AWARD NUMBER: W81XWH-19-1-0373

TITLE: Clinical Assessment of Vertebral Bone Quality Using Direct Biomechanical and Textural Analysis via Digital Tomosynthesis

PRINCIPAL INVESTIGATOR: Yener N. Yeni, Ph.D.

CONTRACTING ORGANIZATION: Henry Ford Health System

REPORT DATE: October 2020

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Oct 2020	Annual	9/15/2019 - 9/14/2020

4. TITLE AND SUBTITLE

Clinical Assessment of Vertebral Bone Quality Using Direct Biomechanical and Textural Analysis via Digital Tomosynthesis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-19-1-0373

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Yener N. Yeni, Ph.D. (PI); D. Sudhaker Rao, MD (Partnering-PI)

E-Mail: yeni@bjc.hfh.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Henry Ford Health System
Bone & Joint Center,
Department of Orthopaedics

HFH/WSU Integrative Biosciences (iBio) Research Bldg.
6135 Woodward Avenue
Detroit, MI 48202

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Osteoporosis (OP) is one of the most common diseases affecting aging individuals and vertebral fractures are the most common type of fracture, complicating osteoporosis. Current techniques for assessing bone strength and osteoporotic fracture risk may be inaccurate and ultimately measure surrogates for biomechanical properties. The proposed research idea is based on the digital volume correlation (DVC) technique, which allows for measurement of displacements inside a porous object such as a vertebra, in response to an applied load. In using DVC combined with digital tomosynthesis (DTS), we can measure the in vivo, patient-specific biomechanical response of vertebrae to load. The overall objective of the proposed research is to clinically validate new DTS-based textural and DVC methods for identification of patients at risk of vertebral fractures in clinically significant cohorts.

15. SUBJECT TERMS

Vertebral fracture, digital tomosynthesis, image analysis, vertebral bone stiffness, cancellous bone texture, osteoporosis, primary hyperparathyroidism, type 2 diabetes

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	18	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

INTRODUCTION

Osteoporosis (OP) is one of the most common diseases affecting aging individuals and vertebral fractures are the most common type of fracture, complicating osteoporosis. In the absence of an existing fracture, the current standard for assessing osteoporosis is bone mineral density (BMD) as measured from dual x-ray absorptiometry (DXA). BMD is useful in predicting bone strength and incident low-trauma fractures but when used alone, predictions can be inaccurate. From a mechanical point of view, the determinants of a bone fracture are bone material quality, its structural organization, and the mechanical loading environment. While helpful in describing the biomechanical integrity of the vertebrae, current techniques fail to take into account at least one of these determinants, and ultimately measure surrogates for biomechanical properties. Complicating this situation is the presence of other metabolic diseases that weaken bone and increase fracture risk, but do not necessarily reduce bone density. Among these diseases, primary hyperparathyroidism (pHPT) and type 2 diabetes are well recognized. Given the spectrum of dramatically different structural and material factors that can result in similar outcomes, it is difficult to assess bone strength and fracture risk using a surrogate measure that can only partially take into account these biomechanical factors. The proposed research idea is based on the digital volume correlation (DVC) technique, which allows for measurement of displacements inside a porous object such as a vertebra, in response to an applied load. In order to bring this technology into the clinical realm, we use DVC combined with digital tomosynthesis (DTS). DTS is a linear cone beam tomographic imaging system with high resolution at a depth of interest within the object and substantially lower radiation exposure than a CT exam. The overall objective of the proposed research is to clinically validate new DTS-based textural and DVC methods for identification of patients at risk of vertebral fractures in clinically significant cohorts.

KEYWORDS

Vertebral fracture, digital tomosynthesis, image analysis, vertebral bone stiffness, cancellous bone texture, osteoporosis, primary hyperparathyroidism, type 2 diabetes

ACCOMPLISHMENTS

What were the major goals of the project?

Major goals as stated in the SOW are detailed below with milestones/target dates (“Target date”) for important activities and phase. Completion dates or percentage of completion are noted in the “Status” column. The responsible PI is noted for each subtask (underlined).

Research-Specific Tasks:	**Months**	**Status**	**Target date**
Major Task 1: IRB, Regulatory Review and Approval Processes	**1-3**	**Complete as of 12/27/2019**	**12/15/2019**
Subtask 1: Prepare, submit and activate local institutional review board (IRB) protocol and informed consent form. (Yeni, Rao)	1	Complete as of 11/12/2019	10/15/2019
Subtask 2: USAMRMC ORP HRPO regulatory review and approval processes. (Yeni, Rao)	2-3	Complete as of 12/27/2019	12/15/2019
Milestone Achieved: Regulatory approval complete in preparation for recruitment	3	Milestone Achieved 12/27/2019	12/15/2019
Specific Aim 1: To determine the extent to which DTS-based texture and biomechanical analyses can separate subjects with established vertebral fractures (fx) from control subjects without a fracture.			
Major Task 2: Recruitment and imaging of vertebral fracture patients and non-fracture control patients	**2-33**	**In progress**	**6/15/2022**
Subtask 1: Identify and recruit subjects *with* prevalent vertebral fracture. Identify and recruit control subjects with low or normal bone mass, but *without* prevalent vertebral fracture for Aims 1-3. (Rao)	2-31	14% complete	4/15/2022

Subtask 2: Coordinate collection of serum and urine biomarker data from clinical tests. (Rao)	2-32	14% complete	5/15/2022
Subtask 3: Coordinate and perform screening of candidate patients using dual x-ray absorptiometry imaging for bone density. Calculate and record lumbar spine BMD and TBS at the time of DXA scans. Perform lateral x-rays for vertebral fracture assessment as needed. (Yeni, Rao)	3-32	14% complete	5/15/2022
Subtask 4: Schedule and perform DTS imaging. (Yeni)	3-33	14% complete	6/15/2022

Target total accrual of patients by quarter (fracture cases: 50 Female, 12 Male, Controls *without* fracture: 50 Female, 24 Male).

