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PRINCIPAL INVESTIGATOR: Melissa McDiarmid, M.D., MPH, DABT

RECIPIENT: University of Maryland, Baltimore

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6. AUTHOR(S) Melissa McDiarmid, M.D., MPH, Stella Hines M.D., MSPH, Joanna Gaitens Email: mmcdiarm@som.umaryland.edu ; shines@som.umaryland.edu ; jgaitens@som.umaryland.edu			5d. PROJECT NUMBER		
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14. ABSTRACT The 'signature' wound of current and recent conflicts in both Iraq and Afghanistan is that incurred via contact with improvised explosive devices (IEDs) and other high kinetic energy weapons. Beyond the traumatic injury inflicted, health risks from wound contamination with toxic metals must be managed, even as risk from these contaminants is not fully known. To provide a scientific evidence base to refine the clinical management of these patients, a multidisciplinary approach using animal models and patient data will be used. A laboratory rat model system (Project 1) will provide bio-kinetic and toxicological data on a variety of military-relevant metals implanted in the rats. (Project 2) will identify biomarkers of early effect in tissues and body fluids of the implanted animals. Using an existing national VA Embedded Fragment Registry of such injured patients, (Project 3) will assess kidney injury --the presumed target of toxic metal exposure-- and (Project 4) will assess pulmonary injury in these Veterans from both systemic metal absorption and presumed blast-induced -baro-trauma at the time of					
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1. INTRODUCTION:

The 'signature' wound of current and recent conflicts in both Iraq and Afghanistan is that incurred via contact with improvised explosive devices (IEDs) and other high kinetic energy weapons. Beyond the traumatic injury inflicted, health risks from wound contamination with toxic metals must be managed, even as risk from these contaminants is not fully known. To provide a scientific evidence base to refine the clinical management of these patients, a multidisciplinary approach using animal models and patient data will be used. A laboratory rat model system (Project 1) will provide bio-kinetic and toxicological data on a variety of military-relevant metals implanted in the rats. (Project 2) will identify biomarkers of early effect in tissues and body fluids of the implanted animals. Using an existing national VA Embedded Fragment Registry of such injured patients, (Project 3) will assess kidney injury --the presumed target of toxic metal exposure-- and (Project 4) will assess pulmonary injury in these Veterans from both systemic metal absorption and presumed blast-induced -baro-trauma at the time of injury.

2. KEYWORDS:

Embedded metal fragments, health effects, military-relevant metals, laboratory rat, toxic metals, transcriptome, registry, exposure

3. ACCOMPLISHMENTS:

What were the major goals of the project?

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

Major Task 1

Experimental Preparation

Year 1/Month 1 to Year 1/Month 6, 100% completed.

Major Task 2

Animal Ordering and Pellet Implantation Surgeries

Year 1/Month 6 to Year 3/Month 8, 100% completed.

Major Task 3

Animal Health Assessments and Urine Collections

Year 1/Month 9 to Year 3/Month 9, 100% completed.

Major Task 4*

Euthanasia and Tissue Collection; Transfer of Research Samples to University of Kentucky

Year 2/Month 8 to Year 3/Month 9, 100% completed.

Major Task 5

Histopathology and Immunohistochemical Analyses

Year 2/Month 5 to Year 3/Month 11, 98% completed.

Major Task 6

Metal Analysis and Tissue Imaging

Year 3/Month 1 to Year 4/Month 12, 80% completed.

Major Task 7

Data Compilation, Statistical Analysis, and Preparation of Final Report

Year 4/Month 1 to Year 5/Month 12, 75% completed.

*(See pg. 8)

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

Major Task 1

Experimental Preparation

Year 1/Month 1 to Year 1/Month 12, 100% completed.

Major Task 2

3M Experimental Group Microarray analyses

Year 2/Month 4 to Year 3/Month 10, 100% completed.

Major Task 3

12M Experimental Group Microarray analyses

Year 2/Month 8 to Year 4/Month 4, 100% completed.

Major Task 4

6M Experimental Group Microarray analyses

Year 3/Month 5 to Year 4/Month 10, 100% completed.

Major Task 5

1M Experimental Group Microarray analyses

Year 3/Month 9 to Year 5/Month 4, 100% completed.

Major Task 6

Data Compilation, Statistical Analysis, and Preparation of Final Report

Year 5/Month 5 to Year 5/Month 12, 35% completed.

PROJECTS 3 & 4:

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3

“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4

“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

The Major Tasks for Year 2 are shared by Projects 3 and 4.

Major Task 1

Questionnaire development

Year 1/Month 1 to Year 1/Month 12, 100% completed.

Major Task 2

Obtain regulatory approvals

Year 1/Month 1 to Year 2/Month 1, 100% completed.

Major Task 3

Recruitment and questionnaire administration

Year 1/Month 1 to Year 4/Month 9, 95% completed.

Major Task 4

Questionnaire analyses

Year 2/Month 1 to Year 5 Month 12, 45% completed

Major Task 5

Collection and analyses of urine specimens

Year 1/Month 1 to Year 4/Month 7, 75% completed.

Major Task 6

Collection analyses of PFT and IOS findings

Year 1/Month 1 to Year 4/Month 6, 85% completed.

Major Task 7

Summarize Metal and Renal Findings

Year 2/Month 1 to Year 5/Month 12, 40% completed.

Major Task 8

Summarize PFT and IOS Findings

Year 2/Month 1 to Year 5/Month 12, 50% completed.

What was accomplished under these goals?

John F. Kalinich, Ph.D., Principal Investigator, Project 1

“Health Effects of Embedded Fragments of Military-Relevant Metals”

In Year 4 of the project histopathological examination of the collected tissues was completed. A table of tumor incidence at the pellet implantation sites, as well as representative photomicrographs can be found in the Appendices. Briefly, all nickel-implanted rats developed tumors at the pellet implantation site, with most identified as rhabdomyosarcomas. In two of these cases, metastases were also found in the lung and bladder. Most of the cobalt-implanted animals also developed tumors at the pellet implantation site; however, these tumors were poorly differentiated and, as such, were classified as undifferentiated spindle tumors. A small percentage of the depleted uranium-implanted rats showed a mild to moderate amount of inflammation and myositis at 12 months along with numerous pigmented macrophages. However, no neoplastic changes in the muscle tissue were observed.

Protein expression analysis of several markers indicative of metal-induced changes in signal transduction mechanisms or muscle damage was also completed this year. The expression of the following proteins were assessed in gastrocnemius isolated from around the area of the implanted pellets: desmin, endothelial Nitric Oxide Synthase (eNOS), inducible Nitric Oxide Synthase (iNOS); matrix metalloproteinase-2 (MMP-2) and cleavage products; matrix metalloproteinase-9 (MMP-9), and hydroxynonenal-modified proteins. As shown in the Appendices, metal exposure affected the expression of several of these proteins although not all metals demonstrated an effect and the effect, if present, was variable. Hydroxynonenal-modified proteins were also increased in metal-implanted gastrocnemius for all metals except W and Ni.

Analysis of urinary metals and biomarkers was also completed in Year 4, with statistical analysis currently underway. Urinary metal levels and a table of the various urinary biomarkers analyzed in Year 4 can be found in the Appendices.

Expression of proteins associated with the blood-brain barrier and neuronal synapse were investigated with respect to implanted metals. The tight junction proteins, ZO-1 and occludin, are involved in proper functioning of the blood-brain barrier while the proteins PSD95, synaptotagmin, and spinophilin are involved in neuronal synapse structure and function. Four brain regions were analyzed: cerebellum, frontal cortex, hippocampus, and amygdala. Representative results can be found in the Appendices.

Analysis of metal levels in the tissues collected upon euthanasia continued in Year 4. The pandemic shutdown and issues with the inductively coupled plasma-mass spectrometer used in the analysis has slowed progress. The remaining tissues will be analyzed in Year 5.

Studies on imaging metal location in the implanted muscle were also continued this year with three sensitive analytical techniques investigated: Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDS), X-ray Photoelectron Spectroscopy (XPS), and Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). It was discovered that LA-ICP-MS performed best at identifying the presence of metal in tissue. A manuscript has been prepared and submitted for publication in *Applied Spectroscopy*.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

During Year 4, data analyses of the completed microarrays of gene expression on all skeletal muscle samples (1, 3, 6 and 12 month time points) for each of the 8 metals and the control metal (tantalum) were performed. The bioinformatic analysis indicate that nickel and cobalt have the most significant impact on skeletal muscle gene expression at early time points, showing 500-1000 differentially expressed genes (DEGs). Analysis of the response to lead across the entire time point showed nearly 300 differentially expressed genes (DEG) at 1 month, which decreased to 40 DEGs by 12 months, suggesting that the muscle is able to mount an adaptive response. The dramatic reduction in DEGs at 12 month prompted us to perform RNA sequencing on muscle samples close to the metal. Results largely mirror the microarray findings. The manuscript reporting these is in press the journal *Physiologic Genomics*.

In year 4 we completed isolation and sequencing of microRNAs from urine and serum from all rats at all time points. Urine microRNA analyses are furthest along, and we have identified microRNAs that are uniquely elevated by specific embedded metals, and some that are common to all metals. A manuscript reporting these findings, with lists of potential biomarkers of metal exposure, will be submitted in the first quarter of year 5. Serum microRNA analyses are underway.

PROJECTS 3 & 4:

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator Project 3
“Biomarker Assessment of Kidney Injury from Metal Exposure in Embedded Fragment Registry Veterans”

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator Project 4
“Respiratory Health in a Cohort of Embedded Fragment Registry Veterans Exposed to Blasts and Metals”

This project consists of two different populations of Veterans who are selected from the VA Toxic Embedded Fragment Registry to either receive an invitation to complete a questionnaire (Study Population #1), or to participate in a clinical assessment visit (Study Population #2).

Study Population #1 – Questionnaire Only Group

During Year 4, we continued mailing out introduction letters, questionnaires, and reminder postcards to randomly selected eligible Veterans. In March 2020, due to COVID-19, we halted mail-

outs because the preparation required several study team members to work in close proximity to one another. Given the potential risks to study team members during this current pandemic, we decided not to continue mail-outs. At this time, we reached 95% of our enrollment goal and additional mailings would not have improved our statistical power to detect a significant difference between blast and bullet injuries. (Our original sample size calculations included over-sampling of Veterans with bullet only injuries. At the time recruitment was halted, mailings had been sent to all bullet-only injured eligible Veterans in the registry, and no additional bullet-only injured Veterans were available for recruitment.) To date, questionnaires have been sent to 9,000 eligible Veterans in batches of 1,000 Veterans at a time. We have received a total of 2,390 completed questionnaires. The overall response rate has been approximately 30%. (Major Task 3 -Recruitment and Questionnaire Administration).

Paper versions of completed questionnaires continue to be entered using a two-step validation process into the study database using DataFax, an optical character reader scanning system for questionnaire data entry. Results from the on-line surveys, created by the VA Albuquerque Cooperative Studies Program (CSP) and VA Automated Information and Technology Center, are automatically captured in the study database. We continue to monitor returned questionnaire responses for blast versus no-blast exposure responses to assure that our sampling strategy was correct to achieve the desired number of bullet-only injuries needed for analytical power. (Major Task 4- Questionnaire Analysis). The small number of paper and electronic questionnaires that continue to trickle in are validated by study staff and payments continue to be processed promptly.

There were two administrative modifications made to the protocol in Year 4. The first modification added a detailed instruction letter for the study participant which accompanies the ClinCard (the updated mechanism of payment). The second amendment added a medical student, Jessica Palmer, to the protocol for a summer internship, and extended the deadline for participants to submit their questionnaires from 5/31/20 to 12/31/20.

Study Population #2 – Clinical Assessment Group

During Year 4, recruitment continued at all sites until March 17, 2020, when the VA mandated all in-person non-essential study visits to be halted. At that time, 380 participants had completed the study across all 6 VA recruitment sites (Major Task 3- Recruitment and Questionnaire Administration). On March 26, 2020, we were required to file an exemption with VA C-IRB which placed all recruitment and in-person visits on hold. Once the hold was lifted by VA, we submitted an amendment which eliminated all in-person study visits from the protocol. The spot urine collection (for metal analysis) and questionnaires can be performed remotely, however the pulmonary testing (PFT/IOS) and renal marker analysis have been omitted due to excessive potential risk created by COVID-19. As PFTs are an aerosol-generating procedure and require the Veteran to come to the VA for an in-person study visit, it was determined that this procedure would place participants and staff at unnecessary risk during this current pandemic. In addition, although we investigated options for collecting renal markers remotely, because azide solution and stabilizing buffer are required to be added to the urine sample immediately upon collection each investigated option also introduced potential risks to the Veteran (i.e., handling of the chemical solutions or requiring the individual to come to the VA for an in-person visit). Therefore, both PFTs and measurement of renal injury markers were eliminated from the protocol. This amendment was submitted on August 7, 2020 before the exemption was set to expire and was approved September 17, 2020. As the recruitment sites receive approval from their local VA ORD, they are beginning to send recruitment letters to eligible Veterans. We anticipate wrapping up recruitment activities on December 31, 2020, if we can successfully enroll 41 more study participants to meet our target.

We have also continued to double-enter data from all study questionnaires into the Clinical Assessment database and initial analyses of the data have led to refinement of the data entry process.

Brian Lambert, data entry specialist, was added to our protocol in March 2020 at the time of our Continuing Renewal submission (Major Task 4 – Questionnaire Analysis).

We have supplied local sites with urine collection kits as needed and the local sites continue to collect and send specimens for metal analyses. We had continued to receive and store urine specimens from the local sites for analyses of the renal markers throughout Year 4. In total over this course of this project, we have shipped urine specimens for 380 individuals to the University of Antwerp Laboratory for renal markers analysis. The first shipment was in June 2019, the second in October 2019, and the final shipment was sent in September of 2020. (Major Task 5- Collection and Analyses of urine specimens).

We continue to enter all urine metal results into the study database and QC checks are performed by Dr. Gaitens. In April 2020, we received renal marker results for the second batch of urine samples to Belgium. These results have been reviewed and entered into our study base for analyses. We anticipate having the final set of renal marker results in late January 2021, allowing final analyses to begin.

During this year, we initiated and continue to have biweekly data meetings, with involvement from the study team's statistician, to review the status of data entry, discuss preliminary findings, and outline plans for upcoming analyses.

Quarterly videoconferences were held with all VA collaborators to review the study protocol and discuss recruitment and any questions that arise. Bi-weekly conference calls continue between all site research coordinators to increase communication and troubleshoot any challenges that arise.

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

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

Nothing to report

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

Nothing to report

PROJECTS 3 & 4:

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator Project 3
“Biomarker Assessment of Kidney Injury from Metal Exposure in Embedded Fragment Registry Veterans”

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator Project 4
“Respiratory Health in a Cohort of Embedded Fragment Registry Veterans Exposed to Blasts and Metals”

Dr. Katherine Chin and Dr. Maxwell Reback, two post-doctoral research fellows who are both currently enrolled in a Pulmonary and Critical Care fellowship training program at University of Maryland Medical Center, continued contributing to the project in Year 4. Similar to their predecessor, Dr. Danielle

Glick, who remained an active member of the research team during her final year of pulmonary training, they have acquired unique expertise and skills in impulse oscillometry testing that they would not have received in their fellowship training otherwise, which will be useful through the rest of their careers. All three research fellows had scientific abstracts accepted for scholarly presentations at national meetings in 2020 and had the opportunity to present the abstracts virtually. Additionally, Jessica Palmer, a third-year medical student at University of Maryland, participated in a summer research internship with our team from May to August 2020. Working with Dr. Hines and Dr. Gaitens on this project, Jessica successfully applied for and received a stipend award from the University's competitive Program for Research Initiated by Students and Mentors (PRISM). She used this opportunity to gain experience in research and had the opportunity to present findings in several different forums.

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?

