Project Name	**A comparison of serum and urine N-telopeptide markers**	
Protocol Title	A comparison of Serum and Urine Bone Marker	
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	Name	**Title, Function**	**Signature**
Prepared by:	Lea El Hage	Principle Investigator	
Reviewed / Approved by:	***		

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1.0 INVESTIGATOR'S RESPONSIBILITIES

The Investigator is responsible for the data collection and the reporting of the clinical study information. The Investigator is also responsible for ensuring that the clinical study is conducted in compliance with all requirements of the clinical study protocol and all requirements as determined by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC).

2.0 INVESTIGATOR'S AGREEMENT

I, the investigator, acknowledge that I have read and understood this protocol. I agree to conduct and supervise this study according to Good Clinical Practices, applicable FDA regulations for protecting the rights, safety, and welfare of subjects under my care, and for the control of devices under investigation.

I agree to start the study only when appropriate institutional review board approval is obtained, if applicable. I will maintain accurate, complete, and current records relating to my participation in the study.

Investigator Signatures:

Investigator Name (Please Print)	Signature	Date

3.0 GLOSSARY

CCF	Cleveland Clinic Foundation
DEXA	Dual-energy X-ray absorptiometry
FRAX	Fracture Risk Assessment Tool
GFR	Glomerular filtration rate
IEC	Independent Ethics Committee
IRB	Institutional Review Board
PI	Principle Investigator
PTH	Parathyroid hormone
RC	Research coordinator
ROC	Receiver Operating Characteristic
SD	Standard deviation
SST	Serum Separator Tube

4.0 OBJECTIVE

This enrollment protocol is an effort to evaluate the correlation between serum and urine values of the bone marker of interest to help establish accuracy of serum values compared to urine.

Data collected in this clinical study may also be used for scientific publication purposes, authored by the study investigators or by scientists collaborating with the study investigator(s), for the advancement of scientific knowledge on bone diseases.

5.0 OVERVIEW

5.1 Background

Osteoporosis is the most common metabolic disorder in the United States and it is estimated that approximately 19% of women and 4% of men over the age 50 years have underlying disease. This number is expected to continue to rise. A silent illness at first, once presenting with fractures, it can lead to increased morbidity, mortality, and decreased quality of life. It carries large financial and societal burdens. Direct annual medical costs are estimated to be approximately 17 to 20 billion dollars in the United States alone [1]. Therefore, it is important to accurately identify those at high risk.

The present gold standard to diagnose osteoporosis is the Dual-energy X-ray absorptiometry (DEXA scan) [2] with a diagnosis based on a T-score of -2.5 SD or below in those without history of fragility fracture. However, many individuals fracture despite having normal or only mildly reduced scores. There are also several barriers within the DEXA technology including accessibility, cost, accurate reference ranges for age and demographic groups that result in missing large groups of people at risk for osteoporosis. The Fracture Risk Assessment Tool (FRAX score) is one tool that identifies those at higher risk of fracture that may benefit from therapy [3]. It was designed and released in 2008 and has been a great asset in clinical practice in stratifying risk and guiding management of osteopenic patients. However, FRAX may miss many individuals that may benefit from therapy due to its limited inclusion criteria [4].

Bone markers have been shown to predict fracture risk in postmenopausal women independent of bone mineral density and may help identify high risk individuals. Amino-terminal cross-linking telopeptides of type I collagen (NTX) reflects osteoclastic bone resorption. NTX can be measured in both the serum and in urine.

The accuracy of the serum NTX is unclear. It may be less sensitive than urine NTX in detecting bone density changes [5]. The urine NTX overcomes circadian rhythm changes to bone density and is less sensitive to dietary collagen intake [6,7]. At present, urine markers need to be checked as a second void of the day which may be cumbersome for patients. Serum levels drawn with other bone labs would be

easier to obtain than second void urine collections. We would like to evaluate the correlation between serum and urine NTX in patients with osteopenia with no prior history of osteoporotic treatment. If the urine and serum markers are equivalent methods, serum levels would be preferred to identify high risk patients at risk of disease due to ease of collection.

