AWARD NUMBER: W81XWH-19-1-0796

TITLE: Serum Protein-Based Indices for the Progression of Fracture Healing and Nonunion

PRINCIPAL INVESTIGATOR: Louis Gerstenfeld

CONTRACTING ORGANIZATION: Boston University, Boston, MA

REPORT DATE: October 2020

TYPE OF REPORT: Annual Report

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2020	Annual	30Sep2019-29Sep2020

4. TITLE AND SUBTITLE

Serum Protein-Based Indices for the Progression of Fracture Healing and Nonunion

5a. CONTRACT NUMBER
W81XWH-19-1-0796

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Louis Gerstenfeld

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

TRUSTEES OF BOSTON UNIVERSITY
BOSTON UNIVERSITY MEDICAL CAMPUS
85 E NEWTON ST M-921
BOSTON MA 02118

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

Medical Problem to Be Addressed: Fractures to the limbs are one of the most common injuries that service men and women will experience both during training and in combat. X-rays and verbal questions about how the injured bone feels (if there is pain and if the person can use the injured limb) are the current approaches used to follow the progress of bone healing. Failed healing as diagnosed by X-ray and pain is made many months after the biology of healing has actually failed. This means that if there was a tool to follow the biology of healing, diagnosis of failed healing could be made at a significantly earlier timepoint, leading to earlier intervention for failed healing and shortening the periods that patients are subjected to pain and disability.
Rationale for Project: A few studies to date have shown that specific proteins associated with the various biological stages of fracture healing, are released from the fractured bone into the serum (blood) and assaying for their presence can be used to follow bone healing. This leads us to believe that we can successfully develop a serum protein based biological diagnostic to both follow the progression of fracture healing and identify failed healing at much earlier times than X-Rays.

15. SUBJECT TERMS
None listed.

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	12	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Medical Problem to Be Addressed: Fractures to the limbs are one of the most common injuries that service men and women will experience both during training and in combat. X-rays and verbal questions about how the injured bone feels (if there is pain and if the person can use the injured limb) are the current approaches used to follow the progress of bone healing. A major deficiency with X-rays and pain assessments is that they do not tell the physician, what is going on biologically over the course of healing and make the diagnosis long after the biology has failed. Objective: The objective of this research proposal is to identify a set of serum proteins that are predictive of progression of the biological processes of human fracture healing and would be diagnostic for failed healing weeks earlier than current diagnostic tools. Study Design*:* A total of 180 patients with an upper arm bone (humerus) fractures, treated with a sling and immobilization will be asked to participate in this study. We will draw blood from these patients across their time course of healing as well as carry out the standard of care X-rays and other clinical assessments of the progression of fracture healing. In this group we anticipate that about 20 patients would experiences failed healing (nonunion) providing us with the ability to identify those proteins in the serum that are diagnostic for failed healing. Outcomes: We will identify those diagnostic proteins that both relate to the biological processes of healing and that predict failure to heal at an earlier time than current clinical tools. These studies will move the diagnosis for failed healing to a much earlier time than current radiological and other clinical assessments. The proposed research if successful will advance optimal treatment and rehabilitation of fractures *by* identifying those patients that progressing to heal vs those needing additional surgical intervention diminishing periods of prolonged pain and debilitation and as well as better predicting timing for rehabilitation and return to work or duty.

2. KEYWORDS:

Limb Fractures, Failure to Progress, Fracture Nonunion, X-Rays, Patient Reported Outcomes, Serum Markers

3. ACCOMPLISHMENTS:

What were the major goals of the project? Goals Summarized from SOW

Major Task 1 Project Administration Set Up: Composed of 8 subtasks.
(Task 1 of the project were to have been performed in 6 months)

1) Selection of METRC Site Participants
2) Coordinate material transfer agreements (MTAs) and COIs
3) Coordinate establishment of enrollment screening protocol, and clinical data collection forms
4) Finalize consent form & human subject's protocols for BUSM and a master protocol at METRC for site distribution
5) Distribute master IRB protocol to participating sites and obtain individual IRB approval
6) HRPO Review and Approval
7) Establishment of Non-Union Adjudication Committee
8) Coordinate with sites for annual IRB report for continuing review

