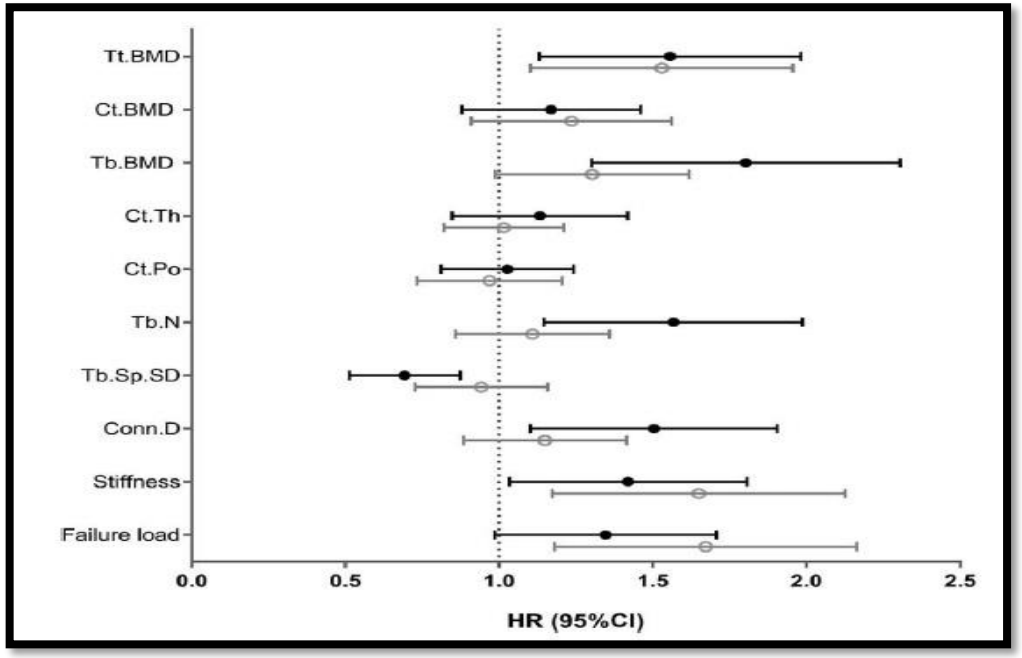


Figure 1: **Distribution of bone mineral density in healthy women aged 30–40 years**

Kanis, John A. *"Diagnosis of osteoporosis and assessment of fracture risk."* ***The Lancet 359.9321 (2002):*** *1929-1936.*

HR-pQCT and Diagnosis of Osteoporosis

- High-resolution peripheral quantitative computed tomography **(HR-pQCT)** is a promising noninvasive method for in vivo **3D characterization** of human bone



Bone Microarchitecture Assessed by HR-pQCT as Predictor of Fracture Risk in Postmenopausal Women: The OFELY Study