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

The focus in Year 5 of the project will be the statistical analysis of the collected data including urinary biomarkers and protein expression results of muscle and brain samples. Metal analysis of the few remaining tissues will be completed, as well as the remaining metal imaging experiments. Several manuscripts are in the planning stage and will be completed and submitted for peer-review publication in Year 5.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

During Year 5 of the project, we will continue bioinformatic analyses of serum microRNAs. The results from urine and serum will be confirmed by real time quantitative polymerase chain reaction (RTqPCR). Our major goal is to integrate our datasets with those generated in project 1 on protein biomarkers in urine and tissue changes associated with microRNA urine markers. We will also correlate our DEG lists in muscle with muscle protein analyses, and muscle tissue metal levels quantified in project 1.

PROJECTS 3 & 4:

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3
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“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

In Year 5, for Study Population #1 we will continue entering data into the study databases. We will also complete our data analysis plan which focuses on 1) characterizing the health and injury experiences of Veterans with retained fragments by examining if those at highest risk for having a retained fragment have worse overall health as well as more self-reported renal systems and 2) examining if exposure to a blast or self-reported metal exposure are associated with self-reported respiratory symptoms.

For Study Population #2, we will continue recruitment and enrollment of Veterans to complete the expanded questionnaire and participate in clinical assessments under the newly amended protocol. Due to COVID-19, we removed all in-person visits from the protocol, including PFT/IOS testing. The new protocol includes collecting urine specimens for metal analyses and completing questionnaires. Additionally, available imaging records continue to be reviewed to determine if the presence of fragments has been documented.

For both populations, data entry via primary and secondary validation will continue.

Year 5 activities will focus heavily on analyses of complete data sets and preparation of scholarly manuscripts for publication. Drs. Gaitens, Hines, and Brown have scheduled and agreed upon timing of analysis and writing tasks to assure that planned publications may be prepared during this year.

We plan to present the results of interim (or final – if available) analyses at national meetings in 2021 of the Society of Toxicology, the American Thoracic Society and the Military Health Systems Research Symposium.

4. IMPACT:

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

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

Nothing to report

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

The initial microarray analyses indicate differential response to different metals and that in some cases, the induced inflammatory response is resolved, suggesting that we will be able to identify metals likely to have the greatest effect on warriors harboring embedded metals. The large array of changes in small RNAs in urine in response to different metals also suggests that biomarkers will be identified.

PROJECTS 3 & 4:

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3
“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

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?

Nothing to report.

5. CHANGES/PROBLEMS:

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

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

Due to covid-19, our laboratory was shut down for over 4 months. This significantly delayed completion of metal analysis work and imaging studies. Some budgetary readjustments within the personnel funding line may be required to complete the study with the 5-year timeframe.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

Nothing to report.

PROJECTS 3 & 4:

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3
“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Due to COVID-19, protocols for both Study Populations were halted in March 2020. As a result, we amended the protocol for Study Population #2 so that study activities can be completed remotely. Pulmonary testing and the collection of renal markers were eliminated. In order to reach our target

enrollment of 421, we would need to recruit 41 more study participants. We are hopeful that this can be achieved by the end of the calendar year.

A total of 2,390 Veterans participated in the Questionnaire mail-out (Study Population #1), allowing us to achieve 95% of our enrollment goal and the decision was made not to proceed with any further mailings to avoid undue risk to study team members and in accordance with maximized telework strategies recommended both by the University of Maryland-Baltimore and the Baltimore VA Medical Center.

Changes that had a significant impact on expenditures

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

Nothing to report

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

Nothing to report

PROJECTS 3 & 4:

Nothing to report.

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:

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3
“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Amendments submitted to IRB and USAMRMC HRPO (Study Population #1)- Protocol #A-19735.1

- **Modification 1** submitted to University of Maryland HRPO 8/18/17 (prior to approval by subsequent IRBs); approved on 9/1/2017; Submitted to DoD HRPO 9/27/17; acknowledged 12/14/17. This modification was submitted due to a change in letterhead logos on recruitment letters and was included in the initial USAMRMC HRPO submission form.
- **Modification 2** submitted to University of Maryland HRPO 3/12/18; approved on 3/28/2018; Submitted to DoD HRPO 3/28/18; acknowledged 4/3/18. This modification was submitted due the

change from use of VA REDCap to VA Cooperative Studies Program-Austin Information Technology Center (CSP-AITC) for capture of electronic survey data.

- **Modification 3** submitted to University of Maryland HRPO on 5/11/2018; approved 6/1/18; Submitted to DoD HRPO 6/5/18; acknowledged 6/13/18. This modification included a slight change to the appearance of the cover letter accompanying the questionnaire providing a better visual representation of instructions for completing the questionnaire online, and information about how an access code and PIN number will be used to log onto the electronic survey site.
- **Modification 4** submitted to University of Maryland HRPO on 7/20/2018 and approved on 7/30/2018; Submitted to DoD HRPO; acknowledged on 8/3/2018. This modification was submitted to replace CTRIC and VA Teleforms with the VA Perry Point Cooperative Studies Program and Datafax for the paper questionnaires.
- **Modification 5** submitted to University of Maryland HRPO on 11/1/2018; approved on 11/25/2018; Submitted to DoD HRPO 11/30/18; acknowledged 12/17/18. This modification was required to update the study coordinator's new extension on all recruitment documents and electronic survey webpage.
- **Modification 6** submitted to University of Maryland HRPO on 7/3/19; approved on 7/12/19; Submitted to DoD HRPO 7/16/19. This modification changed the method of payment to ClinCard by Greenphire, rather than checks. Two new pulmonary fellows were added to the protocol.
- **Modification 7** submitted to University of Maryland HRPO on 8/20/19; approved on 8/21/19; Submitted to DoD HRPO 9/3/19; Acknowledged 12/20/20. This modification was required to include a new mandatory VA warning on the electronic survey homepage.
- **Modification 8** submitted to University of Maryland HRPO on 11/20/29; approved on 11/22/19; Submitted to DoD HRPO 11/22/19. This modification added a letter containing detailed instructions to accompany the participant's ClinCard.
- **Modification 9** submitted to University of Maryland HRPO on 6/9/20; approved 6/22/20; Submitted to DoD HRPO on 6/24/20. This modification added a medical student to the protocol and extended the deadline for participants to submit their questionnaires from 5/31/20 to 12/31/20.

*Please see the "Submissions Tracking Table" in the appendices for a summary of these amendments

Amendments submitted to IRB and USAMRMC HRPO (Study Population #2)- Protocol #A-19735.2

- **Modification 1:** Submitted to VA C-IRB on 6/5/2018; Approved 6/19/18; Submitted to DoD HRPO 6/25/18 and approved on 6/26/18. This modification grants some flexibility in the event that all study procedures cannot be completed during a single visit. In such an event, (i.e. equipment malfunctions in the pulmonary function lab and test not able to be completed), we will be able to bring participants back to complete the protocol. This modification also allows for additional travel pay for the second visit and includes language in the consent form describing this.
- **Modification 2:** Submitted to VA C-IRB on 8/15/18; Approved 11/5/18; Submitted to DoD HRPO 11/20/18; Acknowledged 12/17/18. This modification gives the participant the opportunity to be re-contacted for future studies.
- **Modification 3:** Submitted to VA -CIRB on 9/4/18; Approved 11/5/18; Submitted to DoD HRPO 11/20/18; Acknowledged 12/17/18. This modification was submitted per the IRB's recommendation in the determination of a deviation that occurred at the San Antonio site. This deviation stemmed from a scheduling error which resulted in a study participant performing a research PFT prior to being consented. In this modification, the HIPAA authorization was revised to indicate that PHI will be used to schedule pulmonary function tests prior to obtaining consent and HIPAA.
- **Modification 4:** Submitted to VA -CIRB on 3/27/19; Approved 4/9/19; Submitted to DoD HRPO 4/18/19; Approved 6/13/19. The Phoenix VA was added as 6th recruitment site.

- **Modification 5:** Submitted to VA -CIRB on 5/2/19; Approved 5/28/19; Submitted to DoD HRPO 5/28/19; Approved 7/9/19. The San Antonio LSI, Dr. Catherine Do, was replaced by Dr. John Duch. The Eligibility Criteria were updated to specify location and era in which injury occurred resulting in fragment, and the participant must be able to provide urine specimen without the use of a catheter.
- **Modification 6:** Submitted to VA -CIRB on 12/9/19; Approved 12/17/19; Submitted to DoD HRPO 12/18/19; Acknowledged 12/19/19. This amendment included a minor change to the urine collection protocol and increased target enrollment numbers at 3 sites, but did not increase the overall target number.
- **Modification 7:** Submitted to VA -CIRB on 8/7/20; PI/SC Approved 9/2/20, LSIs Approved 9/17/20; Submitted to DoD HRPO 9/28/20; Acknowledged 10/2/20. The protocol was modified to eliminate all in-person study visits due to COVID-19.

*Please see the “Submissions Tracking Table” in the appendices for a summary of these amendments

Significant changes in use or care of vertebrate animals:

John F. Kalinich, Ph.D., Principal Investigator, Project 1 ***“Health Effects of Embedded Fragments of Military-Relevant Metals”***

Nothing to report

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2 ***“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”***

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications:

John F. Kalinich, Ph.D., Principal Investigator, Project 1 ***“Health Effects of Embedded Fragments of Military-Relevant Metals”***

1. D.E. Smith, T. Todorov, A.P. Defante, J.F. Hoffman, J.F. Kalinich, J.A. Centeno. Spectroscopic and Spectrometric Approaches for Assessing the Composition of Embedded Metals in Tissues. Applied Spectroscopy (under review).
2. Y. Wen, I. Vechetti, A. Alimov, J. Hoffman, V. Vergara, J. Kalinich, J. McCarthy, C. Peterson. Time Course Analysis of the Effect of Embedded Metal on Skeletal Muscle Gene Expression. Physiological Genomics (under review). Submitted in conjunction with Project Team 2.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2

“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

Wen, Y., I.J. Vechetti, A.P. Alimov, J.F. Hoffman, V.B. Vergara, J.F. Kalinich, J.J. McCarthy, C.A. Peterson. Time course analysis of the effect of embedded metal on skeletal muscle gene expression. *Physiol. Genomics*, in press.

PROJECTS 3 & 4

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3
“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Books or other non-periodical, one-time publications.

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

Nothing to report.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

Nothing to report.

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3
“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Nothing to report.

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Nothing to report.

Other publications, conference papers and presentations.

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

1. Seminar: Dr. Jessica Hoffman. Embedded metals alter blood brain barrier and synaptic expression in the rat. AFRRRI Seminar Series, 21 February 2020.
2. Abstract: Diane Smith, Jessica F. Hoffman, John F. Kalinich, Jose A. Centeno. Assessing the effect of embedded metals in tissues using novel spectroscopic techniques. American Chemical Society Spring Meeting, Philadelphia, PA. Note: abstract was selected for symposium talk, but meeting was cancelled due to covid-19 pandemic. Abstracts and presentation slides were made available online by the American Chemical Society in April 2020.
3. Virtual Seminar: Diane Smith, "Exploration of Raman Data: Rat Gastrocnemius Exposed to Ta, W, and Ni". Presentation to the Division of Biological, Chemical, and Material Science of the U.S. Food and Drug Administration, April 28, 2020.
4. Virtual Seminar: Jessica Hoffman, "Hydrophobic Sand: A Novel and Effective Method of Urine Collection in the Rodent". Presentation to the Animal Welfare Information Center (AWIC), Office of Laboratory Animal Welfare (OLAW), June 4, 2020.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2

"Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds"

Nothing to report.

PROJECTS 3 & 4

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3

"Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans"

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4

"Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals"

Scientific abstracts:

1. M. Reback, S.E. Hines, M. McDiarmid, C. Brown, J. Gaitens. (2020, March 15-19). *Biomarkers of acute kidney Injury in metal exposed war-injured Veterans: Preliminary results*, accepted for poster presentation at the Society of Toxicology Conference, Anaheim CA (conference canceled).
2. M. A. Reback, M. McDiarmid, J. Gaitens, C. H. Brown, K. Chin, D. R. Glick, P. S. Sriram, W. E. Lawson, K. Cavanaugh, L. Beck, J. Duch, S. E. Hines. (2020, May 15-20). *Respiratory symptoms in Veterans with embedded metal fragments: An interim analysis*. American Thoracic Society, Philadelphia, PA, U.S. (Conference canceled)
3. D. R. Glick, M. McDiarmid, J. Gaitens, C. H. Brown, M. A. Reback, K. Chin, P. S. Sriram, W. E. Lawson, K. Cavanaugh, L. Beck, J. Duch, S. E. Hines. (2020, May 15-20). *Impulse oscillometry testing among blast-exposed Veterans in the VA's Toxic Embedded Fragment Registry*. American Thoracic Society, Philadelphia, PA, U.S. (Conference canceled)
4. K. H. Chin, M. McDiarmid, J. Gaitens, C. H. Brown, M. A. Reback, D. R. Glick, P. S. Sriram, W. E. Lawson, K. Cavanaugh, L. Beck, J. Duch, S. E. Hines. (2020, May 15-20). *Pulmonary function testing in blast-exposed veterans from Operation Enduring Freedom*

and Operation Iraqi Freedom (OEF/OIF): An interim analysis. American Thoracic Society, Philadelphia, PA, U.S. (Conference canceled)

5. K.H. Chin, D.R. Glick, M. McDiarmid, J. Gaitens, C.H. Brown, M.A. Reback, P.S. Sriram, W.E. Lawson, K.L. Cavanaugh, L. Beck, J.M. Duch, S.E. Hines. (2020, August 24-27). *Use of pulmonary function testing and impulse oscillometry among blast-exposed Veterans in the Veterans Affairs' Toxic Embedded Fragment Registry- An interim analysis.* Military Health System Research Symposium (MHSRS), Orlando, FL, U.S. (Conference canceled)
6. M. Reback, S. E. Hines, M. McDiarmid, C.H. Brown, K. Cavanaugh, W. E. Lawson, P.S. Sriram, L. Beck, J. Duch, and J. Gaitens. (2020, August 24-27). *Systemic metal exposure and kidney injury in war-injured Veterans with embedded metal fragments: An interim analysis.* MHSRS, Orlando. FL, U.S. (Conference canceled)

Research Presentations:

1. M.A. Reback, M.D. "Renal effects of embedded fragments: Interim data review", presented to Project 3 & 4 study investigators and coordinators via web-conference on November 13, 2019.
2. K. Chin, M.D. "Primary blast injury in the deployer: is the lung hiding in plain sight?" presented to Project 3 & 4 study investigators and coordinators via web-conference on December 17, 2019.
3. Danielle Glick, M.D. "Assessment of airways disease in Veterans", presented to Project 3 & 4 study investigators and coordinators via web-conference on January 29, 2020, and at University of Maryland Medical Center Pulmonary Grand Rounds on March 3, 2020.
4. Danielle Glick, M.D., K. H. Chin, M.D. "Use of pulmonary function testing and impulse oscillometry among blast-exposed Veterans in the Veterans Affairs' Toxic Embedded Fragment Registry- An interim analysis", University of Maryland PCCM Virtual Presentation, May 20, 2020.
5. Maxwell Reback, M.D. "Biomarkers of acute injury in metal exposed war-injured Veterans: Preliminary results", University of Maryland PCCM Virtual Presentation, May 18, 2020.
6. Jessica Palmer. "Measuring general health status in the blast fragment survey population", presented virtually to Project 3 & 4 study team members, June 12, 2020.
7. Jessica Palmer. "General health status among Veterans with embedded metal fragments", University of Maryland School of Medicine Summer Research Programs, Virtual Student Research Forum, July 30, 2020.

• Website(s) or other Internet site(s)

John F. Kalinich, Ph.D., Principal Investigator, Project 1
"Health Effects of Embedded Fragments of Military-Relevant Metals"

Nothing to report.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
"Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds"

Nothing to report.

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3

“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Nothing to report.

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Nothing to report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

Nothing to report.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

Nothing to report.

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3
“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Nothing to report.

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Nothing to report.

- **Inventions, patent applications, and/or licenses**

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

Nothing to report.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

Nothing to report.

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Leader/Principal Investigator, Project 3
“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Nothing to report.

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Nothing to report.

- **Other Products**

John F. Kalinich, Ph.D., Principal Investigator, Project 1
“Health Effects of Embedded Fragments of Military-Relevant Metals”

Nothing to report.

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

The muscle microarray and RNAseq data were deposited in the NCBI Gene Expression Omnibus (GEO) repository. The data can be found in Series GSE157931, which includes both the microarray (GSE157921) and the RNA-seq (GSE157929) data.