5.2 Study Site

This is a prospective blood and urine collection study under the supervision of clinical researchers. Subjects will be enrolled at the CCF

5.3 Overall Study Design

This is a prospective specimen collection cohort study to evaluate the correlation between serum and urine values of the bone marker of interest, and their association with baseline DEXA scan measures and fracture risk within 6 months.

Study samples will be obtained longitudinally. One collection of both serum and urine collection will be obtained. The urine will be collected as second void of day and at the same time the blood collection is drawn. Study will continue for a period or 1 year, with plan to enroll around 40 subjects.

5.4 Subject Enrollment

Subjects will be enrolled prospectively, under Institutional Review Board (IRB) approval and patient informed consent. Enrollment will include patients diagnosed with osteopenia on DEXA scan who have not been on any medical therapy in the past. Recruitment will occur via phone, mail, virtual or in-office visit. Subjects will be consented in an office visit. Subjects will be identified either during an in-office visit, referral by colleague or via ICD code EPIC inquiry.

5.5 Inclusion and Exclusion Criteria

Subject Inclusion criteria

- Consent to participate in the study
- Participants limited to subjects in the CCF
- Age between and inclusive of 18 and 85 years of age
- No gender exclusion
- Patients diagnosed with Osteopenia on DEXA scan who have not been on any medical therapy in the past
- Presence of normal vitamin D levels, kidney function, and parathyroid hormone levels (per our reference ranges)

Sample Inclusion Criteria

- Serum sample collected in Serum Separator Tubes (SST) Gold tube and urine sample collected in clean container
- Samples to be collected only at a CCF Laboratory based on standard of care for the CCF
- Samples to be collected at the same time of day. Urine NTX will be collected as the second void of the day

Subject Exclusion criteria

- Prior history of medical therapy for osteopenia
- Prior radiation therapy
- Prior history of bone fracture
- History of high risk medication associated with increased risk of fracture
- Presence of abnormal vitamin D levels, kidney function, and parathyroid hormone
- Pregnancy status (verbal)
- Those with medical co-morbidities that increase the risk of fracture will be excluded and these include but are not limited to: rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, diabetes mellitus, hyperparathyroidism, chronic kidney disease, pituitary disease, multiple myeloma, leukemia, lymphoma, thalassemia major, HIV/AIDS, malabsorption, Inflammatory bowel disease, chronic obstructive pulmonary disease, hypogonadism, chronic liver disease, untreated hyperthyroidism, and those with chronic immobility

Sample Exclusion Criteria

- Grossly hemolyzed (red) or turbid samples
- Samples with visually detected microbial contamination
- Sample not handled or stored as per sample handling instructions, which will be provided to the study sites at the time of the study initiation visit
- Urine sample that is not collected as second void of the day

5.6 Required Information

This section lists the main information that will be collected for each subject by the Principal Investigator (or designee). Data will be captured in RedCap and will adhere to the data management plan as outlined in section 6.0.

At enrollment:

- Demographics
- Age and Gender
- Date of osteopenia diagnosis
- Bone mineral density and T-score values of latest DEXA scan
- Medical history; including history of any relevant concomitant conditions per CRF

- Date and time of blood draw and urine collection
- Calcium supplementation, duration and dosing
- Vitamin D supplementation, duration and dosing
- Laboratory such as:
    - Vitamin D level
    - PTH level
    - Calcium level
    - Alkaline phosphatase level
    - Creatinine level
    - GFR

5.7 Sample Collection

- Consent subjects prior to blood draw and urine collection
- Laboratory tests will only be collected at CCF laboratory facilities per CCF standard of care
- The serial number labels should be affixed to each collection tube or container prior to the collection
- Patient samples will be serum from SST tubes Gold tubes and urine test from clean container. CPT code 82523
- Standard venipuncture must be done in accordance with institutional standards and requirements to collect whole blood
- A 2 mL SST tube of whole blood will be collected at study enrollment onset
- Standard urine collection will be done in accordance with institutional standards. The urine collection will be second void of the day
- A 5 mL clean container of second void of the day urine will be collected at study enrollment onset

5.8 Sample Handling

Will be handled per CCF laboratory standards

5.8.1 Stability

The urine and serum sample can be kept at ambient temperature for 1 day, if refrigerated for 7 days and if frozen for 14 days

5.9 Standard of Care Assessments

The study will collect the results of any procedures performed as per standard of care.