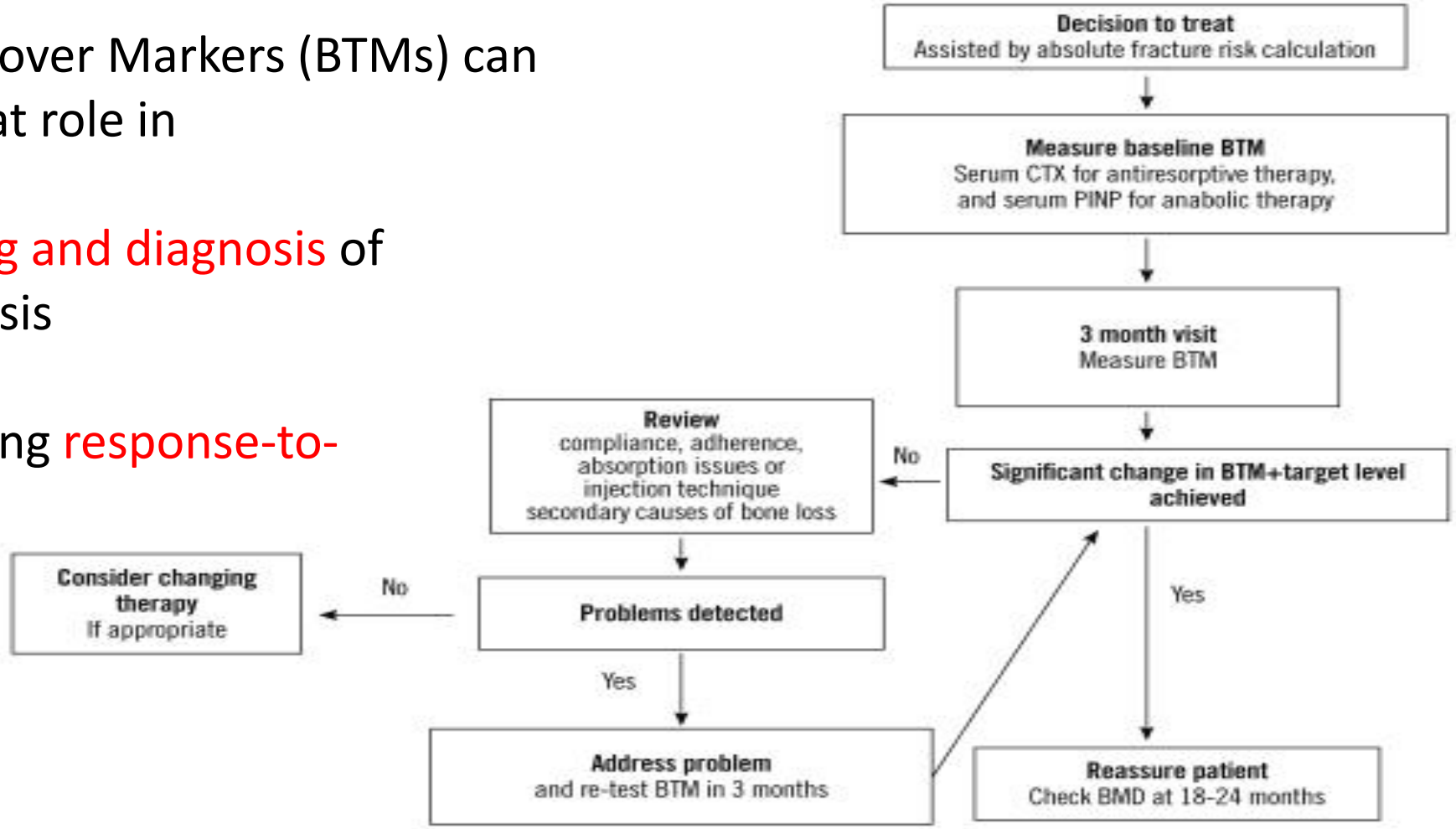
Metcalf, Louis M., et al. "Validation of calcaneus trabecular microstructure measurements by HR-pQCT." ***Bone 106 (2018)****: 69-77.*

Bone Turnover Markers (BTMs) and Diagnosis of Osteoporosis

Bone Turnover Markers (BTMs) can play a great role in

- Screening and diagnosis of osteoporosis

- Monitoring response-to-treatment



Bone Turnover Markers (BTMs)

- **BTMs** largely represent products of **bone proteins**, particularly type I collagen which form during **bone synthesis**

- Other BTMs are **products of bone cells**, reflecting the number of particular cells within the bone environment at any time.

*Khashayar, P., Aghaei Meybodi, H., Amoabediny, G., & **Larijani, B**. (2015). Biochemical markers of bone turnover and their role in osteoporosis diagnosis: a narrative review. Recent patents on endocrine, metabolic & immune drug discovery, 9(2), 79-89.*

BTM concentrations and prediction of fracture risk

- There are significant associations between **bone turnover markers** and incident **fracture risk** (though the association is modest).

- The concentration of **bone resorption** markers are more consistently associated with fracture risk than **bone formation** ones.

Morris, H. A., et al. "Clinical usefulness of bone turnover marker concentrations in osteoporosis." Clinica Chimica Acta467 (2017): 34-41.