Stella Hines, M.D., MSPH, Project Leader/Principal Investigator, Project 4
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Melissa McDiarmid, M.D., Principal Investigator:
“Assessing the Health Effects of Blast Injuries and Embedded Metal Fragments”

Name: Melissa McDiarmid, M.D.
Project Role: Principal Investigator

Nearest Person Month worked: 2.40

Contribution to Project: Dr. McDiarmid oversaw conduct and progress of all four study projects and participated in quarterly project team calls.

Name: Rachel Coates-Knowles, MSM

Project Role: Finance Manager

Nearest Person Month worked: 6.0

Contribution to Project: Maintained and processed all financial transactions and reporting.

Name: Clayton Brown

Project Role: Statistician

Nearest Person Month worked: 1.2

Contribution to Project: Provided input on data entry processes and conducted preliminary data analyses.

Name: Sheila Williams

Project Role: Administrative Assistant

Nearest Person Month worked: 1.20

Contribution to Project: Assisted with procurement, document preparation, and scheduling teleconferences.

John F. Kalinich, Ph.D., Principal Investigator, Project 1:

“Health Effects of Embedded Fragments of Military-Relevant Metals”

Name: John Kalinich, PhD

Project Role: Principal Investigator, Project 1

Researcher Identifier: 0000-0003-1591-9389

Nearest person month worked: 2 (no change)

Contribution to Project: Responsible for overall functioning of this portion of the project.

Funding Support: Federal Government Employee (Department of Defense)

Name: Christine Kasper, PhD RN, FAAN FACS

Project Role: Co-Investigator,

Research Identifier: 0000-0002-7784-2519

Nearest person month worked: 1 (no change)

Contribution to Project: Responsible for experimental planning

Funding Support: Federal Government Employee (Department of Veterans Affairs)

Name: Jessica Hoffman, PhD

Project Role: Co-Investigator

Researcher Identifier: 0000-0003-1858-8394

Nearest person month worked: 5 (no change)

Contribution to Project: Member of the surgical implantation and euthanasia teams. Responsible for protein analysis of collected tissues.

Funding Support: Federal Government Employee (Department of Defense)

Name: Vernieda Vergara, BS
Project Role: Research Assistant
Nearest person month worked: 12 (no change)
Contribution to Project: Responsible for implantation surgeries and animal welfare, sample collection and metal analysis.

Name: Jose Centeno, PhD, FRSC
Project Role: Co-investigator
Nearest Person Month worked: 1 (no change)
Contribution to Project: Oversight of metal imaging experiments and post-doctoral fellow
Funding Support: Federal Government Employee (U.S. Food and Drug Administration)

Name: Diane Smith, PhD
Project Role: Post-doctoral fellow
Nearest person month worked: 12 (no change)
Contribution to Project: Metal imaging experiments under the direction of Dr. Centeno

Charlotte A. Peterson, Ph.D., Principal Investigator, Project 2:
“Biomarkers for Assessing Return-to-Duty Potential of Personnel with Embedded Metal-Fragment Wounds”

Name: Charlotte A. Peterson, PhD
Project Role: Principal Investigator, Project 2
Nearest person month worked: 1.2 (no change)
Contribution to Project: Responsible for overall functioning of this portion of the project.
Funding Support: University of Kentucky

Name: John J. McCarthy, PhD
Project Role: Co-Investigator
Nearest person month worked: 1.2 (no change)
Contribution to Project: Responsible for experimental planning
Funding Support: University of Kentucky

Name: Alexander Alimov
Project Role: Research Scientist II
Nearest person month worked: 12
Contribution to Project: Responsible for exosome isolation and characterization (Western blot analysis) and RNA isolation.

Name: Ivan Vechetti
Project Role: Postdoctoral Scholar
Nearest person month worked: 7
Contribution to Project: Dr. Vechetti accepted a faculty position and his last day on the project was June 30, 2020.

Name: Yuan Wen, MD, PhD
Project Role: Post-doctoral scholar

Nearest person month worked: 3
Contribution to Project: Dr. Wen replaced Dr. Vechetti on the project. He is an accomplished bioinformatician.

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Lead Investigator/ Local Site PI, Project 3:
“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Name: Joanna Gaitens, PhD, MSN/MPH
Project Role: Project Lead Investigator/ Local Site PI
Nearest person month worked: 2.4
Contribution to Project: Responsible for overall functioning of this portion of the project, including overseeing recruitment, enrollment, data collection, specimen collection, regulatory protocols, and project team meetings.

Stella Hines, M.D., MSPH, Project Lead Investigator/ Local Site PI, Project 4:
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Name: Stella Hines, MD, MSPH
Project Role: Project Lead Investigator/ Local Site PI
Nearest person month worked: 2.4
Contribution to Project: Responsible for overall functioning of this portion of the project, including overseeing recruitment, enrollment, data collection, pulmonary testing, regulatory protocols, and project team meetings.

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

Dr. John Kalinich’s CDMRP-funded Gulf War Illness grant has been successfully completed.

Dr. Hines has been awarded special purpose clinical funding from VA Post-deployment Health Services via the Airborne Hazards Center of Excellence to direct the Baltimore VA site of the Post Deployment Cardiopulmonary Evaluation Network. She will spend 2.4 calendar months on this project. She also has been awarded a NIOSH contract to study hospital elastomeric respirator use best practices during COVID-19. She will spend 0.6 calendar months on this new project. Neither project creates support overlap with the CDMRP research.

What other organizations were involved as partners?

Participant Enrollment Sites – Clinical Collaboration

Baltimore VAMC (Site 1)

Joanna Gaitens and Stella Hines are the Local Site Principal Investigators for the Baltimore recruitment site. Their contributions to the projects are listed above.

Name: Kate Agnetti, BS
Project Role: Research Coordinator
Nearest person month worked: 12
Contribution to Project: Interacted with HRPO and regulatory bodies in order to obtain and maintain required approvals; assisted in developing recruitment, enrollment, and scheduling strategies; recruited and enrolled participants, collected data and specimens; organized and participated in quarterly project team calls and biweekly site calls.

Name: Christopher Crayton
Role on Project: Database Manager
Nearest person month worked: 12
Contribution to Project: Data entry and database maintenance

Name: Brian Lambert
Role on Project: Data Entry
Nearest person month worked: 9.6 person months (annually); 2.4 person months (quarterly)
Contribution to Project: Began data entry on April 1, 2020

Nashville (Site 2):

Name: Kerri Cavanaugh, MD MHS
Project Role: Local Site Investigator
Nearest person month worked: 1.2
Contribution to Project: Acquired and maintained required approvals; oversaw local recruitment, enrollment, specimen collection; participated in quarterly project team calls.

Name: William Lawson, MD
Project Role: Local Site Investigator
Nearest person month worked: 0.6
Contribution to Project: Acquired and maintained required approvals; participated in quarterly project team calls.

Name: Audrey Tesi
Project Role: Local Study Coordinator
Nearest person month worked: 12
Contribution to Project: Acquired and maintained required approvals; participated in quarterly project team calls, attended biweekly site calls; recruited and enrolled participants; collected data and specimens.

Gainesville (Site 3):

Name: Perevumba Sriram, MD
Project Role: Local Site Investigator
Nearest person month worked: 0.6 (5% effort)
Contribution to Project: Acquired and maintained required approvals; oversaw local recruitment, enrollment, specimen collection; participated in quarterly project team calls.

Name: Nataliya Kirichenko

Project Role: Local Study Coordinator
Nearest person month worked: 4.5 (37.5% effort)
Contribution to Project: Acquired and maintained required approvals; participated in quarterly project team calls and biweekly site calls; recruited and enrolled participants; collected data and specimens.

Name: Katherine Solis
Project Role: Local Study Coordinator
Nearest person month worked: 4.5 (37.5% effort)
Contribution to Project: Acquired and maintained required approvals; participated in quarterly project team calls, attended biweekly site calls; recruited and enrolled participants; collected data and specimens.

Name: Paige Webb (Formerly Gustad)
Project Role: Local Regulatory Assistant
Nearest person month worked: 1.2 (10% effort)
Contribution to Project: Interacted with local HRPO and regulatory bodies

Oklahoma City (Site 4):

Name: Lisa Beck, MD
Project Role: Local Site Investigator
Nearest person month worked: 1.8
Contribution to Project: Acquired and maintained required approvals; oversaw local recruitment, enrollment, specimen collection; participated in quarterly project team calls.

Name: Vickie Phillips
Project Role: Local Study Coordinator
Nearest person month worked: 7.2
Contribution to Project: Acquired and maintained required approvals; participated in quarterly project team calls, attended biweekly site calls; recruited and enrolled participants; collected data and specimens.

San Antonio (Site 5):

Name: John Duch, MD
Project Role: Local Site Investigator
Nearest person month worked: 1.2 (20% effort)
Contribution to Project: Acquired and maintained required approvals; oversaw local recruitment, enrollment, specimen collection; participated in quarterly calls.

Name: Antonio Anzueto, MD
Project Role: Local Site Investigator
Nearest person month worked: 1.2
Contribution to Project: Acquired and maintained required approvals.

Name: Alex Aguilera
Project Role: Local Study Coordinator
Nearest person month worked: 1.2
Contribution to Project: Acquired and maintained required approvals; participated in quarterly project team calls and biweekly site calls; recruited and enrolled participants.

Name: Myra Mireles
Project Role: Local Study Coordinator
Nearest person month worked: 12

Contribution to Project: Acquired and maintained required approvals; participated in quarterly project team calls, attended biweekly site calls; recruited and enrolled participants; collected data and specimens.

Phoenix (Site 6):

Name: Paska Permana, PhD
Project Role: Local Site Investigator
Nearest person month worked: 1.2 (15% effort annually; Person months reflects site approved as of April 9, 2019.)

Contribution to Project: Acquired and maintained required approvals; oversaw local recruitment, participated in quarterly calls.

Name: Samuel Aguayo, MD
Project Role: Local Site Investigator
Nearest person month worked: 1.2 (15% effort annually; Person months reflects site approved as of April 9, 2019.)

Contribution to Project: Acquired and maintained required approvals; oversaw local recruitment, participated in quarterly calls.

Name: Kelli Bingham
Project Role: Local Study Coordinator
Nearest person month worked: 12
Contribution to Project: Acquired and maintained required approvals; participated in quarterly project team calls, attended biweekly site calls; recruited and enrolled participants; collected data and specimens.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to report.

QUAD CHART

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John F. Kalinich, Ph.D., Principal Investigator, Project 1:
“Health Effects of Embedded Fragments of Military-Relevant Metals”

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Projects 3 & 4

Joanna Gaitens, Ph.D., MSN/MPH, RN, Project Lead Investigator/ Local Site PI, Project 3:
“Biomarker assessment of kidney injury from metal exposure in embedded fragment registry veterans”

Stella Hines, M.D., MSPH, Project Lead Investigator/ Local Site PI, Project 4:
“Respiratory health in a cohort of embedded fragment registry veterans exposed to blasts and metals”

Poster Presentations:

1. K.H. Chin, D.R. Glick, M. McDiarmid, J. Gaitens, C.H. Brown, M.A. Reback, P.S. Sriram, W.E. Lawson, K.L. Cavanaugh, L. Beck, J.M. Duch, S.E. Hines. (2020, August 24-27). *Use of pulmonary function testing and impulse oscillometry among blast-exposed Veterans in the Veterans Affairs’ Toxic Embedded Fragment Registry- An interim analysis.* Military Health System Research Symposium (MHSRS), Orlando, FL, U.S. (Conference canceled)
2. M. Reback, S.E. Hines, M. McDiarmid, C. Brown, J. Gaitens. (2020, March 15-19). *Biomarkers of acute kidney Injury in metal exposed war-injured Veterans: Preliminary results,* accepted for poster presentation at the Society of Toxicology Conference, Anaheim CA (conference canceled).
3. M. A. Reback, M. McDiarmid, J. Gaitens, C. H. Brown, K. Chin, D. R. Glick, P. S. Sriram, W. E. Lawson, K. Cavanaugh, L. Beck, J. Duch, S. E. Hines. (2020, May 15-20). *Respiratory symptoms in Veterans with embedded metal fragments: An interim analysis.* American Thoracic Society, Philadelphia, PA, U.S. (Conference canceled)

Table 1: Tumor Incidence at the Pellet Implantation Sites

Experimental Groups				
Metal	1 Month	3 Month	6 Month	12 Month
Ni	8 of 8	8 of 8	8 of 8	8 of 8
Co	0 of 8	4 of 8	6 of 8	7 of 8
Cu	1 of 8			

Data represent the number of animals with visible tumors surrounding the embedded metal pellets at the time of euthanasia. No other experimental metals resulted in tumor formation at the pellet implantation site.

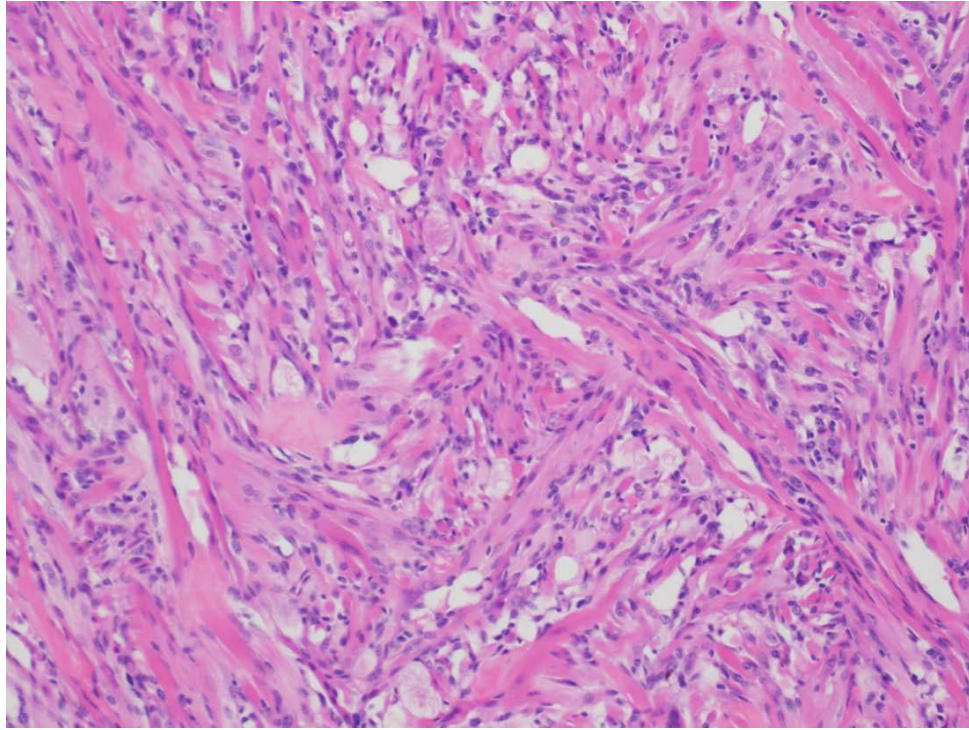
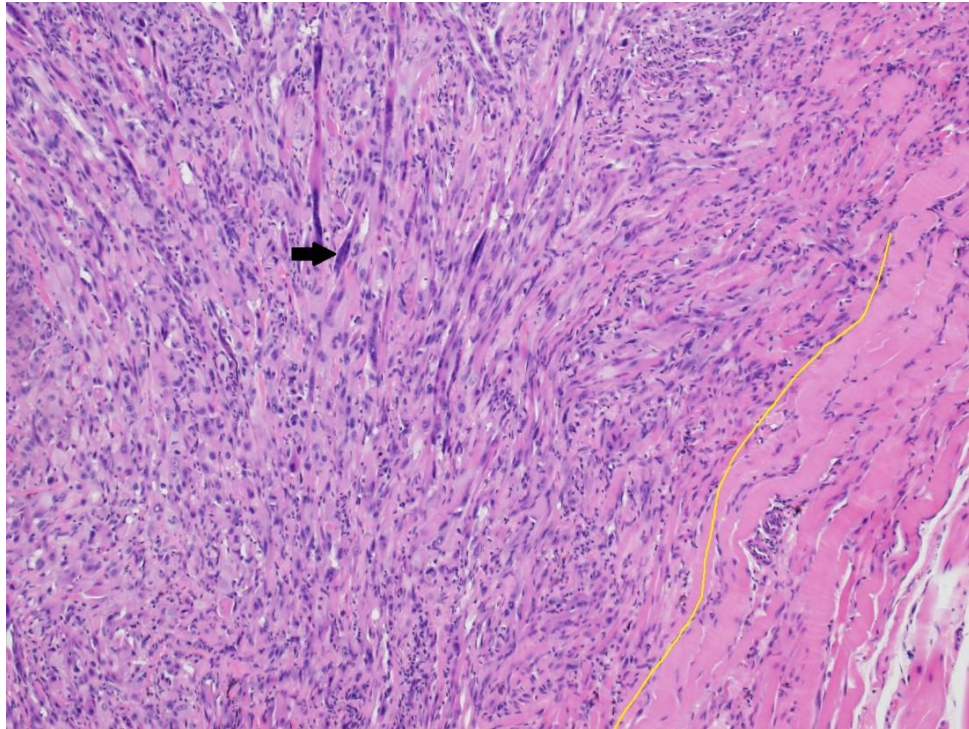


Figure 1: Photomicrograph of H&E-stained gastrocnemius from Ni-implanted rat.