	Y1Q1	Y1Q2	Y1Q3	Y1Q4	Y2Q1	Y2Q2	Y2Q3	Y2Q4	Y3Q1	Y3Q2	Y3Q3	Y3Q4
Fracture planned	-	6	12	18	24	30	36	42	48	54	62	-
Fracture current status	-	4	5	8								
No Fracture planned	-	-	8	16	24	32	40	48	56	64	74	-
No Fracture current status	-	10	10	11								

Milestone Achieved: Aim 1 patient recruitment and imaging complete.	33	In progress	6/15/2022
Milestone Achieved: Aim 1 BMD, TBS and biomarker data collection complete.	33	In progress	6/15/2022
Major Task 3: Image analysis: Fracture patients and non-fracture control patients	**4-33**	**In progress**	**6/15/2022**
Subtask 5: Perform Vertebral texture and geometry analyses. (Yeni)	4-32	14% complete	5/15/2022
Subtask 6: DVC preprocessing (Align images via rigid body registration, segmentation, prepare analysis volumes, create DVC parameter files). (Yeni)	4-31	14% complete	4/15/2022
Subtask 7: DVC analysis: Execute DVC analysis. Post-process DVC displacements to produce segmented volumetric displacement maps. (Yeni)	6-32	14% complete	5/15/2022
Subtask 8: Calculate mechanical parameters from DVC displacements. (Yeni)	6-33	14% complete	6/15/2022
Milestone Achieved: Aim 1 image analysis and data collection complete.	33	In progress	6/15/2022

Specific Aim 2: To determine the extent to which DTS can detect differences between subjects (without fracture) who have established primary hyperparathyroidism (pHPT) and normal controls.			
Major Task 4: Recruitment and imaging of pHPT patients	**2-33**	**In progress**	**6/15/2022**
Subtask 1: Identify and recruit subjects with pHPT, but without fracture. (Rao)	2-31	7% complete	4/15/2022
Subtask 2: Coordinate and perform screening of candidate hyperparathyroidism patients using dual x-ray absorptiometry imaging for bone density. Lumbar spine BMD and TBS will be calculated. Perform lateral x-rays for	3-31	7% complete	4/15/2022

vertebral fracture assessment as needed. (Yeni, Rao)			
Subtask 3: For those patients without vertebral deformities, schedule and perform DTS imaging. (Yeni)	3-33	7% complete	6/15/2022

Target total accrual of hyperparathyroidism patients (50 male, 20 female) by quarter.												
	Y1Q1	Y1Q2	Y1Q3	Y1Q4	Y2Q1	Y2Q2	Y2Q3	Y2Q4	Y3Q1	Y3Q2	Y3Q3	Y3Q4
pHPT planned	-	7	14	21	28	35	42	49	56	63	70	-
pHPT current status	-	2	2	4	5							

Milestone Achieved: Aim 2 patient recruitment and imaging complete.	33	In progress	6/15/2022
Milestone Achieved: Aim 2 BMD, TBS, biomarker data complete.	33	In progress	6/15/2022
Major Task 5: Image analysis: pHPT patients	**4-33**	**In progress**	**6/15/2022**
Subtask 4: Perform Vertebral texture and geometry analyses. (Yeni)	4-32	7% complete	4/15/2022
Subtask 5: DVC preprocessing (Align images via rigid body registration, segmentation, prepare analysis volumes, create DVC parameter files). (Yeni)	4-31	7% complete	4/15/2022
Subtask 6: DVC analysis: Execute DVC analysis. Post-process DVC displacements to produce segmented volumetric displacement maps. (Yeni)	6-32	7% complete	5/15/2022
Subtask 7: Calculate mechanical parameters from DVC displacements. (Yeni)	6-33	7% complete	6/15/2022
Milestone Achieved: Aim 2 image analysis and data collection complete.	33	In progress	6/15/2022

Specific Aim 3: To determine the extent to which DTS can detect differences between subjects (without fracture) who have established diabetes and normal controls.			
Major Task 6: Recruitment and imaging of diabetic patients	**2-33**	**In progress**	**6/15/2022**
Subtask 1: Identify and recruit patients with established type 2 diabetes. (Rao)	3-31	11% complete	4/15/2022
Subtask 2: Coordinate and perform screening of candidate diabetic patients using dual x-ray absorptiometry imaging for bone density. Lumbar spine BMD and TBS will be calculated. Perform lateral x-rays for vertebral fracture assessment as needed. (Yeni, Rao)	3-32	11% complete	5/15/2022
Subtask 3: For those patients without vertebral deformities, schedule and perform DTS imaging. (Yeni)	3-33	11% complete	6/15/2022

Target total accrual of diabetic patients (50 male, 20 female) by quarter.												
	Y1Q1	Y1Q2	Y1Q3	Y1Q4	Y2Q1	Y2Q2	Y2Q3	Y2Q4	Y3Q1	Y3Q2	Y3Q3	Y3Q4
DM planned	-	7	14	21	28	35	42	49	56	63	70	-