Advantages of measurement of BTMs for assessment of fracture risk

- Samples of blood and urine easily collected
- Relative specificity for bone resorption or bone formation
- Variety of assays available
- Complementary information to BMD
- Changes in bone turnover markers occur earlier than changes in BMD

Morris, H. A., et al. "Clinical usefulness of bone turnover marker concentrations in osteoporosis." Clinica Chimica Acta467 (2017): 34-41.

Different types of BMTs and their clinical applications

- **BTMs that predict Bone Loss**
  - Bone Formation Markers: P1NP, BALP, OC
  - Bone Resorption Markers: DPD, NTX

- **BTMs that predict Fracture**
  - Bone Formation Markers: BALP, P1CP, P1NP, OC
  - Bone Resorption Markers: TRAP, CTX, NTX

BTMs to screen bisphosphonates adherence

- The IOF working group recommends measuring **PINP and CTX** at **baseline and 3 months** after starting therapy to check for a decrease above the least significant change (decrease of >38% for PINP and 56% for CTX).

- Detection rate for **PINP** is 84%, for **CTX** 87% and, if variation in at least one is considered when measuring both, the level of detection is 94.5%.

*Diez-Perez, Adolfo, et al. "International osteoporosis foundation and european calcified tissue society working group. Recommendations for the screening of adherence to oral bisphosphonates. " **Osteoporosis International 28.3 (2017)**: 767-774.*

Limitations of BMD and Measurement of BTM through ELISA and Similar Techniques

- **BMD (bone mineral density) :**
  - X-ray based
  - Detection only when BMD significantly decreased
  - Provides no information about the cause of osteoporosis (bone resorption / formation);
  - 2 years interval between tests needed to see significant changes in monitoring treatment

- **ECLIA & ELISA:**
  - Expensive
  - Not available everywhere
  - Skilled technician needed
  - Long waiting time in centralized laboratories

Diagnositc value of measurement of PINP and CTX

A 2014 meta-analysis found a modest, but significant association between both **PINP** and **CTX** concentrations at baseline and **fracture risk**

Studies of bone turnover markers to predict fractures in men and women not on treatment for osteoporosis.

Study	Population and setting	Age (years)	Expression of risk	Length of follow-up	Fracture type	Outcome
Johansson [11]	Meta-analysis, 6 prospective cohort studies, middle-aged or older men (2 studies) and women (4 studies)	>50	HR for fracture per SD in BTM (GR)	From 2 to 6.5 years	Different between studies: hip, non-vertebral, osteoporotic	HR per SD (95% CI). Different settings for adjustment. Fracture combined (hip, non-spine, osteoporotic, any, low-trauma) PINP HR = 1.23 (1.09–1.39) CTX HR = 1.18 (1.08–1.29) HR = 1.19 (1.05–1.34) (if women only) HR = 1.17 (1.04–1.31) (if adjusted for age) HR = 1.12 (0.97–1.29) (if adjusted for BMD) Hip fracture CTX HR = 1.23 (1.04–1.47) HR = 1.17 (0.95–1.44) (If women only)
Yoshimura [13]	307 middle-aged and elderly Japanese recruited by age- and gender –stratification in the Taiji cohort (147 men and 160 women), 32 with fractures	40–79	HR per SD	10 years	Osteoporotic (spine, pelvis, ribs, distal radius, forearm, humerus and hip)	HR per SD. However, HR are not shown in article, as no significant associations were found s-OC, s-tOC, s-BAP,s- PICP, s-PINP, s-ICTP, s-beta-CTX, s-NTX, u-PYR, u-DPD
Chubb [12]	4028 community-dwelling older men from Perth, Australia enrolled in the population-based Health In Men Study (HIMS), 114 with hip fractures, 3896 in control group	70–89	OR per SD in BTM	From 8 to 11 years	Hip fractures	OR per SD (95% CI) Log10(tOC) 1.20 (1.00–1.42) (after adjustment for age and GC use) Log10(PINP) and Log10(CTX-I) not significantly associated with incident hip fracture after adjustment for age and GC use ($P > 0.17$)

ICTP: C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinase; BAP: bone-specific alkaline phosphatase; beta-CTX: beta-isomeri… linking telopeptide of type I collagen; BTM: bone turnover marker; CI: confidence interval; CTX: C-terminal cross-linking telopeptide of type I collagen; DPD: deoxypyr… of collagen; GC: glucocorticoid; GR: gradient of risk; HR: hazard ratio; NTX: N-terminal cross-linking telopeptide of type I collagen; OC: intact osteocalcin; OR: odds ratio; PICP: C-terminal propeptide of type I collagen; PINP: N-terminal propeptide of type I collagen; PYR: pyridinoline cross-links of collagen; SD: standard deviation; tOC: total osteocalcin.