Image shows rhabdomyosarcoma, magnification 200X.

A



B

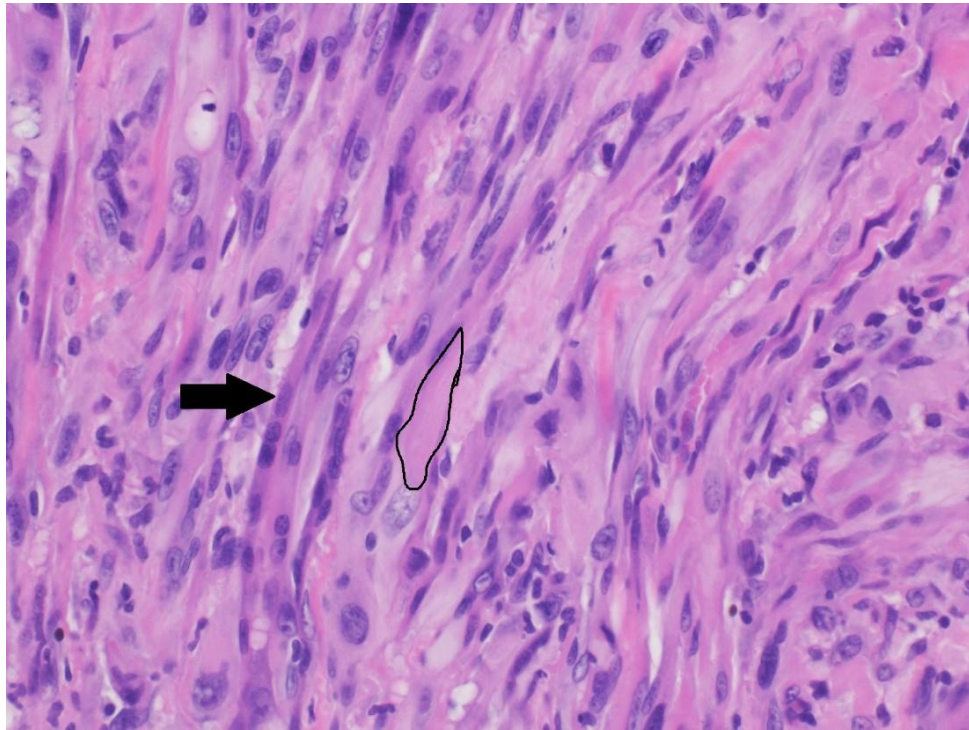


Figure 2: Photomicrograph of H&E- stained gastrocnemius from Ni-implanted rat.

Panel A shows rhabdomyosarcoma at 100X magnification with black arrow indicating strap cells and yellow line differentiating tumor from muscle. Panel B shows 400X magnification of rhabdomyosarcoma with black arrow indicating strap cells and black circle showing eosinophilic cytoplasm.

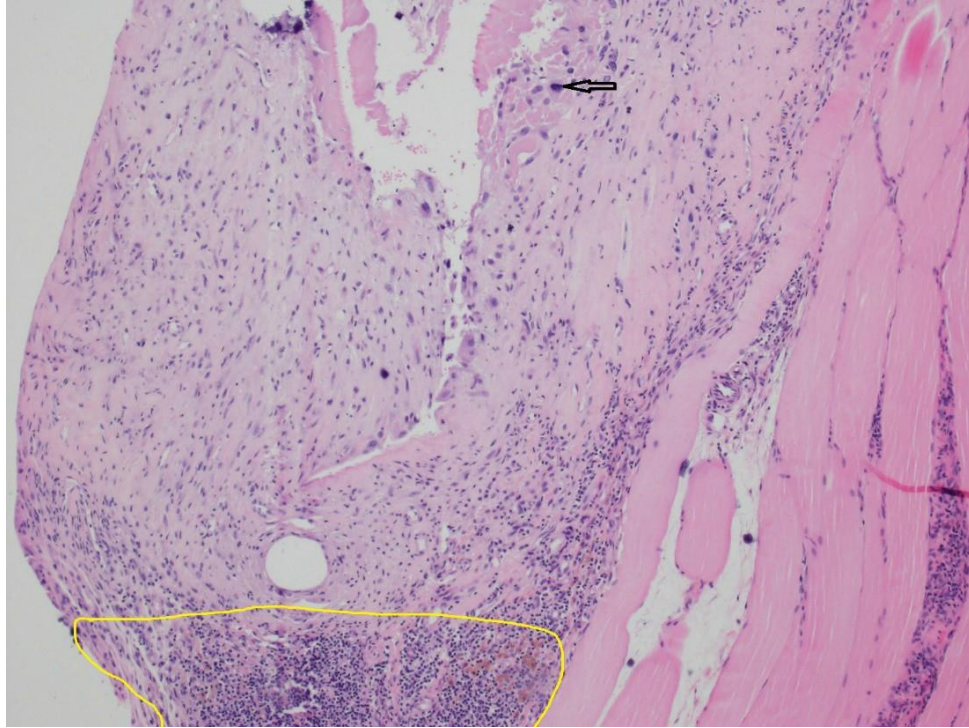


Figure 3: Photomicrograph of H&E-stained gastrocnemius of Co-implanted rat. Image shows a spindle cell neoplasm at 100X magnification. Black arrow is an abnormal mitotic cell and yellow line marks an area of lymphocytes and pigmented macrophages.

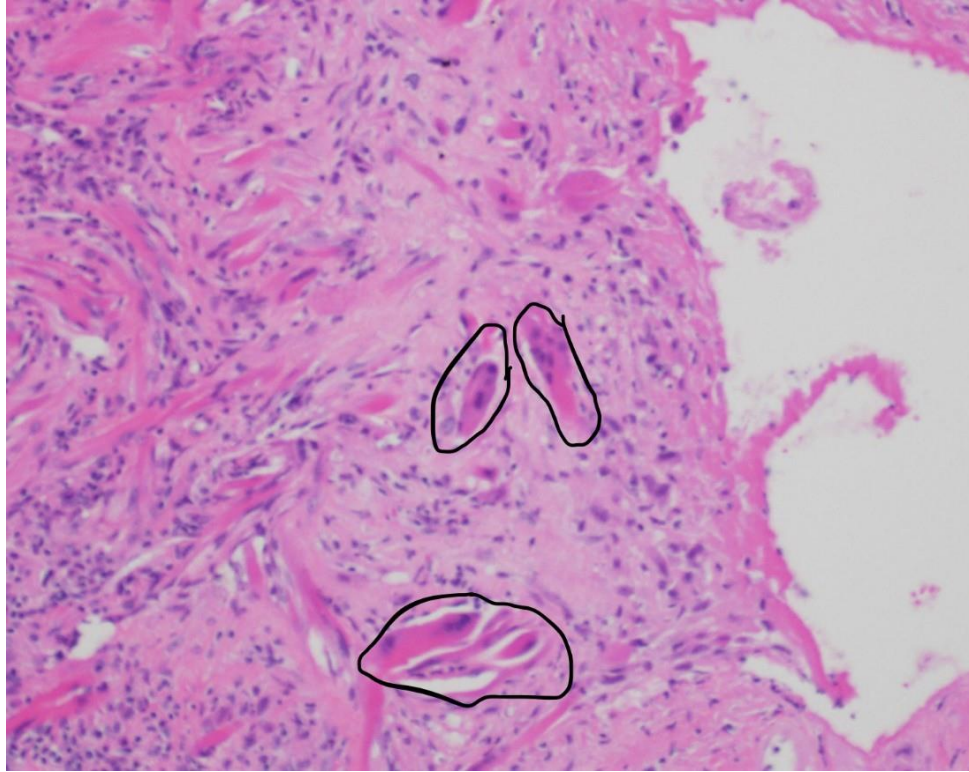


Figure 4: Photomicrograph of H&E-stained gastrocnemius from Co-implanted rat. Image shows granuloma in muscle with large multinucleated muscle cells in circled areas which may be early neoplastic cells or muscle cells attempting to regenerate in the inflammatory region.

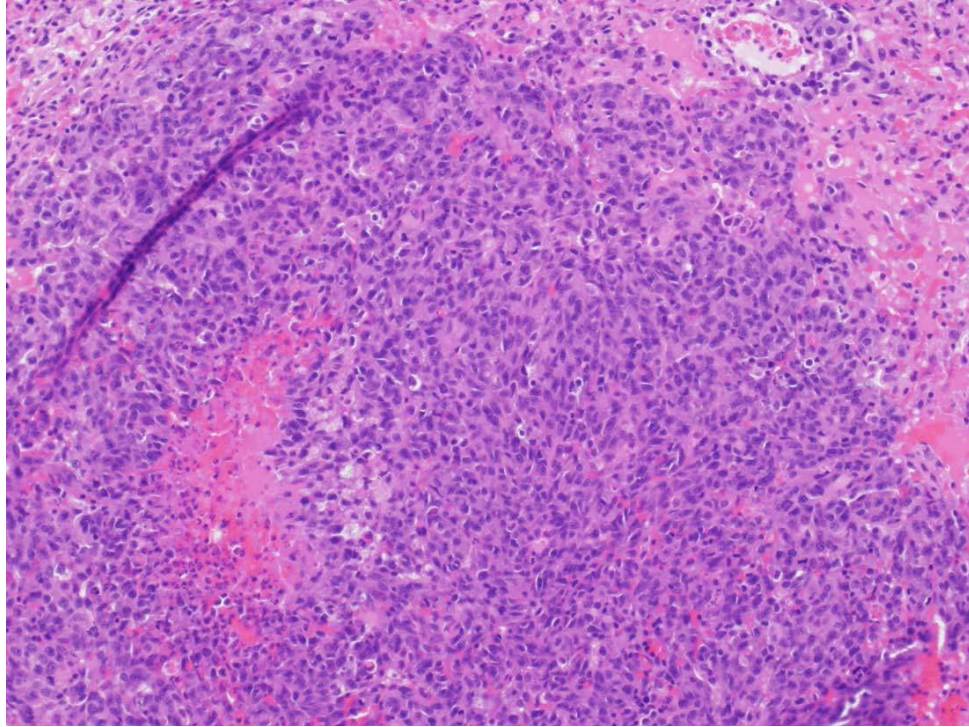


Figure 5: Photomicrograph of H&E-stained lung from Ni-implanted rat. Image shows tumor identified as rhabdomyosarcoma that had metastasized from tumor in gastrocnemius. 200X magnification.

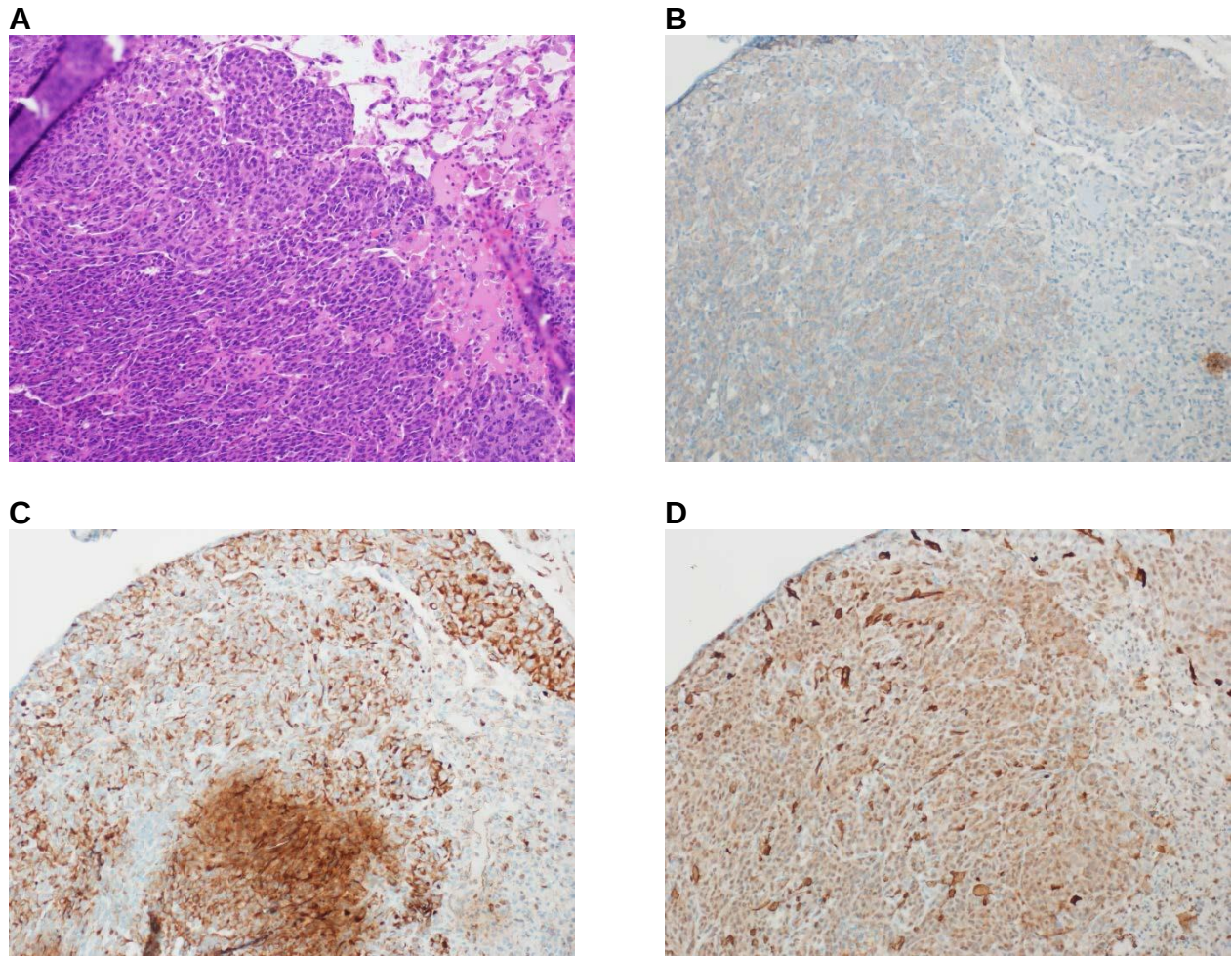


Figure 6: Photomicrographs of lung tumor from Ni-implanted rat. Panel A – H&E stained rhabdomyosarcoma lung tumor; Panel B – lung tumor probed with anti-cytokeratin antibody; Panel C - lung tumor probed with anti-vimentin antibody; Panel D - lung tumor probed with anti-muscle-specific actin antibody. Immunohistochemistry results suggest lung tumor metastized from rhabdomyosarcoma in gastrocnemius muscle. 200X magnifications.