DM current status	-	7	7	8								

Milestone Achieved: Aim 3 patient recruitment and imaging complete	33	In progress	6/15/2022
Milestone Achieved: Aim 3 BMD, TBS, biomarker data complete.	33	In progress	6/15/2022
Major Task 7: Image analysis: Diabetic patients	**4-33**	**In progress**	**6/15/2022**
Subtask 4: Perform Vertebral texture and geometry analyses. (Yeni)	4-32	11% complete	5/15/2022
Subtask 5: DVC preprocessing (Align images via rigid body registration, segmentation, prepare analysis volumes, create DVC parameter files). (Yeni)	4-32	11% complete	5/15/2022
Subtask 6: DVC analysis: Execute DVC analysis. Post-process DVC displacements to produce segmented volumetric displacement maps. (Yeni)	6-32	11% complete	5/15/2022
Subtask 7: Calculate mechanical parameters from DVC displacements. (Yeni)	6-33	11% complete	6/15/2022
Milestone Achieved: Aim 3 image analysis and data collection complete.	33	In progress	6/15/2022

Major Task 8: Data analysis (Yeni, Rao)	**34-35**	**Not started**	**8/15/2022**
Milestone Achieved: Statistical analysis complete and data prepared for publication and report to funding agency.	35	Not yet complete (0% complete)	8/15/2022
Major Task 9: Publications, reports and proposal writing (Yeni, Rao)	**35-36**	**Not started**	**9/15/2022**
Milestones Achieved: Writing of manuscript(s) for dissemination of the findings, writing of a final report to funding agency.	36	Not yet complete (0% complete)	9/15/2022

What was accomplished under these goals?

Major Task 1: IRB, regulatory review and approval processes for the project (Yeni/Rao).
IRB and HRPO approvals have been granted for the research project. HRPO review of human subjects protocols and approval took slightly longer than anticipated (recruitment was delayed until December 27).

Major Tasks 2, 4, & 6: Recruitment and imaging of participants (Yeni/Rao).

Recruitment (Rao): Thirty two participants have been recruited. The current breakdown by aim is as follows. Aim 1: 8 fracture patients and 11 non-fracture control patients. Aim 2: 5 pHPT patients. Aim 3: 8 Diabetic patients. A complete breakdown of the testing goals by group and current population is shown below (**Tables 1-2**). Collection of serum and urine biomarker data is current where appropriate for recruited participants.

During the reporting period, Dr. Rao and Ms. Warner have identified and performed screening (chart review) of 3,205 patients' medical records. From the 3,205 patients screened, 2090 were directly contacted for followup to determine eligibility. At the time of this report, there are currently an additional 40 participants identified through these efforts who are scheduled for upcoming testing dates through Y2Q1. An additional 33 participants have been confirmed as eligible and are pending scheduling for the coming weeks.

Due to the COVID-19 pandemic, both patient recruitment and imaging were halted in March, 2020 and did not resume until August 10, 2020. During the interim period, our study coordinator continued screening charts and identified over 100 likely eligible candidates for the study. We are currently testing patients as frequently as possible while following rules set forth by the institution. At the current rate of 4 patients per week, we anticipate rejoining the proposed cumulative recruitment target by the end of the upcoming quarter (Y2Q1).

Table 1: Participants recruitment goals and current population breakdown by aim.

Aim	Group	Goal	Current
1	Fracture Females	50	6
	Fracture Males	12	2
	Normal BMD Females	25	1
	Normal BMD Males	12	1
	Low BMD Females	25	8
	Low BMD Males	12	1
2	Hyperparathyroid Females	50	5
	Hyperparathyroid Males	20	0
3	Diabetes Females	50	5
	Diabetes Males	20	3
	Total	276	32

Table 2: Demographics of recruited participants

Demographics	Count
Male	7
Female	25
White	25
Black	7
Hispanic	0
Mean Age	64

Imaging (Yeni): All thirty two recruited participants have been imaged using two imaging techniques. First, bone mineral density (BMD), vertebral fracture analysis (VFA), and trabecular bone score (TBS) were measured using Dual Energy X-Ray Absorptiometry (DXA). Four images were taken of each participant using DTS: standing with an anterior view, standing with a lateral view, laying with an anterior view and laying with a lateral view. The collimation window is centered about T12 vertebra during imaging. There is a ten minute rest period between the standing and laying. There have been no adverse effects related to this study and participants have reported feeling comfortable throughout all procedures.

Before testing, all participants signed informed consent and HIPAA forms, which are being stored in a locked cabinet in the PI's office. Questionnaire forms used to determine eligibility are stored in a locked cabinet in the PI's office. All participant data (imaging metadata, imaging reports, and research data spreadsheets) stored on BJC computers have been deidentified and labeled only by unique study specific participant IDs. BJC computers and file cabinets are located in a locked room within the iBio building; the iBio building requires two levels of card access and an additional passcode. All HFHS computer systems are password-protected and data is protected using a 256-bit AES encryption algorithm. Data is backed up to a HFHS maintained NAS server with access permissions limited to the study team.

To ensure patient and personnel safety during the COVID-19 pandemic, we received approval from the IRB for a planned change indicating additional precautions to minimize transmission risk. These changes include, for example, screening participants by phone prior to arrival, taking temperatures of participants and staff daily, washing hands upon arrival, wearing masks at all times and gloves as appropriate, social distancing, adherence to disinfection of high touch surfaces, and post-visit monitoring.

Major Tasks 3, 5, & 7: Image analysis of tested participants (Yeni).