Save PDF to Evernote

Diez-Perez, Adolfo, et al. "International osteoporosis foundation and european calcified tissue society working group. Recommendations for the screening of adherence to oral bisphosphonates." ***Osteoporosis International 28.3 (2017)****: 767-774.*

Relationship between BTMs and Fracture Risk

- There is a significant association **between s-PINP and fracture risk**. The HR per SD increase in s-PINP (gradient of risk) was 1.23 (95% CI 1.09 -1.39) for men and women combined unadjusted for BMD.
- There is a significant association between **s-CTX and fracture risk**, GR = 1.18 (95% CI 1.05 – 1.34) unadjusted for BMD.
- For **hip fracture** as the outcome, the association between s-CTX and fracture risk was slightly higher, 1.23 (95% CI 1.04 – 1.47)

*Johansson, Helena, et al. "A meta-analysis of reference markers of bone turnover for prediction of fracture." **Calcified tissue international 94.5 (2014)**: 560-567.*

BTM concentrations for monitoring of treatment

- In the case of clinical trials for osteoporosis treatment the clinical end- point is **fracture** and the **surrogate biological markers are BTM**.

- **Effectiveness of treatment** following administration of alendronate, risedronate, zoledronic acid, raloxifene, and strontium ranelate anc be followed through **measurement of BTMs** during the first year of treatment.

- BTMs can be effectively used for monitoring **adherence** to **bisphosphonates** treatment

Jacques, Richard M., et al. "Relationship of changes in total hip bone mineral density to vertebral and nonvertebral fracture risk in women with postmenopausal osteoporosis treated with once-yearly zoledronic acid 5 mg: The HORIZON-Pivotal Fracture Trial (PFT)." ***Journal of Bone and Mineral Research*27.8 (2012):** 1627-1634.

Measurement of Response-to-treatment Using BTMs

- The change in PINP can effectively reflect **response-to-treatment** in:
  - Vertebral fracture
  - Hip fracture

- The effect explained by PINP was independent of that explained by total hip BMD, so the results of BMD and PINP in monitoring the **success of treatment** interventions are **complimentary.**

Studies of bone turnover markers following initiation of osteoporosis treatment.

Treatment	Trial	Author	N	BTM	Months	Change, %	Duration, yr	Fracture	Treatment effect explained
Zoledronic acid	HORIZON	Jacques [14]	1132	PINP	12	56	3	Vertebral	58%
Bazedoxifene (all)	International	Bruyere [16]	5244	CTX	12	CTX (46), OC (37)	3	Vertebral	CTX, 18% (3–41)
20 mg daily				OC		CTX (49), OC (39)			OC, 14% (0–46)
									CTX, 20% (4–44)
40 mg daily									OC, 4% (0—21)
									CTX, 25% (3–68)
									OC, 29% (0–85)

BTM abbreviations are as described for Table 1.

Jacques, Richard M., et al. "Relationship of changes in total hip bone mineral density to vertebral and nonvertebral fracture risk in women with postmenopausal osteoporosis treated with once-yearly zoledronic acid 5 mg: The HORIZON-Pivotal Fracture Trial (PFT)." ***Journal of Bone and Mineral Research*27.8 (2012):** 1627-1634.