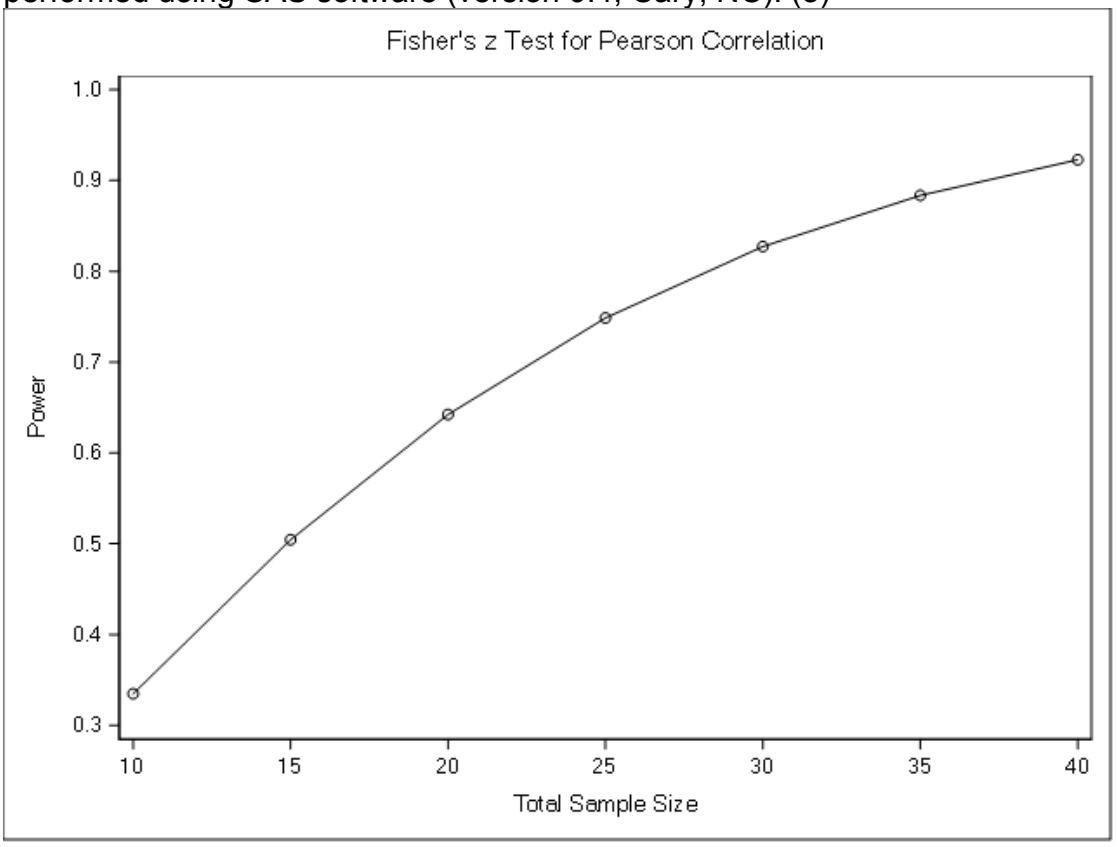
5.10 Statistical Analyses

Pearson or Spearman correlations will be used to measure the association between serum and urine markers. Similar correlation measures will be used to measure associations with DEXA scan results, and Comparisons of the correlations were performed using the cocor package in R software (version 4.0;

Vienna, Austria) using the methods by Meng, Rosenthal, and Rubin (1992) to compare the correlations and calculate 95% confidence intervals for their difference. As an exploratory analysis, associations between serum and urine levels with fracture risk will be evaluated using two-sample t-tests and, if feasible, ROC analyses will be used to compare the predictive ability of each marker. If the number of fractures is small, this analysis will not be performed. If the follow-up through 6 months cannot be measured consistently across patients, alternate methods including time to event analysis may be used to account for differential follow-up.

5.11 Sample Size Justification

The primary sample size calculation was based on the ability to detect a moderate correlation (r=0.5) between serum and urine levels. With 40 patients, there will be 90% power to detect moderate correlations based on a two-sided test of the Fisher's Z test of the Pearson correlation (Image 1). Power calculations were performed using SAS software (version 9.4; Cary, NC). (8)



6.0 DATA MANAGEMENT

The Principal Investigator (PI) is responsible for ensuring that all data are submitted in a timely fashion.