Three sets of image analysis are used for each participant: digital volume correlation (DVC), textural, and geometric analyses. Currently all tested participants have been analyzed using DVC for both anterior views and lateral views. Textural and geometric analyses have also been performed for all currently tested patients.

During DVC of the anterior views, a minimum of the T11, T12, and L1 vertebrae are analyzed for each participant. Based on the imaging results, sometimes the T10 or L2 vertebrae are also able to be processed. We have processed the DVC results for all available vertebrae from all currently tested participants. During DVC of the lateral views, a minimum of the T12 and L1 vertebrae are analyzed for each participant. Based on the imaging results, sometimes the T10, T11 or L2 vertebrae are also able to be processed.

Texture analysis of the images includes fractal analysis, line fraction deviation (LFD) and mean intercept length (MIL) analysis. These tests are being used to process all vertebrae included in DVC analyses, for both anterior and lateral views. During the reporting period, existing texture analysis protocols (i.e., those used for cadaver specimens) were adapted for in vivo images of the spine. These changes were necessary due to issues with bone orientation in the 3D image space. For example, when imaging human vertebrae in vitro, textural analyses are typically performed using a cuboid volume of interest for which anatomical axes are well-aligned with image axes. Using the same approach is not practical *in vivo*, for example due to spine curvature. For *in vivo* spine images anatomical axes of each vertebra are different for each vertebral level, owing to the natural curvature of the spine. During the current reporting period, a protocol has been developed for preparing analysis volumes from *in vivo* spine images and coded into a semi-automated image cropping procedure. This protocol is described below (in section “Efforts during COVID-19 period”) and has been applied to images from all currently tested patients.

Geometric analysis of the images includes measures of vertebral size derived from the DTS images, including vertebral height, width, area, and endplate depth. Similar to textural analysis, geometric analysis required establishing the method for dealing with spine curvature. During the reporting period, standard in vitro analysis protocols were coded into a semi-automated image analysis procedure for in vivo images. This protocol is described below (in section “Efforts during COVID-19 period”) and has been applied to images from all currently tested patients.

Image quality for tomosynthesis reconstructions, and in turn calculated displacement distributions, were as expected based on previous experience. Image quality was also sufficient to calculate displacement and stiffness values (**Figure 1**).

For the 32 participants considered in this report, the primary study variables measured from both AP and LM acquisitions (i.e., S_{DVC}, vertebral stiffness; and D_{DVC}, overall displacement) agree with the reference range established during our previous studies (**Figure 1**). The range of displacements is generally larger for the current work. This is not surprising, as the current project includes a more diverse cohort of subjects.

As in the reference data, which were acquired in AP view, small negative mean displacements (i.e., elongation rather than compression) were observed for some bones (9/120) in AP acquisitions and for a larger number of bones (45/104) in LM acquisitions. These resulted in large negative stiffness values (note stiffness has a reciprocal relationship with displacement). We are currently examining image quality for these bones including presence of artifacts and appropriate definition of analysis volume. It is possible that displacements as small as measurement error are recorded as negative values. If we conclude this is the case, we will consider defining a threshold displacement below which the vertebra can be categorically considered to be strong with low risk of fracture. Alternatively, it is possible to observe a small negative mean displacement (elongation) if excessive rotations occur during standing, as in the case of forward bending. The higher number of observations for the negative occurrences in the LM view may be because such rotations are more visible in this view. We are quantifying in- and out-of plane rotations in addition to axial displacements to better interpret these occurrences.

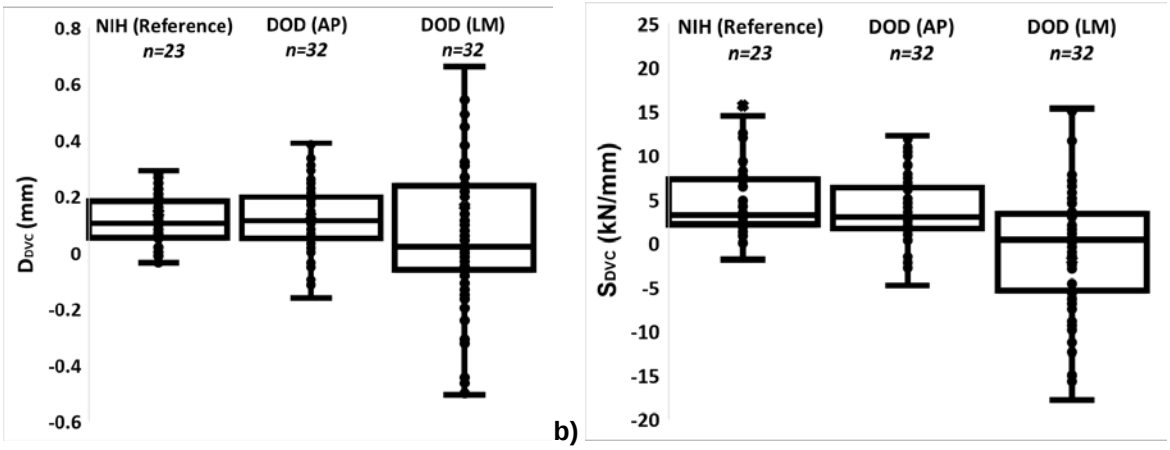

Figure 1. (a) Distribution of vertebral displacement (D_{DVC}) and (b) distribution of vertebral stiffness (S_{DVC}) from 32 participants. The values include vertebral levels from Thoracic 9 through Lumbar 2, and are presented separately for AP and LM acquisitions. The displacement and stiffness values agree with those obtained from an independent group of participants imaged in AP acquisition in a previously conducted pilot study shown as "NIH (Reference)".