Genetics and Osteoporosis

- The **heritability of osteoporosis** is estimated at **60% to 80%** in families and twins
- Certain **genetic determinants** contribute to **osteoporosis** and enhance the risk of fracture
- **Genomewide association** studies have identified polymorphisms in several genes related to low bone density and **osteoporosis**
- **In Iran**, several studies are being conducted to demonstrate the link between particular **genes and osteoporosis**

*Collet, Corinne, et al. "Primary Osteoporosis in Young Adults: Genetic Basis and Identification of Novel Variants in Causal Genes." **JBMR Plus 2.1 (2018):** 12-21.*

Proteomics in Bone Research

- Unlike imaging techniques such as dual-energy x-ray absorptiometry, (DXA), measurement of bone turnover markers allows for a **dynamic assessment** of bone remodeling

- The application of **proteomics** has led to discovery of new and sensitive bone turnover markers (BTMs) , which provide **unique information** for clinical diagnosis and treatment of patients with bone diseases

- **Quantitative proteomics** can be employed for detection of signaling dynamics, biomarkers which leads to **discovery of therapeutic targets**.

*Zhang, Hengwei, et al. "Proteomics in bone research." **Expert review of proteomics** 7.1 (2010): 103-111.*

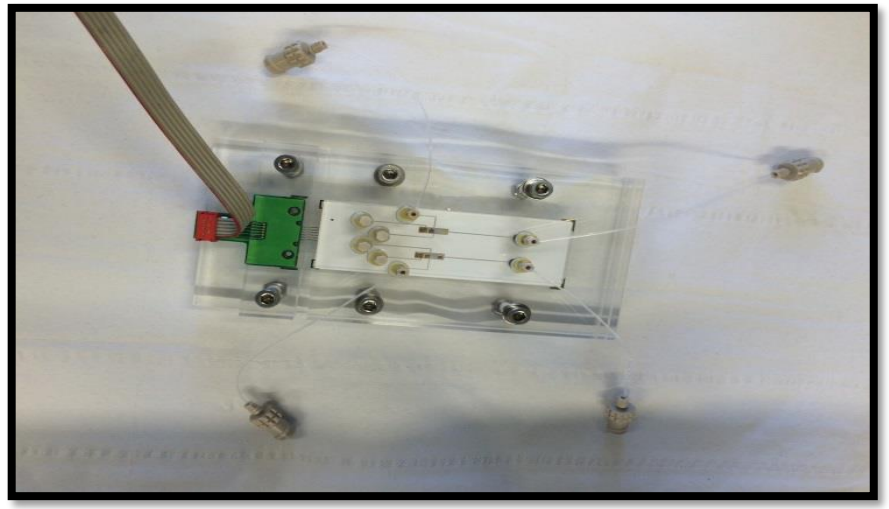
MicroRNAs and Osteoporosis

- MicroRNAs have been shown to play an **important role** in **bone homeostasis** and development of **osteoporosis**
- **Measurement of circulating microRNAs** is demonstrated to be a great diagnostic tool for diagnosis of several human diseases including **osteoporosis**
- In **osteoporotic patients**, up-regulation of **specific microRNAs** has been reported in *in-vivo* assessments
- It is suggested that modulation of specific microRNA expressions may have a key role in future **targeted therapies** of musculoskeletal diseases.

*Perez-Sanchez, Carlos, et al. "Circulating microRNAs as Potential Biomarkers of Disease Activity and Structural Damage in Ankylosing Spondylitis Patients." **Human molecular genetics (2018).***

Point-of-care diagnostic tools

- Recently, a **microfluidic chip** is invented in **Iran** for preventive screening and treatment monitoring for osteoporosis management