Major Task 2 Technical Aim 1: Learning Set: Identification of Initial Non-union Markers: Composed of 5 subtasks
(Tasks 2 of the project were to run from 6 months through 21 months)

1) METRC will coordinate enrollment at the five sites including BUSM with a target to complete the serum collection and a complete clinical data set of 40 patients by 12 months
2) Initiate data simulation and testing of computational models for learning phase
3) Review and coordination of clinical functional data collection and facilitation of RUST analysis of discovery group
4) Collection and coordination of the serum transfer to BUSM. At least 90 specimens to complete the learning set analysis of which 10 will be determined to be fractures that have failed to progress
5) Computational analysis, development, and identification of the learning set proteins

Major Task 3 Technical Aim 2: Validation Set: Refinement and Validation of Non-union Markers; Composed of 3 subtasks
(Tasks 3 of the project were to run from 21 months through 48 months)

1) METRC will coordinate enrollment at the five sites including BUSM with a target to complete the serum collection and a complete clinical data set of an additional 10 patients that have been determined to be fractures that failed to progress
2) Review and coordination of clinical functional data collection and facilitation of RUST analysis of validation group
3) Computational analysis of the validation group: comparison of results between the validation and learning sets from for predictive accuracy, retest third time against full data set of 60 (20 Non-unions and 40 Unions) with further refinement of the diagnostic marker group.

What was accomplished under these goals?

The primary milestones under Task 1 included 8 subtasks: All these elements were to be completed in six months. Tasks 1-4 are completed. Task 5 is 90% completed with the Master protocol distributed to all participating with the final sIRB in review. Task 6, the HRPO submission, is ready on the approval of the final sIRB. Task 7 is initiated and will be completed in the next two weeks from submission to this report.

1) Selection of METRC Site Participants (completed)
2) Coordinate material transfer agreements (MTAs) and COIs (completed)
3) Coordinate establishment of enrollment screening protocol, and clinical data collection forms (completed)
4) Finalize consent form & human subject's protocols for BUSM and a master protocol at METRC for site distribution (completed)
5) Distribute master IRB protocol to participating sites and obtain individual IRB approval (90% completed)
6) HRPO Review and Approval (incomplete: awaiting final approval from JHU sIRB)
7) Establishment of Non-Union Adjudication Committee (Initiated 50%)
8) Coordinate with sites for annual IRB report for continuing review (incomplete: awaiting final approval from JHU sIRB)
9)

The primary milestones under Task 2 included 5 subtasks. : All these elements were to be completed from 6 months to 21 months. Tasks 1 and 2 have not been initiated. Tasks 3 and 4 are 25% complete.

1) METRC will coordinate enrollment at the five sites including BUSM with a target to complete the serum collection and a complete clinical data set of 40 patients by 12 months (We have been unable to initiate subtasks 1 and 2 because of the initial shut down of all human clinical activities due to COVID-19 from March to September, 2020. We are now surveying the participating sites to determine their abilities to now initiate patient-based studies.)
2) Initiate data simulation and testing of computational models for learning phase
3) Review and coordination of clinical functional data collection and facilitation of RUST analysis of discovery group (We have completed 25% of subtask 3. The clinical guidance documents to carry out this subtask have been set up, the standard clinical data forms (case report forms) have been developed, and the REDCap database and data base file sharing structures between the participant sites for the this study have been set up and piloted.)
4) Collection and coordination of the serum transfer to BUSM. At least 90 specimens to complete the learning set analysis of which 10 will be determined to be fractures that have failed to progress (We have completed 25% of the work on this subtask. We have set up the guidance documents for the blood draws serum and blood cell processing and shipping procedure to send the blood cells and serum to BUSM for analysis.)
5) Computational analysis, development, and identification of the learning set proteins

The primary Milestones under Task 2 included 1-3 subtasks. All these elements are slated to be initiated by the 21-month of the project. None of these elements have been started.