BMD measurements performed using standard clinical techniques for the 32 patients included in this report fall expected reference ranges and are presented in **Table 3**.

Table 3: Range of BMD, BMC, T-Score, and TBS values by study group (minimum - maximum).

Patient group	BMD (g/cm^2)	BMC (g)	T-Score	TBS
Type 2 Diabetes	0.739 to 1.319	39.69 to 116.33	-2.8 to 1.9	1.188 to 1.491
Primary hyperparathyroidism	0.711 to 1.163	38.77 to 58.98	-4 to 0.8	1.238 to 1.465
Non-fracture control	0.752 to 1.016	35.17 to 60.08	-2.8 to -0.4	1.151 to 1.438
Vertebral Fracture	0.684 to 0.965	34.67 to 56.08	-3.4 to -0.7	1.05 to 1.327

Measurements of vertebral geometry from AP (**Table 4**) and LM (**Table 5**) acquisitions for the 32 participants included in this report are in line with expectations. Variables such as vertebral width, height, area, and endplate depth, as expected, increased with vertebral level in the supero-inferior direction. Measured parameters generally agree with literature values, for example, in terms of vertebral height (19.3 - 24.3 vs reference range 22.2±1.6 - 24.7±3.6 for T10-L2 vertebral bodies, [1]), minimum vertebral width (24.125 - 34.699 vs reference range 29.07±2.82 - 34.31±2.96 using a caliper measurement of T10-T12 vertebral bodies, [2]).

Table 4: Average value of geometric parameters by level measured using coronal supine (AP) acquisitions

Vertebral Level	T10	T11	T12	L1	L2
LM Width (mm)	28.86	31.08	33.35	34.93	34.70
Inner endplate-endplate distance (mm)	15.88	18.00	18.33	20.58	20.59
Outer endplate-endplate distance (mm)	22.65	24.49	25.80	27.72	28.02
Average endplate-endplate distance (mm)	19.27	21.24	22.06	24.15	24.30
AP projection area (mm^2)	739.3	885.0	988.3	1082.4	1074.4
Endplate depth Av (Superior EP) (mm)	1.95	1.80	1.91	1.68	2.04
Endplate depth SD (Superior EP) (mm)	0.68	0.63	0.78	0.73	0.84
Endplate depth Av (Inferior EP) (mm)	2.30	2.76	3.34	4.00	4.01
Endplate depth SD (Inferior EP) (mm)	0.65	0.65	0.77	0.73	0.69

Table 5: Average value of geometric parameters by level measured using lateral decubitus (LM) acquisitions.

Vertebral Level	T10	T11	T12	L1	L2
AP Width (mm)	24.13	26.10	25.97	26.45	25.48
Inner endplate-endplate distance (mm)	16.43	18.15	18.53	21.44	19.97
Outer endplate-endplate distance (mm)	22.36	24.54	26.32	28.70	28.47
Average endplate-endplate distance (mm)	19.40	21.34	22.42	25.07	24.22
LM projection area (mm^2)	645.0	754.7	822.5	926.4	902.0
Endplate depth Av (Superior EP) (mm)	2.12	2.19	2.67	2.56	2.84
Endplate depth SD (Superior EP) (mm)	0.67	0.78	1.07	0.81	1.07
Endplate depth Av (Inferior EP) (mm)	1.72	2.39	2.63	2.69	2.71
Endplate depth SD (Inferior EP) (mm)	0.51	0.56	0.61	0.65	0.77

Textural measurements from AP and LM unloaded (**Tables 6,7**) and standing (**Tables 8,9**) acquisitions for the 32 participants included in this report are in line with expectations. The average range of fractal dimension (FD=2.678-2.751), lacunarity (λ=0.068-0.092), and slope lacunarity (S_λ=0.057-0.071) fall within the expected range from a separate in vivo cohort of 36 patients with multiple myeloma (For T7 and T11 vertebral levels, FD=2.646-2.775, λ=0.057-0.091, and S_λ=0.057-0.123, [3]) and another in vivo cohort of 23 patients (Unpublished data for T10-L4 vertebral levels measured in unloaded and standing configurations, FD=2.618-2.870, λ=0.032-0.160, and S_λ=0.046-0.113). Likewise, the average range of LFD (0.831-1.632) and MIL-derived degree of anisotropy (DA=1.162-1.354) fall within the expected range from the same cohort of 23 patients described above (LFD=0.516-3.450 and DA=1.056-1.446).

Table 6: Average value of textural parameters by level measured using coronal supine (AP) acquisitions.

Level	**T10**	**T11**	**T12**	**L1**	**L2**
Line fraction deviation	1.379	1.118	1.133	1.049	1.046
Anisotropy	1.300	1.311	1.297	1.278	1.269
Fractal dimension	2.697	2.716	2.714	2.721	2.715
Lacunarity	0.080	0.078	0.079	0.079	0.081
Slope Lacunarity	0.071	0.063	0.063	0.058	0.059

Table 7: Average value of textural parameters by level measured using lateral decubitus (LM) acquisitions.

Level	**T10**	**T11**	**T12**	**L1**	**L2**
Line fraction deviation	1.297	1.478	1.220	0.831	0.841
Anisotropy	1.195	1.252	1.306	1.350	1.354
Fractal dimension	2.678	2.696	2.717	2.742	2.739
Lacunarity	0.092	0.092	0.079	0.073	0.069
Slope Lacunarity	0.070	0.062	0.063	0.058	0.060

Table 8: Average value of textural parameters by level measured using coronal standing (AP) acquisitions.