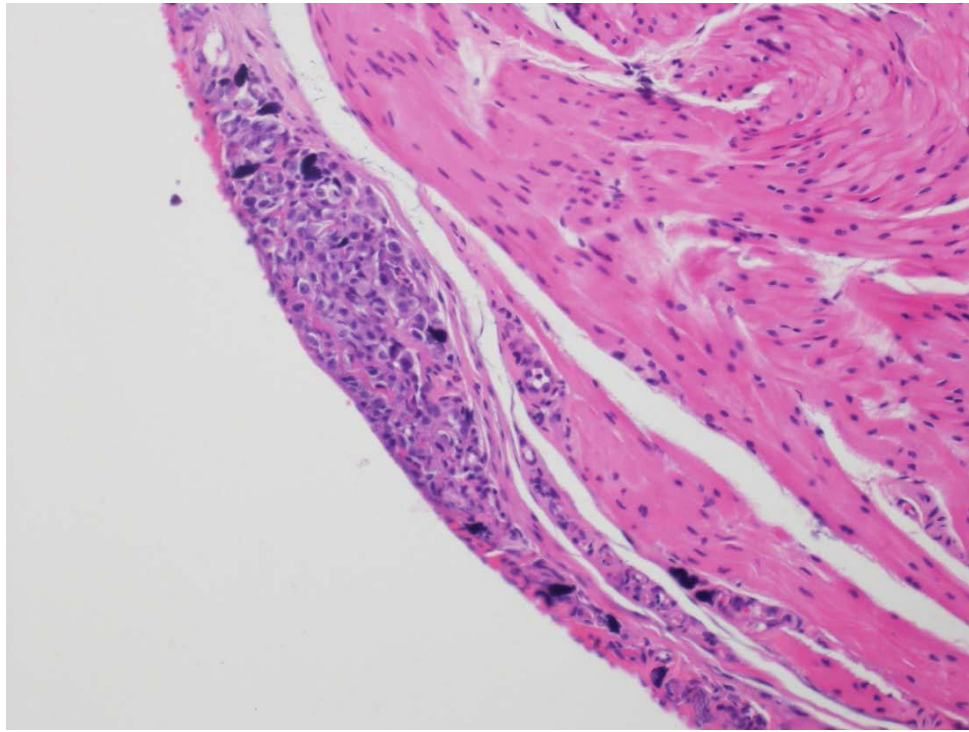


Figure 7: Photomicrograph of urinary bladder tumor in Ni-implanted rat.

Image (200X magnification) shows bladder tumor from Ni-implanted rat that also had an identified rhabdomyosarcoma in the gastrocnemius and lung (see Figure 8). Although tentatively identified as a rhabdomyosarcoma, immunohistochemistry validation was not conclusive on this bladder tumor.

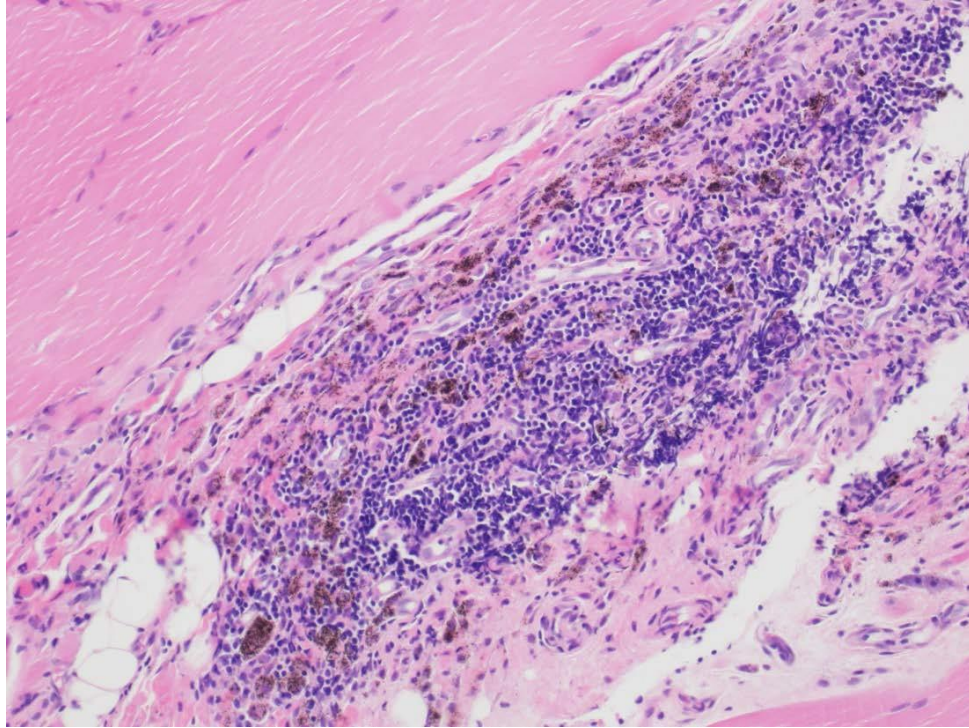


Figure 8: Photomicrograph of H&E stained gastrocnemius from DU-implemented rat. Image (200X magnification) shows moderate amount of inflammation primarily composed of lymphocytes and pigmented macrophages.

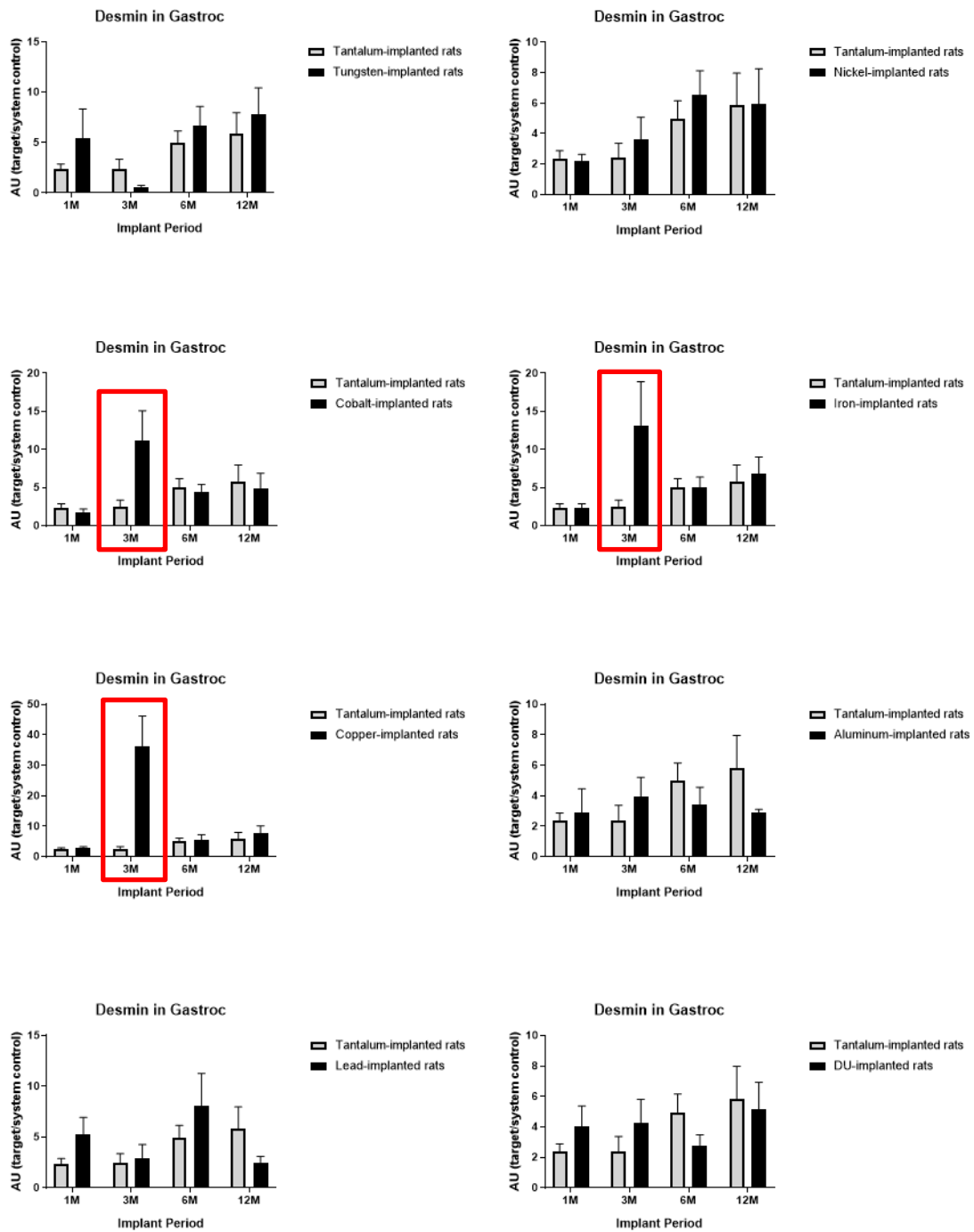


Figure 9: Desmin expression in gastrocnemius muscle implanted with metal pellets for various times. Tissue was dissected from an area around the implanted pellet, homogenized, and analyzed using the Protein Simple Wes System and appropriate antibody against rat desmin. Red box indicates a statistically significant difference when compared to tantalum control.

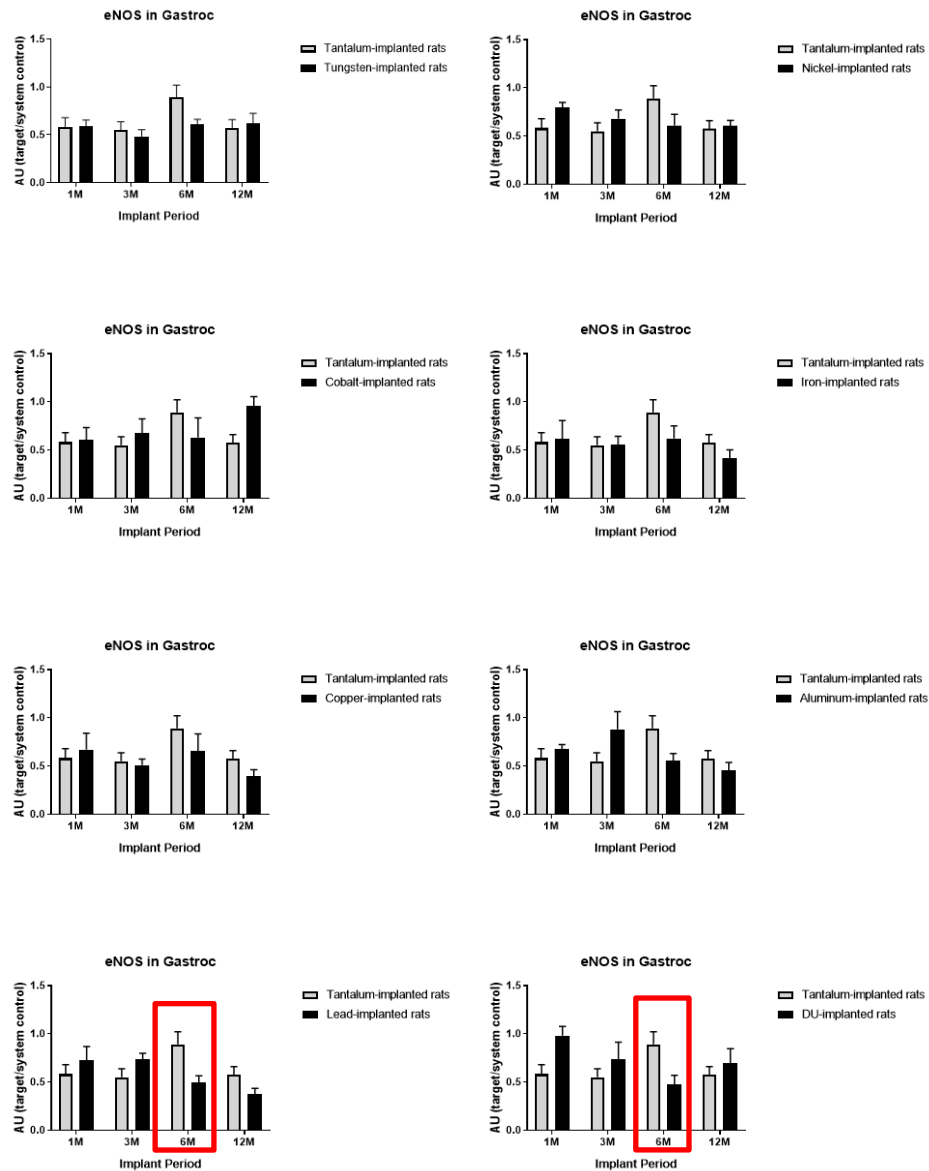


Figure 10: Endothelial Nitric Oxide Synthase (eNOS) expression in gastrocnemius muscle implanted with metal pellets for various times. Tissue was dissected from an area around the implanted pellet, homogenized, and analyzed using the Protein Simple Wes System and appropriate antibody against rat eNOS. Red box indicates a statistically significant difference when compared to tantalum control.

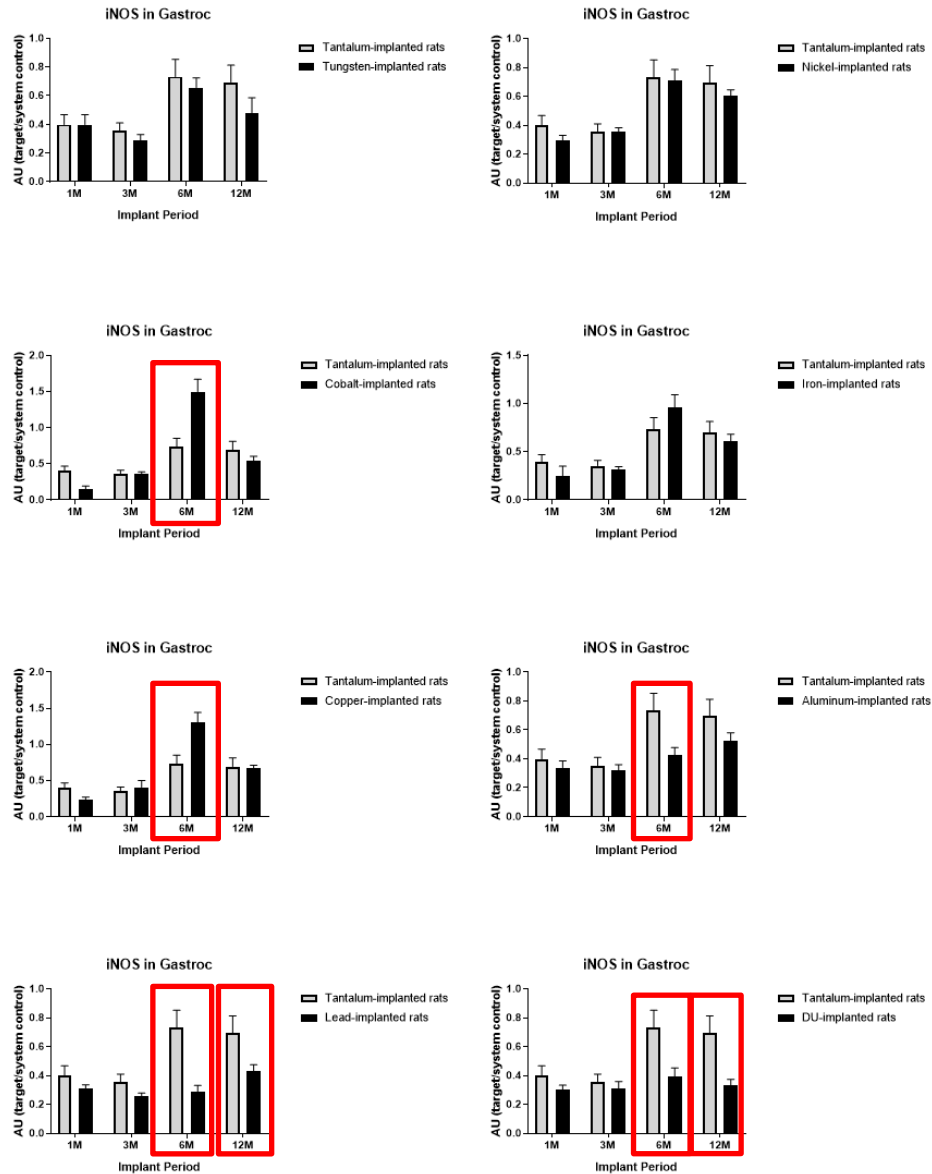


Figure 11: Inducible Nitric Oxide Synthase (iNOS) expression in gastrocnemius muscle implanted with metal pellets for various times. Tissue was dissected from an area around the implanted pellet, homogenized, and analyzed using the Protein Simple Wes System and appropriate antibody against rat iNOS. Red box indicates a statistically significant difference when compared to tantalum control.