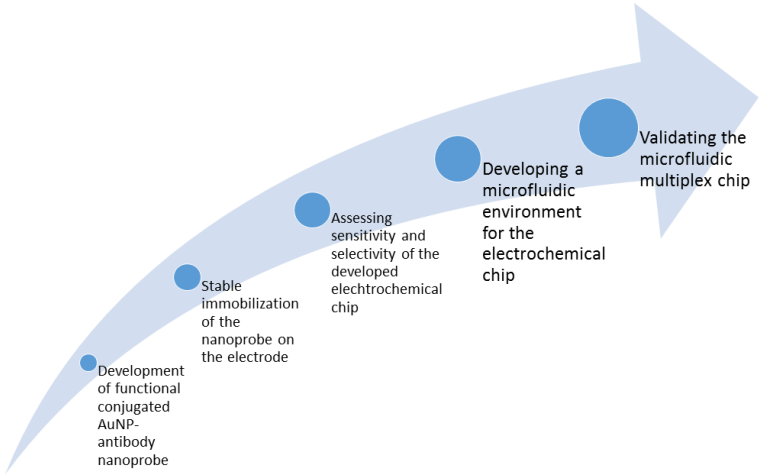
- This **point-of-care** (PoC) device, is **cheap** and capable of performing **rapid** analysis, with small volumes of sample, minimum number of assay steps, and **no need for highly skilled personnel** for routine check up and patient screening.

Limitations of the device

- Strength of the association between BTMs and the rate of bone loss largely depends on the **accuracy of their measurement** which is not high in POC devices

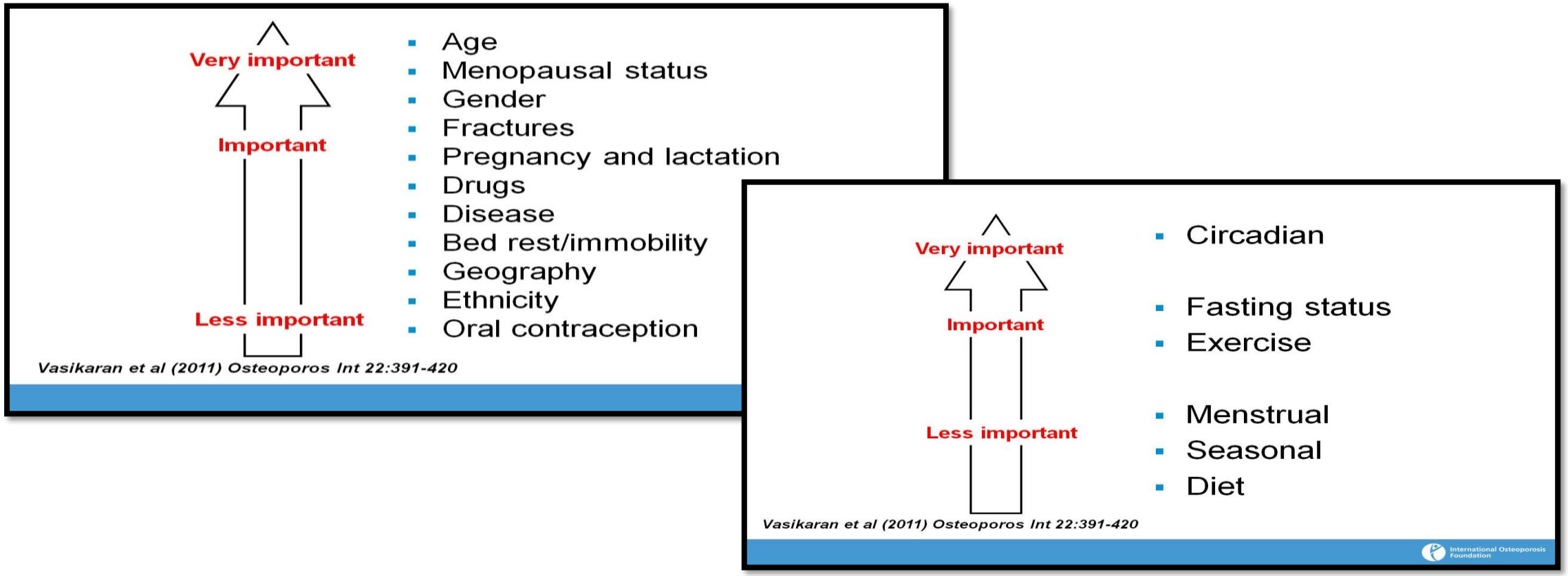
- Factors such as **age, daytime, underlying conditions, past drug** history can influence the results of measurement of BTMs

- Measurement of BTMs does not provide **information on structural abnormalities** of bone