Vertebral Level	**T10**	**T11**	**T12**	**L1**	**L2**
Line fraction deviation	1.317	1.125	1.087	1.042	1.052
Anisotropy	1.295	1.313	1.306	1.293	1.276
Fractal dimension	2.699	2.714	2.716	2.727	2.724
Lacunarity	0.079	0.076	0.078	0.075	0.077
Slope Lacunarity	0.070	0.066	0.063	0.058	0.057

Table 9: Average value of textural parameters by level measured using lateral standing (LM) acquisitions.

Level	T10	T11	T12	L1	L2
Line fraction deviation	1.632	1.606	1.451	0.866	0.884
Anisotropy	1.162	1.241	1.310	1.342	1.350
Fractal dimension	2.679	2.700	2.727	2.751	2.748
Lacunarity	0.084	0.086	0.078	0.068	0.071
Slope Lacunarity	0.067	0.063	0.061	0.058	0.059

Efforts during COVID-19 period: Due to the COVID-19 pandemic, patient enrollment and imaging studies were temporarily halted under guidance of the governor of Michigan (Michigan's "Stay Home, Stay Safe" executive order) and institutional phased contingency plans for COVID-19. Despite the delays with patient enrollment associated with the COVID-19 pandemic, team members were able to focus on different aspects of the project to ensure continuity of productivity during the period of Y1Q2-Y1Q3.

- **Study coordinator:** Ms. Warner and Dr. Rao spent Y1Q2-Y1Q3 identifying over one hundred potentially eligible patients to recruit upon resumption of imaging activities.
- **Technical staff:** Dr. Drost, Mr. Oravec spent the first half of this period working on followup analysis of existing image data. A considerable effort was dedicated in the second half of the period to process improvements and optimization of methods to increase data processing throughput when it was possible to continue data acquisition. Specific examples are presented below:
    - **Textural analysis:** As described above, for previous cadaveric experiments, the cancellous bone analysis region of interest (ROI) for LFD, MIL, and fractal analyses is typically cropped to a rectangular prism with bounds orthogonal to image axes. Due to the natural lordosis of the spine *in vivo*, anatomical axes do not necessarily align with the image axes. Dr. Drost developed a semi-automated procedure for cropping analysis regions which accounts for slice-to-slice variability in the position of the cancellous centrum. The refined method involves defining landmarks at remote slices in the image stack to account for vertebral body tilt, after which the texture cropping code automatically crops ROIs of a fixed dimension centered in the vertebral volume. These regions are then analyzed using existing methods for LFD, MIL, and fractal analyses.
    - **Geometry analysis:** Mr. Oravec developed a semi-automated procedure for applying established methods for analysis of vertebral bone geometry. In short, the 3D DTS segmentation mask is first interactively rotated to align anatomical and image axes, and landmarks are placed in order to automatically calculate and tabulate standard vertebral geometry metrics such as bone size (projection area), vertebral width and height, and endplate depth.
    - **Image registration:** Dr. Drost and Mr. Oravec worked in conjunction with Mr. Zauel on several programming efforts to reduce processing time within the DTS-DVC workflow. The first was related to two of the most time-consuming tasks in the DTS-DVC workflow: alignment and registration steps which occur prior to DVC calculation. Alignment and registration were previously cumbersome due to reliance on 3 different plugins implemented within open source third-party code (Align3_TP and TransformJ ImageJ and Optimized Automatic Registration in MIPAV). Mr. Zauel implemented the alignment and registration workflow in a single code ("Register3D"), which is summarized as follows.
        - The program loads the reference volume, the mask volume and the deflected volume.
        - The program requires the operator to estimate the location of the "center" of the region corresponding to the geometric center of the mask in the deflected volume. This is just so it can make a starting guess for the search. This step, together with those that follow, eliminate the need for pre-alignment prior to registration.
        - The search is a simple (minus epsilon, zero, plus epsilon) guess on each of the six axes, resulting in a total of 3^6 (729) correlations for one pass. The program takes the best correlation coefficient of the 729 guesses and starts over with that guess at the center for the next pass. The program implements a simple crawl toward better correlation. When a full pass does not make any improvement to the correlation, the program multiplies epsilon by 0.75 and reevaluates.
        - When epsilon goes below 0.001 voxel spaces and 0.001 degrees of rotation, optimization is complete.