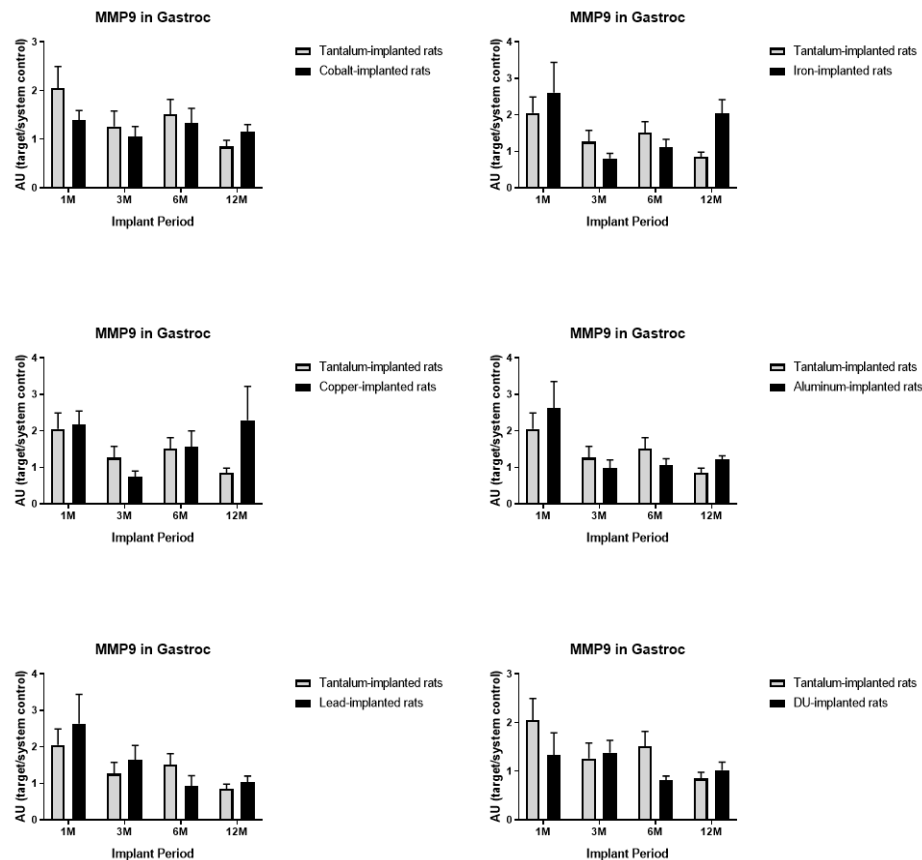


Figure 12: Matrix Metalloproteinase 9 (MMP-9) expression in gastrocnemius muscle implanted with metal pellets for various times. Tissue was dissected from an area around the implanted pellet, homogenized, and analyzed using the Protein Simple Wes System and appropriate antibody against rat MMP-9. Red box indicates a statistically significant difference when compared to tantalum control.

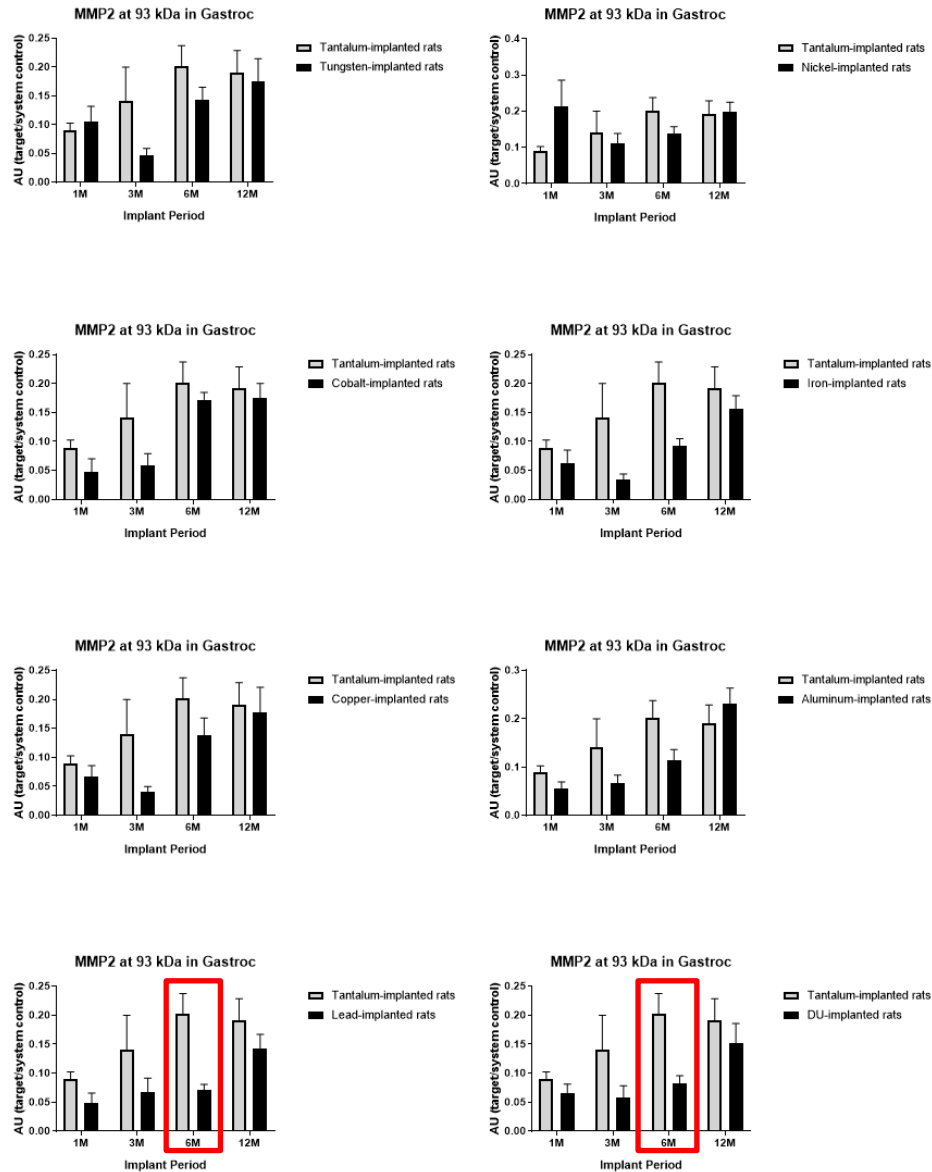


Figure 13: Matrix Metalloproteinase 2 (MMP-2) expression in gastrocnemius muscle implanted with metal pellets for various times. Tissue was dissected from an area around the implanted pellet, homogenized, and analyzed using the Protein Simple Wes System and appropriate antibody against full-length rat MMP-2 (93 kDa). Red box indicates a statistically significant difference when compared to tantalum control.

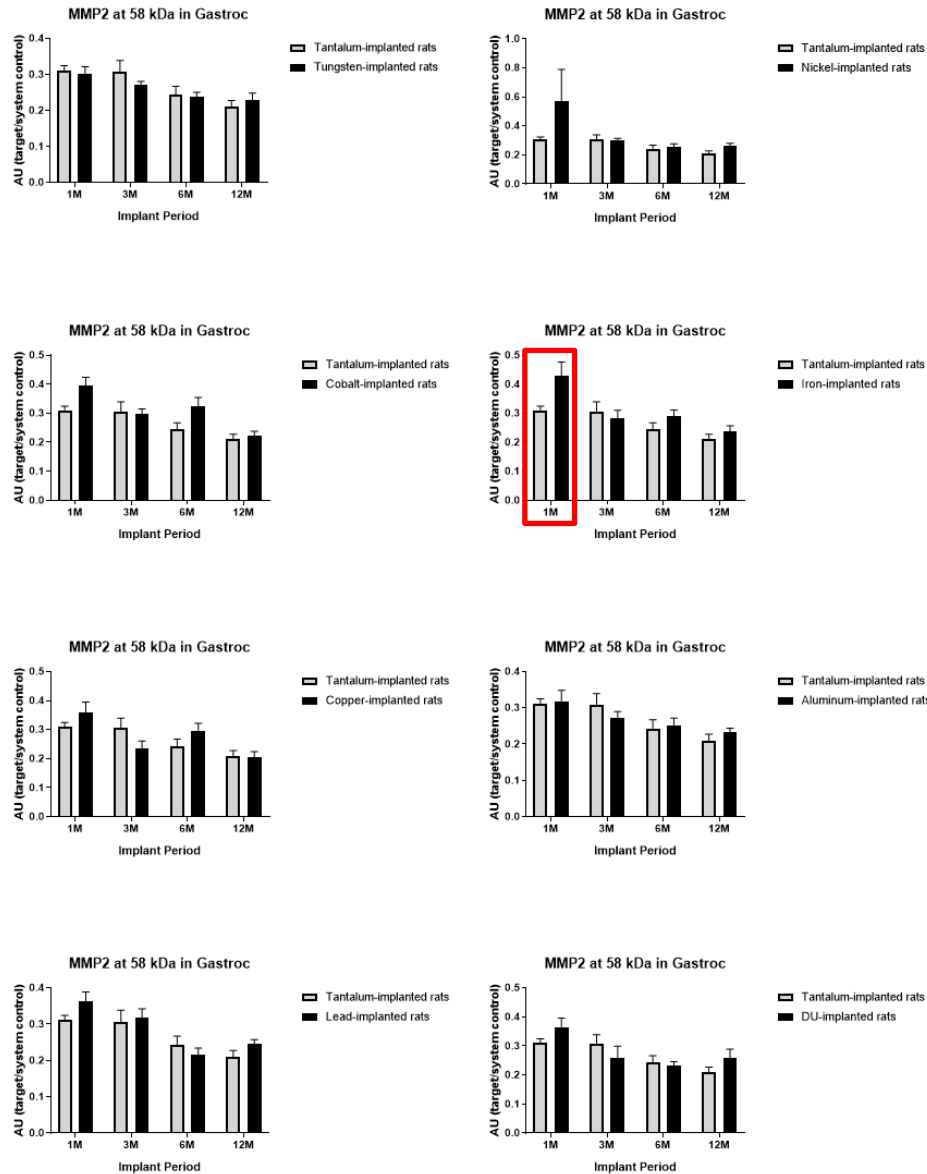


Figure 14: Matrix Metalloproteinase 2 (MMP-2) 58 kDa cleavage product expression in gastrocnemius muscle implanted with metal pellets for various times. Tissue was dissected from an area around the implanted pellet, homogenized, and analyzed using the Protein Simple Wes System and appropriate antibody against 58 kDa MMP-2 cleavage product. Red box indicates a statistically significant difference when compared to tantalum control.

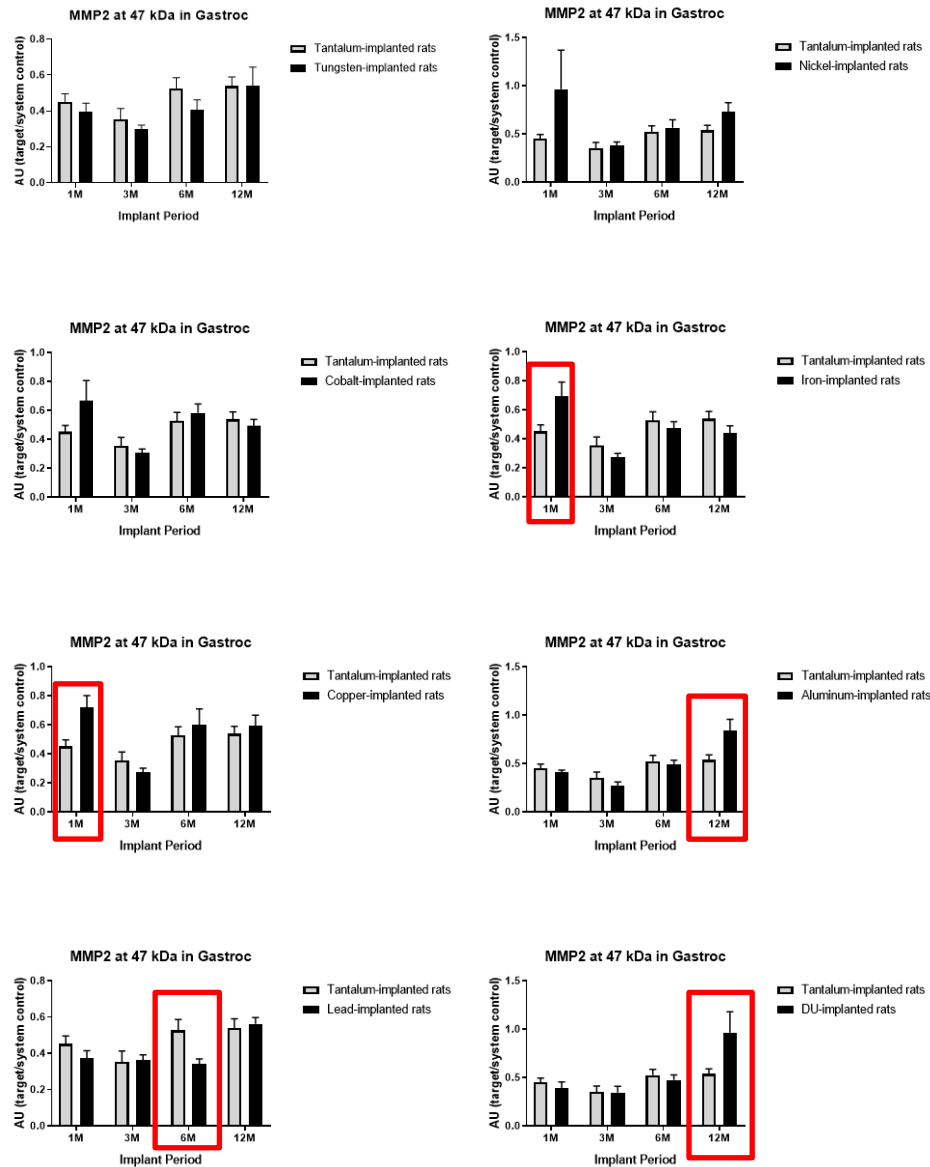


Figure 15: Matrix Metalloproteinase 2 (MMP-2) 47 kDa cleavage product expression in gastrocnemius muscle implanted with metal pellets for various times. Tissue was dissected from an area around the implanted pellet, homogenized, and analyzed using the Protein Simple Wes System and appropriate antibody against 47 kDa MMP-2 cleavage product. Red box indicates a statistically significant difference when compared to tantalum control.