What opportunities for training and professional development has the project provided?

None was provided in year one

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?

We have prioritized putting in place all of our clinical guidance documents and all administrative elements that will allow us to initiate the clinical aspects of Tasks 2 and 3 once hospital conditions related to COVID-19 allows clinical research to resume. Currently we are waiting on the survey of our participating sites to determine the timeline of study launch at each site. Upon the survey results that we determine that enough sites are available to initiate Task 2, we will plan on staring the clinical parts of our studies in November 2020.

Once we determine the study launch date at each site, we will provide clinical guidance documents and site training. Each site will be certified to begin screening and enrollment when they have the local IRB approval.

We will also continue to work on preparing the adjudication committee and the SOP.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

We have made no major changes to the project since we obtained our funding.

We are awaiting site specifics reviews for the timeline of study launch at each site which is dependent on the conditions relative to COVID-19.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

All delays to the project initiation are due to COVID-19. We have not made any purchases of the antibody microarray chips as of date since this purchase is time sensitive to the shelf-life of the chips and when we would use of the chips for the proteomic analysis.

Changes that had a significant impact on expenditures

We have not begun our graduate student recruitment process at Boston University School of Public Health as we do not wish to expend any funds until we have initiated the clinical data collection phase addressed in Tasks 2 and 3. Again, we do not what to make any expenditures of funds prior to our initiation of the project.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Significant changes in use or care of human subjects

One significant change relates to the method of collecting the Patient Reported Outcomes (PROs). We will use electronic tablets or online survey format to collect PROs as that would limit patient contact with any research or clinical staff.

Significant changes in use or care of vertebrate animals

Not applicable

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS: Publications, conference papers, and presentations

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

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Louis Gerstenfeld, PhD
Project Role:	Principal Investigator
Researcher Identifier:	https://orcid.org/0000-0002-0477-1211
Nearest person month worked:	1.0
Contribution to Project:	Developed Protocol for primary grant, recruited members of the PC committee and participating centers.
Name:	Paul Tornetta III, MD
Project Role:	Site Principal Investigator
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-6448-8864
Nearest person month worked:	.25 Cal months
Contribution to Project:	Dr. Tornetta assisted in developing protocol.
Name:	Serkalem Demissie, PhD/
Project Role:	Primary Study Statistician
Researcher Identifier (e.g. ORCID ID):	https:// orcid.org/ 0000-0002 -8009-0987
Nearest person month worked:	.75 Cal months
Contribution to Project:	Dr. Demissie assessed study design effects of missing data and reviewed redcap data base
Name:	Renan Castillo, PhD
Project Role:	Coordinating Center Co-Principal Investigator
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.23 Cal months
Contribution to Project:	Dr. Castillo oversaw coordinating center activities during this period.
Name:	Suna Chung , MPH
Project Role:	Coordinating Center Project Director
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.45 Cal months
Contribution to Project:	Ms .Chung has assumed Ms DeLuca's responsibilities in the project. She is responsible for study planning meetings, drafting the study protocol and working with the MCC team to establish the overall study timeline.
Name:	Trisha Chaffee, MS
Project Role:	Coordinating Center Study Manager
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.30 Cal months
Contribution to Project:	Ms. Chaffee corresponded with participating centers, organized site survey responses, and drafted the consent documents and case report forms.

Name:	Manisha Kumar, MBA, MA
Project Role:	Coordinating Center Financial Manager
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.09 Cal months
Contribution to Project:	Ms. Kumar set up the study account and prepared subaward paperwork for participating centers.
Name:	Jack Dagg
Project Role:	Coordinating Center Data Analyst
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.15 Cal months
Contribution to Project:	Mr. Dagg supports the analysis of the data under the supervision of the study investigators

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS
None
QUAD CHARTS : Updated Quad uploaded

9. APPENDICES: None