        - Like MIPAV, the correlation uses tri-linear interpolation and the program only searches the masked ROI for correlation. Once it has found the best fit it uses the same transformation matrix to resample the entire deflected volume and writes out the registered volume.
        - The program also writes out a qualitative RGB audit volume that encodes the reference volume as red plus 50% blue and the registered deflected volume as green plus 50% blue. Where there's a perfect match it comes out gray to white. Where there's a mismatch it comes out reddish or greenish.
        - Register3D was validated to assess agreement of the resulting DVC solution with MIPAV and solutions were deemed indistinguishable. Register3D also produced a considerably lower rate of registration failure than previous methods.
        - Register3D reduced the total time required for alignment and registration from 2-4 hours to less than one minute. The registration code was written to run using a variable number of threads on a given workstation. This further reduced solution time for a test case from approximately 2.5 minutes (single threaded) to 30 seconds (using 40 threads).
        - In addition, Mr. Oravec created a front-end to open the images, interactively create a masking region to define where to limit the registration in Register3D, and generate a batch file including the arguments required to run Register3D from the command prompt.
    - **Multi-threaded implementation of DVC:** Our DVC software was previously written in the interest of large-memory applications (i.e., micro-CT based DVC). To allow efficient processing of a large number of vertebrae in the current project, DVC was modified by Mr. Zauel to operate using a variable number of threads on a given workstation.
      These changes reduced solution time from approximately 2 hours to 20 minutes. The calculations were extensively validated and were identical at all voxels to solutions calculated using the previous, single threaded code. We anticipate these changes will be important to increase data processing throughput in the coming periods as we increase accrual rate to account for recruitment delays to the COVID-19.
      In addition, an option was added to run DVC using standard .TIF stacks as input rather than the previous input format (relatively uncommon HFH format image sequence). This further reduces time spent in format conversion prior to running DVC.
    - **Multi-threaded implementation of DVC post-processor:** Calculation of the Cauchy strain tensor was previously performed using a single thread. Mr. Zauel modified the code to run as a multithreaded process. These changes reduced post-processing time from approximately 20 minutes to 80 seconds. The calculations were extensively validated and were identical at all voxels to solutions calculated using the previous, single threaded code. We anticipate these changes will be important to increase data processing throughput in the coming periods as we increase accrual rate to account for recruitment delays to the COVID-19.

REFERENCES

[1] Busscher I, Ploegmakers JJ, Verkerke GJ, Veldhuizen AG. Comparative anatomical dimensions of the complete human and porcine spine. Eur Spine J. 2010;19:1104-14.
[2] Singh R, Srivastva SK, Prasath CSV, Rohilla RK, Siwach R, Magu NK. Morphometric measurements of cadaveric thoracic spine in Indian population and its clinical applications. Asian spine journal. 2011;5:20.
[3] Oravec D, Yaldo O, Bolton C, Flynn MJ, van Holsbeeck M, Yeni YN. Digital Tomosynthesis and Fractal Analysis Predict Prevalent Vertebral Fractures in Patients With Multiple Myeloma: A Preliminary In Vivo Study. Am J Roentgenol. 2019:W1-W7.

Major Tasks 8-9: Statistical analysis and publication (Yeni/Rao).
We have performed interim descriptive analyses for quality control purposes, but the statistical analysis of final data and publication of results will begin as soon as full set of data is available for any given aim. This is likely after all participants have been recruited, tested, and processed.

What opportunities for training and professional development has the project provided?
This project provided training opportunities for the following individuals:

- Joshua Drost, Ph.D: Michigan State University, Post-doctoral fellow

    - Digital volume correlation analysis, patient imaging (DTS, DXA), DXA and TBS analysis, ImageJ and MATLAB scripting, image textural and geometry analysis.
    - Clinical research experience and working with patients.
    - Project design, management, presentation skills towards becoming independent investigator.

- Daniel Oravec, MSc: Tampere University of Technology, Finland, Senior Research Engineer
    - Digital volume correlation analysis, patient imaging (DTS, DXA), DXA and TBS analysis, ImageJ and MATLAB scripting, image textural and geometry analysis.
    - Clinical research experience and project coordination skills through interactions with the partnering project.

How were the results disseminated to communities of interest?

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period, we will continue to identify and enroll participants (Rao), test participants and calculate relevant outcomes (DVC, texture, geometry) (Yeni). By the end of the next reporting period (Y2Q4), we will recruit and test an additional 156 participants. At the current rate of 4 patients per week, we anticipate rejoining the proposed cumulative recruitment target by the end of the upcoming quarter (Y2Q1).

Since resuming enrollment, at the current accrual rate we have been able to remain current with the most time consuming analysis task (i.e., DVC) as a result of our efforts in the last two quarters in terms of workflow process improvements and optimization of methods to reduce data processing time. As processing bottlenecks are identified, we will continue to identify potential targets for workflow optimization. Finally, we will continue to monitor the incidence of and establish the underlying mechanism for negative top endplate displacements as described in “Major Tasks 3, 5, & 7”.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

There is nothing to report from the past period beyond science and technology, except for the training and job opportunities this project offered to several individuals, and education opportunities on osteoporotic fractures to the participants. At the conclusion of the project, this research is likely to make an impact in establishing the utility of a clinically viable method for directly measuring the patient-specific biomechanical response of vertebrae to load. This method is expected to more accurately identify bones devoid of mechanical integrity and individuals at risk of fracture. These individuals can be helped by timely initiation of preventative therapies. Prevention of osteoporotic fractures would have significant impact on patients’ suffering caused by a fracture (which may even lead to death) and on the enormous economic burden associated with the treatment of fractures and related morbidities. We expect that the ability to assess the mechanical behavior of vertebral bone under load may additionally be relevant in other clinically significant issues such as low back pain associated with vertebral fractures, implant stability, degenerative and congenital diseases of the skeletal

system resulting in deformities, skeletal response to drug, exercise and disuse can be addressed more effectively.

CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report. There were no changes to the objectives or scope of the project in the reporting period.

Actual or anticipated problems or delays and actions or plans to resolve them

No significant problems or adverse events have occurred in terms of patient testing. The process to obtain approval for the research project took longer than expected leading to a later date for starting participant testing. However, recruitment and participant testing went faster than expected initially. Prior to COVID-19 pandemic, based on cumulative accrual, we were ahead of schedule in terms of the timelines laid out in the statement of work.