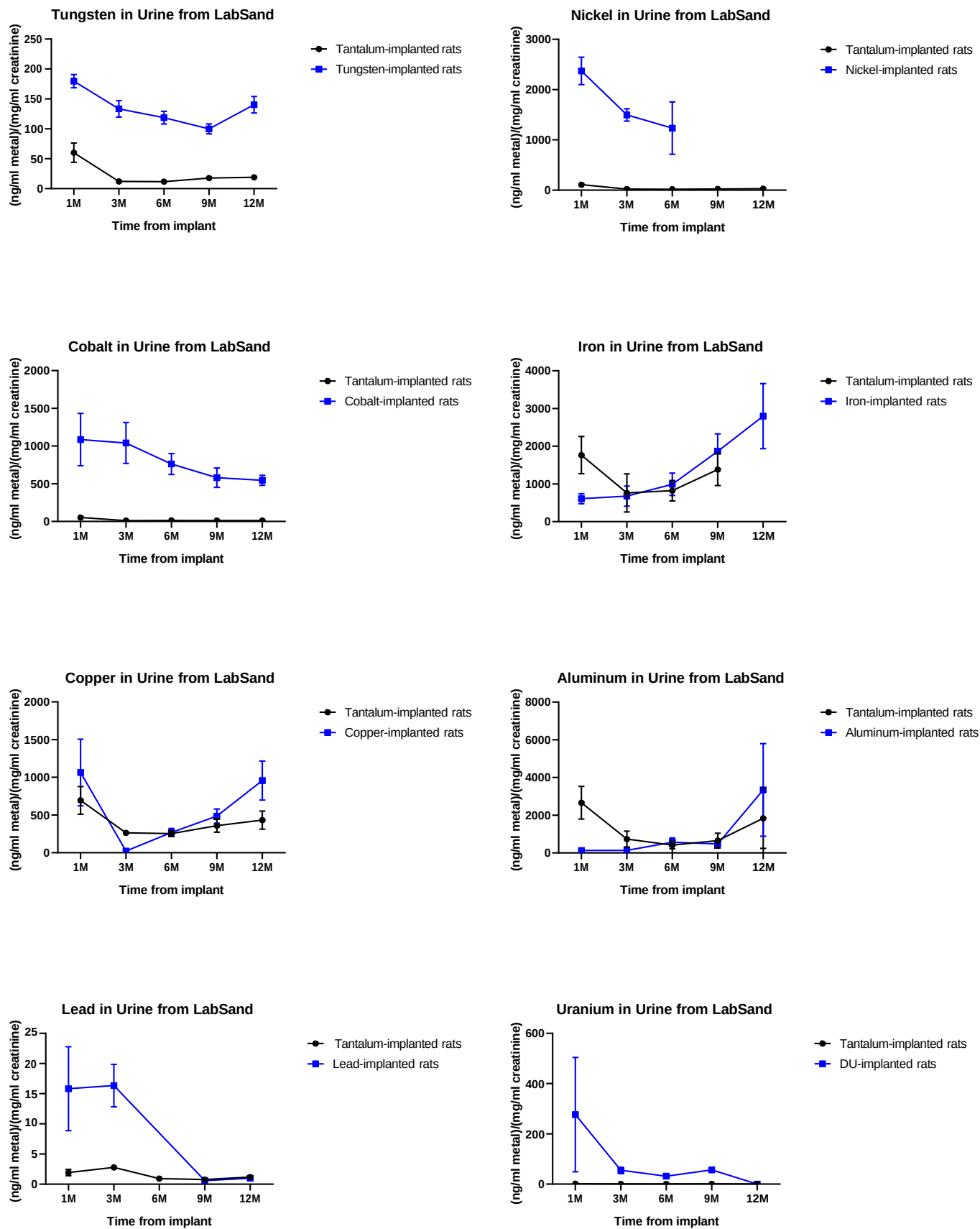


Figure 16: Urinary metal levels from implanted rats.

Table 2: Experimental Groups with Enhanced Hydroxynonenal-modified Proteins in the Gastrocnemius as a Result of Metal Implantation.

Protein Molecular Weight (kDa)		
35 kDa	55 kDa	95 kDa
12 M Co 12 M Cu 6 M DU	6M Co 3M Fe 12M Fe 12M Cu 12M Al 6M DU	3M Fe 6M Fe 12M Fe 12M Pb 3M DU
40 kDa	62 kDa	144 kDa
6M Fe 12M Fe 12M Pb 12M DU	12M Cu 12M Pb	3M Fe 12M Fe 3M Pb 12M Pb

Tissue was dissected from an area around the implanted pellet, homogenized, and analyzed using the Protein Simple Wes System and an antibody against hydroxynonenal. Hydroxynonenal is a highly reactive aldehyde derived from the decomposition of peroxidation products of omega-polyunsaturated fatty acids such as arachidonic and linoleic. Increased production of hydroxynonenal is an indication of oxidative stress and lipid peroxidation. The most highly modified proteins were characterized by molecular weight (in kDa) and experimental groups compared to tantalum control. Data in the table represent those metal implantation groups that had higher levels of hydroxynonenal-modified proteins, at that particular molecular weight, when compared to tantalum control group.

Table 3: Urinary Biomarkers Being Assessed

- Alkaline Phosphatase (ALP)
- N-Acetyl-Glucosaminidase (NAG)
- Retinol Binding Protein (RBP)
- Alpha-2-Macroglobulin (α -2-M)
- Beta-2-Microglobulin (B-2-M)
- IL-18
- Creatinine
- Total Protein
- NGAL (lipocalcin-2)
- Osteopontin
- Albumin
- KIM-1 / Tim-1
- Urea Nitrogen
- Specific Gravity

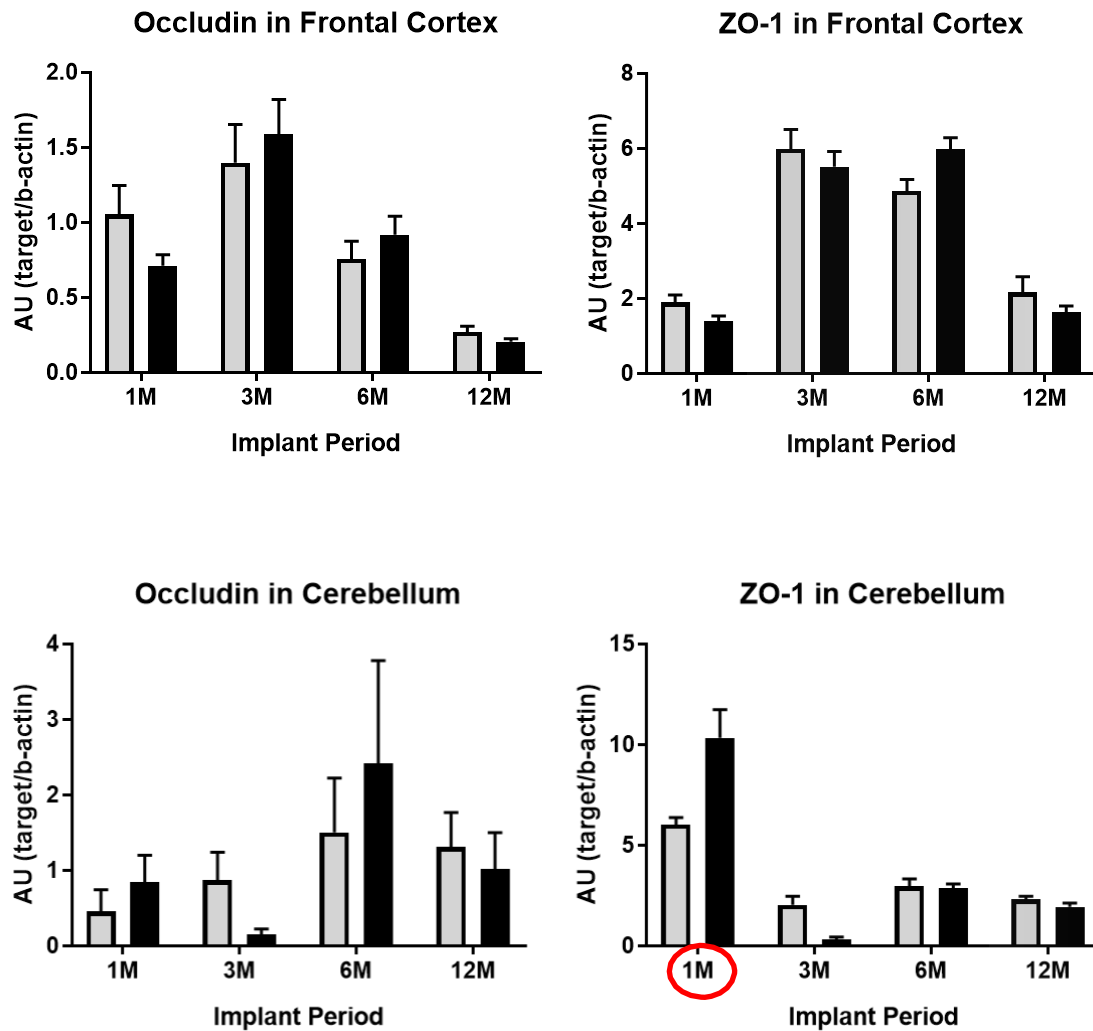


Figure 17: Blood-brain barrier markers in aluminum-implanted rats. Gray bars represent tantalum control rats while black bars represent aluminum-implanted rats. Red circle indicates statistically significant difference between tantalum control and aluminum-implanted rats.

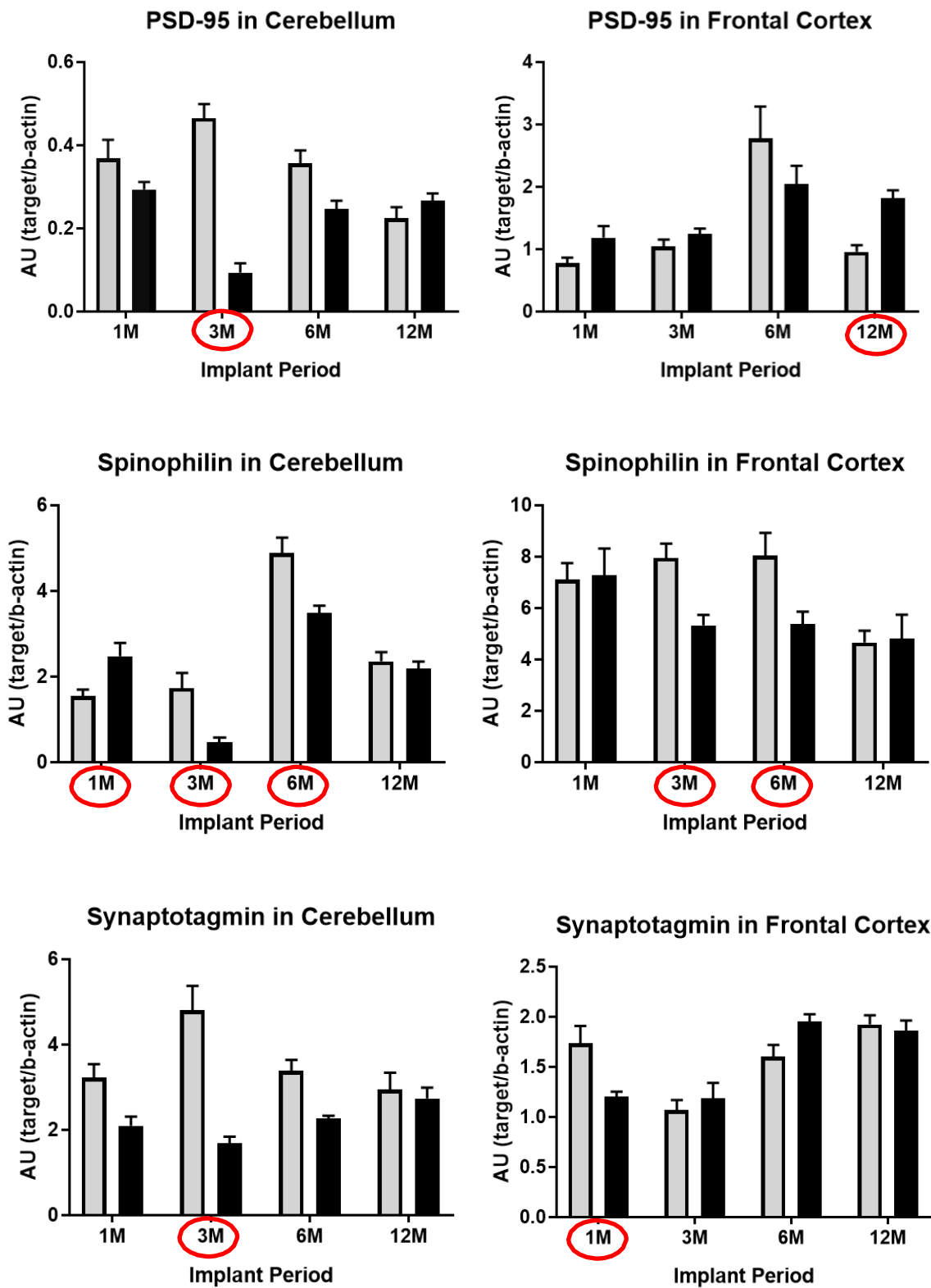


Figure 18: Synaptic markers in aluminum-implanted rats. Gray bars represent tantalum control rats while black bars represent aluminum-implanted rats. Red circle indicates statistically significant difference between tantalum control and aluminum-implanted rats.



Use of Pulmonary Function Testing and Impulse Oscillometry Among Blast-Exposed Veterans in the Veterans Affairs' Toxic Embedded Fragment Registry – An Interim Analysis

D.R. Glick, MD¹, K.H. Chin, MD¹, M. McDiarmid, MD, MPH¹, J. Gaitens, PhD, RN¹, C.H. Brown, PhD¹, M.A. Reback, MD¹, P.S. Sriram, MD², W.E. Lawson, MD³, K.L. Cavanaugh, MD³, L. Beck, MD⁴, J.M. Duch, MD⁵, S.E. Hines, MD, MSPH¹

¹Baltimore VAMC, Baltimore, MD, ²Malcolm Randall VAMC, Gainesville, FL, ³Tennessee Valley Healthcare System VAMC, Nashville, TN, ⁴Oklahoma City VAMC, Oklahoma City, OK, ⁵Audie L. Murphy VA Hospital, San Antonio, TX



Background

- Signature wound of recent military conflicts in Iraq and Afghanistan results from improvised explosive devices (IEDs) and other high kinetic energy weapons
- Toxic Embedded Fragment registry (TEF) – US veterans of these conflicts who have injuries related to explosions or bullets and resultant embedded fragments
- Blast exposure represents an additional mechanism of injury and has the potential to cause damage to both the small airways due to high pressure shearing injury as well as the parenchyma
- Previous studies in similar populations support the complementary use of impulse oscillometry (IOs) in the evaluation of the small airways and identification of abnormalities not otherwise appreciated on traditional pulmonary function testing (PFT)
- We used sought to interrogate the effect of blast injury on the small airways in a subset of patients from the TEF registry through the use of both PFTs and IOs

Methods

- Veterans enrolled in VA's TEF registry completed clinical evaluation at 5 VA sites as part of a research protocol
- Interim analysis of 242 of a planned 421 participants, all of whom completed medical history and exposure questionnaires, traditional PFT and IOs examinations.
- Blast exposure captured by self-reported mechanism of injury, blast (+) vs no blast (-, bullet only), and veterans were stratified accordingly
- The following outcomes for PFTs and IOs were obtained:
 - Spirometry**
 - Forced Expiratory Volume (FEV1)
 - Forced Vital Capacity (FVC)
 - FEV1/FVC ratio
 - Impulse Oscillometry**
 - Resistance at 5 Hz (R5)
 - Resistance at 20 Hz (R20)
 - Area of Reactance (AX)
 - Frequency Dependence of Resistance (R5-20)
 - Resonant Frequency (Fres)
- *all resistance and spirometry values were pre-bronchodilator
- Mean trends in PFT and IOs parameters were observed, and IOs values were compared to accepted norms
- The relationships between blast exposure, performance on PFT and IOs were examined and adjusted for height, age, race, weight, and smoking status through multi-variate regression.

Results

Table 1. Demographics for the interim group

DEMOGRAPHICS	N = 242
Gender, Male, n (%)	234 (96.7%)
Age, years (SD)	42 (7.6)
Height, cm (SD)	177 (7.6)
Weight, kg (SD)	97.6 (20.2)
Race, N (%)	
White/Caucasian	145 (60%)
Black/African American	20 (8.3%)
Hispanic	52 (17.4%)
Other (American Indian, Alaskan Native, Pacific Islander, etc.)	35 (14.5%)
Ever Smoker, N (%)	129 (53.3%)
Years since injury, years (SD)	12.7 (3.2)
Military Service Branch, N (%)	
Army	186 (76.9%)
Navy	14 (5.8%)
Marine Corps	43 (17.8%)
Air Force	7 (2.9%)

Table 2. Means IOs parameters between the blast exposed and no blast groups.