6.1 Data Management

We will use RedCap database, only CCF MRN as identifier, assign serial number to each unique patient and store data on a Cleveland Clinic Shared Drive which will be accessed on workstations requiring login credentials. Once data are collected, we will de-identify the rows and use only serial numbers.

6.2 Data Clarification

During the conduct of the study, if a question regarding the data arises, the Monitor and/or Delegate will work with the site to resolve the issue. All communications will be documented in a query via the electronic database.

7.0 ADVERSE EVENT REPORTING

Unanticipated Non-Serious Adverse Event Reporting:
Unanticipated non-serious adverse events assessed by the PI as being either caused by, or related to, the study procedures, will also be reported using the Adverse Event Report form.

The following adverse events are anticipated as a result of the blood collection required per the study procedures: pain, bruising, bleeding or infection at the site where the blood was collected from the subject. Fainting may also occur as a result of blood collection. Because these events are anticipated, they do NOT need to be reported using the Adverse Event Report form as long as they are not assessed as serious. If assessed as serious, then they need to be reported.

7.1 Date of Awareness

A test site becomes aware of an MDR reportable event when medical personnel who are employed by or otherwise formally affiliated with the facility acquire information that reasonably suggests that a reportable event has occurred. Medical personnel include persons who are licensed, registered, or certified to administer healthcare, who have received a diploma or a degree in a professional or scientific discipline, or who are responsible for receiving medical complaints or adverse event reports or who supervise such persons.

8.0 ADMINISTRATION

8.1 Ethics Committee or Institutional Review Board Review

It is the responsibility of the Investigator to provide the Study Monitor or designee with written documentation demonstrating that this study has been approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC). A copy of the signed documentation must be returned to the study monitor or designee.

8.2 Investigator Delegation of Responsibilities

The Investigator's decision to delegate study related responsibilities to his study personnel will be recorded on the ***Principal Investigator Delegation of Responsibilities and Signature Log***.

8.3 Disposition of Data

All data and information obtained during this evaluation are the property of CCF and may be used in support of regulatory submissions.

8.4 Record Retention

The Principal Investigator will ensure that study records are maintained in accordance with IRB/IEC
At a minimum the following documentation will be maintained:

- All correspondence with the IRB/IEC, study monitor or designee, including required reports
- The protocol and regulatory documentation
- Copies of all data

8.5 Protocol Amendments

Amendments to this protocol will be signed by the Investigator, and approved or exempted by IRB/IEC as required, prior to implementation.

8.6 Deviations from the Agreed Protocol

Deviations from the written protocol should be avoided. If deviations occur, they must be reported immediately to the Study Monitor or designee and recorded in a Protocol Deviation Memorandum.

9.0 REFERENCES

1. Becker, D.J., M.L. Kilgore, and M.A. Morrisey, The societal burden of osteoporosis. Curr Rheumatol Rep, 2010. 12(3): p. 186-91.
2. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser, 1994. 843: p. 1-129.
3. Kanis, J.A., et al., FRAX and its applications to clinical practice. Bone, 2009. 44(5): p. 734-43.
4. Silverman, S.L. and A.D. Calderon, The utility and limitations of FRAX: A US perspective. Curr Osteoporos Rep, 2010. 8(4): p. 192-7.
5. Abe Y, Ishikawa H, Fukao A (2008) Higher efficacy of urinary bone resorption marker measurements in assessing response to treatment for osteoporosis in postmenopausal women. Tohoku J Exp Med 214:51–59

6. Cremers S, Garnero P (2006) Biochemical markers of bone turnover in the clinical development of drugs for osteoporosis and metastatic bone disease: potential uses and pitfalls. Drugs 66:2031–2058

7. Clemens JD, Herrick MV, Singer FR et al (1997) Evidence that serum NTx (collagen-type I N-telopeptides) can act as an immunochemical marker of bone resorption. Clin Chem 43:2058–2063

8. Meng XL, Rosenthal R, Rubin DB. Comparing Correlated Correlation Coefficients. Psychol Bull. 1992; 111:172-175.