Starting March 15, 2020, on order from the State of Michigan, Henry Ford Hospital has placed temporary restrictions on visitors to the hospitals within the Health System. In addition, as of March 17, 2020, Research Administration at Henry Ford Health System asked investigators to temporarily delay human subjects (no new enrollment or visits for studies requiring face to face interaction) for Tier 3 protocols (i.e., those with low direct benefit to research participants). On March 23, 2020 Michigan Governor Gretchen Whitmer signed the “Stay Home, Stay Safe” Executive Order (EO 2020-21), to temporarily suspend in-person operations that were not necessary to sustain or protect life for 3 weeks. For these reasons, patient recruitment and imaging were halted in March, 2020 and did not resume until August 10, 2020.

It is impossible to predict if a resurgence in cases will result in additional delays. However, we are testing patients as frequently as possible while following rules set forth by the institution. At the current rate of 4 patients per week, we anticipate rejoining the proposed cumulative recruitment target by the end of the upcoming quarter (Y2Q1). We dedicated considerable effort in optimizing workflow processes to increase data processing throughput as we increase accrual rate to match accrual targets.

Changes that had a significant impact on expenditures

As a result of reduced accrual during the COVID-19 pandemic, a portion of the budget allocated for patient testing (e.g., imaging fees and patient compensation) remains unspent. The funds allocated for these purposes will be used for these purposes in the coming period as we increase accrual rate to account for the delay in patient recruitment and testing.

Significant changes in use or care of human subjects

To ensure patient and personnel safety during the COVID-19 pandemic, we received approval from the IRB for a planned change indicating additional precautions to minimize transmission risk. These changes include, for example, screening participants by phone prior to arrival, washing hands upon arrival, wearing masks at all times and gloves as appropriate, social distancing, adherence to disinfection of high touch surfaces, and post-visit monitoring. The IRB approval was communicated to HRPO. However, there is no actual change in the test procedures or to the informed consent form, and so they are not considered major changes or significant deviations.

PRODUCTS

Publications, conference papers, and presentations

- **Journal publications.**
  - Nothing to report.
- **Books or other non-periodical, one-time publications.**
  - Nothing to Report.

- **Other publications, conference papers, and presentations.**
    - Nothing to Report.

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques
Nothing to Report

Inventions, patent applications, and/or licenses
Nothing to Report

Other Products

Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."
Example:

Name:	Yener N. Yeni
Project Role:	PI
Researcher Identifier (e.g. ORCID ID)	
Nearest person month worked:	4
Contribution to Project:	He has managed the entire project, designed all experiments, and assisted with their execution. Dr. Yeni is responsible for writing manuscripts and reports and is responsible for data quality and analysis.
Funding Support:	PR180156 (Yeni)

Name:	Sudhaker Rao, M.B.; B.S., PACP, FACE
Project Role:	Partnering PI
Researcher Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to Project:	Dr. Rao recruited the human subjects for the study and evaluated clinical test results (x-rays, DXA and biochemistry assays). He is jointly responsible for the interpretation of the data and preparation of the manuscripts.
Funding Support:	PR180156P1 (Rao)

Name:	George Divine, Ph.D.
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID)	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Divine is a Senior Research Biostatistician. He participated in the study design and has overseen all aspects of data analysis. Dr. Divine also oversaw preparation of data reports submitted to the research monitor. He is jointly responsible for interpretation of all results and preparation of manuscripts.
Funding Support:	PR180156 (Yeni)

Name:	Michael J. Flynn, Ph.D.
Project Role:	Co-investigator
Researcher Identifier (e.g. ORCID ID)	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Flynn oversaw the scheduling, acquisition, and archiving of all image data on live human subjects. He is jointly responsible for preparation of the manuscripts.
Funding Support:	PR180156 (Yeni)

Name:	Daniel Oravec, M.Sc.
Project Role:	Senior Research Engineer
Researcher Identifier (e.g. ORCID ID)	
Nearest person month worked:	12
Contribution to Project:	Mr. Oravec acquired the DXA and the specialized DTS images of patients. He has led protocol development, and improvements to the image processing workflow. He has also assisted the post-doctoral fellow with image processing and measurements on the acquired images.
Funding Support:	PR180156 (Yeni)

Name:	Roger Zauel
Project Role:	Software Development
Researcher Identifier (e.g. ORCID ID)	N/A
Nearest person month worked:	1
Contribution to Project:	Mr. Zauel assisted in the further development of software tools and overseeing all computer operations.
Funding Support:	PR180156 (Yeni)

Name:	Joshua Drost, Ph.D.
Project Role:	Post-doctoral Fellow
Researcher Identifier (e.g. ORCID ID)	
Nearest person month worked:	12
Contribution to Project:	Dr. Drost performed all image processing and measurements on the acquired images. These tasks include image registration, segmentation, DVC solutions, post-processing of displacement distributions for the DTS-DVC method, and image preprocessing, texture analysis and postprocesing for DTS-based textural methods. Dr. Drost is responsible for data reduction, analysis, and preparation of manuscripts, abstracts and reports.
Funding Support:	PR180156 (Yeni)

Name:	Elisabeth Warner
Project Role:	Study coordinator
Researcher Identifier (e.g. ORCID ID)	N/A
Nearest person month worked:	12
Contribution to Project:	Ms. Warner coordinated the identification and screening of patients, scheduling of the exams, patient assistance and record keeping.
Funding Support:	PR180156P1 (Rao)

SPECIAL REPORTING REQUIREMENTS

Quad Chart and Award Chart

APPENDICES

None