	Oc	CTX
R^2	0.93	0.98
Reference Range	9-42 ng/mL	18-1008 ρg/mL
Dynamic Range	1-100 ng/mL	1-1000 ρg/mL
LOD	1.94 ng/mL	206.7 ρg/mL
LOQ	5.89 ng/mL	626.5 ρg/mL
Correlation	0.96	0.98

Factors which can influence BMT measurement



Measurement of PINP concentrations for diagnosis of osteoporosis

- Several studies have demonstrated great values for **PINP assays** as a BTM
- However, PINP values are not reliable in **bedridden** patients and those with **renal failure**
- Variation between **plasma or serum specimens** may affect the results

Regression equations describing the relationships of CTX values from the IDS automated assay and the IDS ELISA.

Method 1 (x)	Method 2 (y)	n	Slope (95% CI)	Intercept (95% CI) (ng/L)	Reference
ELISA	iSYS	156	1.266 (1.192–1.337)	−108.6 (−132.9 to −78.8)	Chubb et al. [53][a]
ELISA	iSYS	93	0.94 (0.81–1.10)	−5.91 (−54.47–42.69)	Huvelle et al. [55]

[a] Note: EDTA plasma specimens were used for these analyses.

Eastell, Richard, et al. "DIAGNOSIS OF ENDOCRINE DISEASE: Bone turnover markers: are they clinically useful?."*European journal of endocrinology* 178.1 (2018): R19-R31.

PoCOsteo project

- The objective of the **PoCOsteo project** is the development, clinical validation and preparation of a **Point-of-Care tool** for **osteoporosis** prevention, detection and treatment

- The consortium is consisted of **5 research partners** working on a well considered **workplan** devised.

- The base of the project is consisted of **proteomic and genomic electrochemical sensors**, available from 2 consortium partners

Main Objectives of PoCOsteo

- Filling **two important gaps** in the clinical armamentarium of osteoporosis management:
  - Early-stage identification of high-risk individuals who would best benefit from intervention
  - A low cost, accessible monitoring solution for those affected.

- The final device, which brings together **biomarker measurement**, profiling of **genetic variations** and assessment of the **underlying risk factors**, would be used as an **in-office tool** by physicians to enhance the predictive accuracy of fracture prognosis and to provide the affected individuals with **personalized care**.

POCOSTEO partners

Engineering Partners (Research Centers)

Clinical Partners

Engineering Partners (SMEs)

Industrialization and manufacturability

Industrial proof-of-concept demonstrating mass manufacturability at acceptable cost.

Integration

1 Requirements

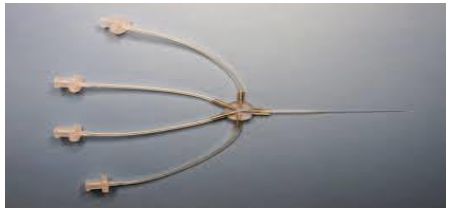
2 **Proteomic Sensor Development**

Fabricate a cost-effective, reproducible and robust electrode array to be used in electrochemical sensing of biomarkers

3 **Genomic Sensor Development**

Fabricate a cost-effective, reproducible and robust electrode array to be used in electrochemical sensing of genetic profile

4 **Microfluidic Manifold Development**



■ Proteomi

- CTX
- Oc
- P1NP
- BALP

■ Genomi

- Fx SNPs
- BMD SNPs
- miRNAs

■ Clinical Risk Facto

- FRAX

+

■ BM

- Hip BMD
- Lumbar BMD

Novel Multifaceted Approach to Screening and Diagnosis of Osteoporosis

- For provision of accurate picture of bone status a **multifaceted approach** and **personalized strategy** is necessary which should contain different information such as:
  - FRAX
  - Imaging
  - Proteomic
  - Genomic
  - CRF data

Conclusions

- Osteoporosis is a common disease all across the world with disastrous complications
- Diagnosis of osteoporosis is usually concurrent with different complications such as fractures and fall-related injuries
- All available techniques face serious limitations and shorcomings
- Novel approaches capable of combining different clinical, imaging, proteomics, and genomics data are necessary for a multifaceted and personalized approach to osteoporosis management

Thank you