	No Blast N = 16 Mean ± SD	Blast N = 224 Mean ± SD	P value*
IMPULSE OSCILLOMETRY			
Resistance			
R5, kPa/L/sec	0.38 ± 0.11	0.42 ± 0.14	0.35
R5, % predicted	140.9 ± 43.6	155.5 ± 51.1	0.23
R20, kPa/L/sec	0.33 ± 0.10	0.34 ± 0.09	0.70
R20, % predicted	142.6 ± 44.5	145.4 ± 39.6	0.61
R5-20, %	13.8 ± 8.9	16.7 ± 10.4	0.53
Reactance			
X5, kPa/L/sec	-0.11 ± 0.06	-0.11 ± 0.08	0.70
AX, kPa/L/sec	0.49 ± 0.33	0.63 ± 0.71	0.90
Fres, Hz	13.2 ± 4.1	14.6 ± 4.8	0.36

*P value adjusted for age, height, weight, race, and smoking. Obstruction defined as either R5 >150% predicted with normal R20 or R5-20 >20% with normal R5.

Table 3. Mean PFT parameters between blast exposed and no blast groups.

	No Blast N = 16 Mean ± SD	Blast N = 224 Mean ± SD	P value*
PULMONARY FUNCTION			
Spirometry			
FEV1, L	3.69 ± 0.50	3.73 ± 0.82	0.28
FVC, L	4.64 ± 0.60	4.76 ± 0.97	0.11
FEV1/FVC, %	79.6 ± 4.9	78.3 ± 6.1	0.40
FEF25-75, L/s	3.48 ± 0.87	3.51 ± 1.12	0.56
Lung Volumes			
TLC, L	6.36 ± 1.17	6.40 ± 1.17	0.63
FRC, L	2.91 ± 1.12	2.80 ± 0.81	0.44
RV, L	1.63 ± 0.74	1.57 ± 0.63	0.30
RV/TLC, %	24.9 ± 6.4	24.5 ± 8.3	0.29
DLCO, mL/min/mmHg	28.5 ± 5.1	28.1 ± 6.2	0.66

*P value adjusted for age, height, weight, race, and smoking. Obstruction on spirometry defined as FEV1/FVC <70%

Discussion

- Mean PFT and IOs values were similar between groups with detection of differences limited by small cohort without blast exposure
- Mean IOs values among veterans of the Iraq and Afghanistan conflicts were elevated, suggesting possible small airways disease, consistent with previous reports of increased IOs resistance values in similar populations

Future Directions

- Following completion of data collection (target 421 veterans), we will reanalyze the data to observe differences in the trends reported here, in addition to the following:
 - Correlation of IOs abnormalities with:
 - Respiratory symptoms
 - PFT abnormalities compared to population reference norms
 - If IOs abnormalities are present, what pattern of injury do the abnormalities represent (small vs large airways, parenchymal injury)?

Acknowledgments

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- Clinical work supported by the University of Maryland General Clinical Research Center



Respiratory Symptoms in Veterans with Embedded Metal Fragments: An Interim Analysis

Reback M^{1,2}, McDiarmid M^{1,2}, Gaitens J^{1,2}, Brown C², Chin K^{1,2}, Glick D^{1,2}, Sriram P³, Lawson W⁴, Cavanaugh K⁴, Beck L⁵, Duch J⁶, Hines S^{1,2}

¹Baltimore VA Medical Center, Baltimore, MD; ²University of Maryland School of Medicine, Baltimore, MD; ³Malcom Randall VA Medical Center, Gainesville, FL; ⁴Tennessee Valley Healthcare System VAMC, Nashville, TN; ⁵Oklahoma City VAHCS, Oklahoma City, OK; ⁶Audie L. Murphy VA Hospital, San Antonio, TX



Background

- The conflicts in Iraq and Afghanistan have exposed US service members to trauma from explosions, frequently resulting in retained metal fragments of unknown composition.
- Metal mobilization from fragments leads to systemic absorption and metal particles have been found in lung tissue of deployers.
- Systemic exposure to certain metals has been associated with decreased lung function.
- The impact of long-term embedded metal fragments on veterans' pulmonary health is as-yet unknown. We therefore investigated whether veterans with significantly elevated urine metal concentrations showed increased levels of dyspnea and other respiratory symptoms.

Methods

- Drawing from the Department of Veterans Affairs' (VA) Toxic Embedded Fragment (TEF) registry, 242 veterans were evaluated during a 1-day visit to one of five VA medical centers.
- Veterans completed medical history and exposure questionnaires, and spot urine collection.
- Concentrations of 14 urine metals were measured by the Joint Pathology Center using inductively coupled plasma mass spectrometry and dichotomized as elevated or normal based on established reference values (Table 2).
- Self-reported respiratory outcomes were derived from the ATS-DLD-78 questionnaire and included the presence of chronic bronchitis, asthma, cough, and shortness of breath.
- The relationship between elevated metal concentration and respiratory symptoms was assessed via chi squared analysis, while controlling for smoking history.

Results

Table 1. Select characteristics of the study population (N = 242).

Demographics		Military History	
Gender, male, N (%)	234 (96.7)	Service Branch, N (%)	*
Age, years (SD)	42.1 (7.6)	Army	186 (76.9)
Race, N (%)		Navy	14 (5.8)
White	145 (59.9)	Air Force	7 (2.9)
Black/African American	20 (8.3)	Marines	43 (17.8)
Hispanic	42 (17.4)	Active Duty, N (%)	226 (93.4)
Other	35 (14.5)	Location of Injury, N (%)	*
Smoking History, N (%)		Iraq	190 (78.5)
Current	48 (19.8)	Afghanistan	64 (26.5)
Former	81 (33.5)	Time Since Injury, years (SD)	12.7 (3.2)
Never	113 (46.7)	*Percentages may add up to more than 100% due to multiple deployments.	
Worked >1 year in dusty job, N (%)	118 (48.8)		

Table 2. Urine metal concentrations in µg/g creatinine (N = 242). 139 (57.4%) subjects had a significant elevation in at least one urine metal.

Metal	Upper limit of normal	Subjects above upper limit, N (%)	Mean (SD)	Min-Max
Aluminum	30	36 (14.9)	18.9 (12.5)	4.4-94.2
Arsenic	60	3 (1.2)	7.1 (12.1)	1.2-131.9
Cadmium	1	2 (0.8)	0.2 (0.2)	0.1-1.9
Chromium	2	7 (2.9)	0.6 (0.8)	0.03-6.6
Cobalt	1	6 (2.5)	0.4 (0.4)	0.1-5.0
Copper	40	2 (0.8)	5.7 (15.4)	0.9-189.3
Iron	250	0	18.4 (20.0)	4.4-226.7
Lead	2	5 (2.1)	0.4 (0.7)	0.01-7.0
Manganese	2	4 (1.7)	0.5 (0.4)	0.1-3.9
Molybdenum	122	2 (0.8)	31.3 (22.8)	4.7-207.9
Nickel	8	0	1.7 (0.8)	0.4-4.7
Tungsten	0.4	11 (4.5)	0.2 (0.5)	0.01-7.1
Uranium	0.04	3 (1.2)	0.01 (0.02)	0.001-0.3
Zinc	1100	116 (47.9)	1267.4 (889.0)	353.9-10229.5

Highlight = known pulmonary toxicants

Table 3. Prevalence of self-reported respiratory symptoms (any level of severity vs asymptomatic) in subjects with vs without elevated urine metals. There was no significant difference between the prevalence of any measured respiratory outcomes with regard to metal levels, even after adjusting for smoking history.

Respiratory problem, N (%)	Elevated urine metal		Odds Ratio (95% CI)
	Yes (N=63)	No (N=179)	
Short of breath...			
hurrying on flat ground	38 (60.3)	135 (75.4)	0.9 (0.5-1.5)
walk slower than peers	22 (34.9)	80 (44.7)	0.9 (0.5-1.5)
walking on flat ground	18 (28.6)	70 (39.1)	1.1 (0.6-1.9)
Cough	29 (46.0)	99 (55.3)	1.2 (0.7-2.0)
Phlegm	36 (57.1)	93 (52.0)	1.2 (0.7-2.1)
Dyspnea from wheezing	16 (25.4)	53 (29.6)	1.4 (0.8-2.6)
Chronic bronchitis	4 (6.3)	20 (11.1)	1.2 (0.5-2.8)
Asthma	7 (11.1)	29 (16.2)	1.2 (0.6-2.5)

Discussion

- Despite reporting indwelling fragments, only about half of veterans had elevated urine metal concentrations. Reasons may include a low body metal burden or metal fragment stability over the time period assessed.
- A high percentage of the study population reported respiratory symptoms, asthma, or chronic bronchitis above levels previously reported in other veteran cohorts (Rivera, et al. 2018; Morris, et al. 2020). Nearly one-third of subjects reported any shortness of breath even with walking on flat ground.
- While there was no significant association between elevated urine metal levels and respiratory symptoms, other injury features of this population including blast trauma or inhaled non-circulating metals may be contributing to the high prevalence of respiratory symptoms and will be explored in further analyses.

References

- Morris MJ, et al. Chest 2020; S0012-3692 Epub.
- Rivera, et al. Am J Epidemiol 2018;187:2136-44.

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Disclaimer: The views expressed in this poster are those of the authors and do not reflect the official policy of the Department of Veterans Affairs, Department of the Army, Department of Defense, or U.S. Government.



Biomarkers of Acute Kidney Injury in Metal Exposed War-Injured Veterans: Preliminary Results

Reback M^{1,2}, Hines S^{1,2}, McDiarmid M^{1,2}, Brown C², Gaitens J^{1,2}

¹Department of Veterans Affairs Medical Center, Baltimore, MD; ²University of Maryland School of Medicine, Baltimore, MD



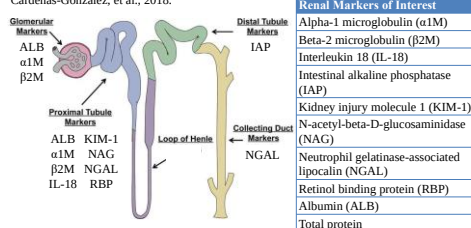
Background

- The conflicts in Iraq and Afghanistan have exposed US service members to trauma from blasts and explosions, often resulting in retained metal fragments of unknown composition.
- Retained metal fragments were previously believed to be inert and left in place however new evidence shows mobilization of metal ions and systemic absorption.
- Acute and long-term exposure to certain metals is known to be nephrotoxic, and co-exposure to multiple metals has been shown to have a greater impact than single metals alone.
- Urine protein markers (Figure 1) may herald kidney injury long before any symptoms of kidney disease or elevation in serum creatinine.
- The impact of long-term embedded metal fragments on veterans' kidney function is as-yet unknown. We therefore investigated whether veterans with significantly elevated urine metal concentrations showed derangements in urine markers of early kidney injury.

Methods

- Drawing from the Department of Veterans Affairs' (VA) Toxic Embedded Fragment (TEF) registry, 120 veterans were evaluated during a 1-day visit to one of five VA medical centers.
- Veterans completed medical history and exposure questionnaires, and spot urine collection.
- Concentrations of 14 urine metals were measured by the Joint Pathology Center using inductively coupled plasma mass spectrometry and dichotomized as elevated or normal based on established reference values (Table 2).
 - After observing an unusually high number of subjects with Zn elevations (Table 2), Zn was excluded from further analyses due to concerns about potential contamination.
- Urine concentrations of 10 protein markers of kidney injury (Figure 1, Table 3) were measured by the University of Antwerp as previously described (McDiarmid, et al., 2013) and compared to clinical laboratory reference values.
- The relationship between elevated metal concentration and urine protein levels was assessed via linear regression, while controlling for known CKD risk factors of age, NSAID use, diabetes, and hypertension, as well as a prolonged time since injury.

Figure 1. Nephron location involved with examined urine proteins. Modified from Cardenas-Gonzalez, et al., 2018.



Results

Table 1. Select characteristics of the study population (N = 120).

Demographics	N (%)	Military History	N (%)
Gender, male	116 (96.7)	Service Branch*	
Race		Army	92 (76.7)
White	81 (67.5)	Navy	6 (5.0)
Black/African American	7 (5.8)	Air Force	3 (2.5)
Hispanic	20 (16.7)	Marines	20 (16.7)
Other	12 (10.0)	Active Duty	109 (90.8)
Routine NSAID use	77 (64.2)	Location of Injury*	
History of hypertension	31 (25.8)	Iraq	96 (80.0)
History of diabetes	9 (7.5)	Afghanistan	28 (23.3)
	Mean (SD)		Mean (SD)
Age, years	41.2 (7.0)	Time Since Injury, years	12.4 (2.6)

*Percentages may add up to more than 100% due to multiple deployments.

Table 2. Urine metal concentrations in µg/g creatinine (N = 120). 56.7% of subjects had a significant elevation in at least one urine metal, 19.2% if excluding zinc.

Metal	Upper limit of normal	Subjects above upper limit, N (%)	Mean (SD)	Min-Max
Aluminum	30	8 (6.7)	15.4 (12.53)	5.0-94.2
Arsenic	60	3 (2.5)	8.3 (12.08)	1.2-131.9
Cadmium	1	0	0.2 (0.17)	0.1-0.7
Chromium	2	3 (2.5)	0.6 (0.75)	0.1-6.6
Cobalt	1	1 (0.8)	0.4 (0.35)	0.1-5.0
Copper	40	1 (0.8)	5.8 (15.36)	0.9-189.3
Iron	250	0	18.4 (19.96)	4.4-226.7
Lead	2	2 (1.7)	0.4 (0.73)	0.01-3.8
Manganese	2	2 (1.7)	0.4 (0.43)	0.1-3.9
Molybdenum	122	1 (0.8)	32.3 (22.81)	5.5-135.4
Nickel	8	0	1.6 (0.81)	0.4-4.4
Tungsten	0.4	4 (3.3)	0.2 (0.47)	0.02-7.1
Uranium	0.04	2 (1.7)	0.01 (0.02)	0.001-0.06
Zinc	1100	60 (50)	1323.8 (889.01)	393.1-10229.5

Table 3. Urine protein concentrations (N = 119). Most protein levels fell within normal limits with the exception of IL-18.

Marker (unit/g creatinine)	Upper limit of normal	Subjects above upper limit, N (%)	Mean (SD)	Min	Max
α1M (mg)	12	6 (5.0)	4.2 (3.9)	0.6	24.0
β2M (mg)	0.2	5 (4.2)	0.1 (0.2)	0.003	2.1
IL-18 (ng)	20	66 (55.5)	29.6 (26.8)	0.2	128.0
IAP (U)	2	8 (6.7)	0.6 (0.7)	0.01	3.8
KIM-1 (ng)	560	10 (8.4)	279.5 (179.6)	31.5	954.0
NAG (U)	5	3 (2.5)	1.7 (1.1)	0.1	7.4
NGAL (ng)	1200	30 (25.2)	1199.3 (1708.0)	14.8	10429.9
RBP (µg)	300	2 (1.7)	46.7 (60.2)	11.9	500.4
Albumin* (mg)	25	21 (17.8)	18.7 (24.2)	3.4	174.2
Total protein* (g)	0.12	3 (2.5)	0.03 (0.03)	0.01	0.3

*N = 118

Results (continued)

Table 4. Association (β coefficient) of any elevated metal on kidney markers, adjusted for CKD risk factors* (N=119). Fit was improved via log transformation of all protein concentrations, with the exception of IL-18 which was square root transformed.

Marker	Estimate (SE)	P-Value
α1M	0.60 (0.18)	0.001
β2M	0.23 (0.22)	0.30
IL-18	0.38 (0.56)	0.51
IAP	-0.29 (0.27)	0.29
KIM-1	0.06 (0.18)	0.74
NAG	0.28 (0.15)	0.07
NGAL	0.40 (0.24)	0.09
RBP	0.27 (0.14)	0.0482
Albumin**	0.73 (0.19)	0.0002
Total protein**	0.46 (0.13)	0.0004

*Age, NSAID use, hypertension, diabetes, time since injury of at least 10 years

**N = 118

Highlight = $p < 0.05$

Discussion

- While most subjects had normal renal protein levels, the on-going mobilization of metals from retained fragments and their excretion from the systemic circulation chronically presents metals to the kidney.
- Despite reporting retained fragments, a minority of veterans had elevated urine metal concentrations. Reasons may include a difference in surface area and metal fragment stability over the time period assessed.
- The significant association between the presence of an elevated metal level and four of ten renal markers (α1M, RBP, albumin, and total protein), even after adjusting for known CKD risk factors, suggests that long-term monitoring to assess renal injury may be warranted in veterans who have retained fragments.

References

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