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TITLE: Identification and Validation of Established and Novel Biomarkers for Infections in Burns

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RECIPIENT: The University of Texas Medical Branch at Galveston, Galveston, TX

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Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Hypothesis: Plasma proteins, clinical data, and patient characteristics can be used to prospectively identify severely burned patients who are at risk for developing sepsis and other infections. Measurement of already identified biomarkers alongside novel biomarkers identified with discovery proteomics can improve identification of risk for infection and identify the early stages of infection prior to clinical detection. This multicenter study will enable us to identify novel biomarkers, validate whether the already identified biomarkers are appropriate, and establish a predictive model. Rationale: Our prior work has shown that severely burned patients who die from sepsis can be identified via their serum protein expression profile at the time of admission, that in the days prior to septic death there is an increase in serum biomarker expression, and that the use of both clinical and proteomic information as biomarkers improves the accuracy of patient survival prediction. Others have shown that procalcitonin is a good candidate marker of sepsis in burn patients. Clinical indices such as heart rate, mean arterial pressure, base deficit, temperature, and glucose levels more accurately identify sepsis in the burn patients than does the ABA consensus definition. Methods: 200 patients will be enrolled at four sites within the Burns Research in Texas Consortia. Blood samples will be taken daily, and clinical data recorded. Specific Aims: 1. Determine plasma proteomic biomarkers for the prediction and diagnosis of sepsis using mass spectrometry techniques; use stable isotope techniques to detect proteins for which assays do not exist. 2. Validate already identified markers of infection in a multicenter study 3. Develop a model of prediction of infection using clinical data and proteomic information. Relevance: 5% of combat-sustained casualties are burn injuries; ~20% of burn patients develop sepsis. This is a life-threatening disease which needs to be treated as early as possible. The studies described here will improve clinical care for the severely burned Wounded Warriors and other burn victims.

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**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	9	**19b. TELEPHONE NUMBER** *(include area code)*

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1. INTRODUCTION

This was designed as a two-pronged study to identify and validate potential biomarkers for infections in severely burned patients, with the intent to confirm already identified biomarkers of infection in a multi-center study and at the same time, to identify novel proteins associated with infections in severely burned patients.

Severe burns trigger marked hypermetabolic and inflammatory responses, inducing dramatic protein degradation that compromises the function of multiple organ systems. Impaired organ function frequently results in multiorgan failure, the leading cause of mortality in burn patients. Advances in clinical care, including early burn wound excision, improvements in resuscitation, and refinement of antibiotic and antimycotic treatment protocols, have reduced mortality following severe burns. The next major advance in burn care will be the ability to predict clinical outcomes, such as infections, so that clinical therapies can be individualized to maximize patients' chance of survival.

Different proteins have been proposed to use as biomarkers of infection, but these perform with varying degrees of efficacy/accuracy for predicting patient outcome. Those biomarker panels remain largely unvalidated. Our intent for this project was to validate the already proposed biomarkers and discover new targets using techniques with which we have experience such as discovery proteomics.

2. KEYWORDS

Burns, biomarkers, sepsis, infection

3. ACCOMPLISHMENTS

a. What were the major goals of the project? (Goals to be accomplished and status.)

Primary Objective: To evaluate prospectively identify and validate biomarkers of infection in patients with massive burns

Secondary Objective: To develop a model of prediction of infection using clinical data and proteomic information

Major Task 1: Develop study protocol, including operations manual, set of standard outcome definitions, and case report forms.

- STATUS: completed

Major Task 2: Obtain IRB approval for all five participating sites.

- STATUS: completed

Major Task 3: Conduct trial to enroll 200 subjects

- STATUS: not complete.

Major Task 4: Sample analysis.

- STATUS: partially complete Y7Q3.

Major Task 5: Data analysis.

- STATUS: yet to start.

Major Task 6: Maintain accurate and responsible budget.

- STATUS: completed

Major Task 7: Publish research data.

- STATUS: yet to start

b. What was accomplished under these goals? (Detailed progress and results.)

Primary Objective: To evaluate prospectively identify and validate biomarkers of infection in patients with massive burns.

Secondary Objective: *To develop a model of prediction of infection using clinical data and proteomic information*

Major Task 1: *Develop study protocol, including operations manual, set of standard outcome definitions, and case report forms.*

- STATUS: completed

Major Task 2: *Obtain IRB approval for all five participating sites.*

- STATUS: completed

Major Task 3: *Conduct trial to enroll 200 subjects*

- STATUS: not complete. Enrollment at the original sites was slower than we predicted based on prior burn unit census numbers. We recruited the Shriners Hospitals for Children Galveston as an enrollment site due to their potential enrollment capability. Study enrollment was halted soon thereafter, however, so target enrollment of 200 was not reached. 41 patients were enrolled.

Major Task 4: *Sample analysis.*

- STATUS: approximately 40% completed Y7Q3. Samples for mass spectrometry were selected (but without being able to include consideration as to whether patient developed infections as originally planned due to unanticipated problems and delays detailed in section 5) and run. We have a list of proteins that were expressed in each patient sample that was run. Once clinical data has been fully obtained, we will be able to determine which proteins were associated with presence or absence of infection. At that point, we will be able to validate the findings in the full set of patient samples as well as to measure previously proposed biomarkers. The additional sample analysis to be performed once the proteins that differ between infected and non-infected patients once patient status is known is needed to validate the results of the measurements already performed.

Major Task 5: *Data analysis.*

- STATUS: yet to start. The proteomic data cannot be analyzed until clinical data has been obtained (see above). Extraction of the clinical data is in progress. Once clinical data is obtained, the first round of proteomic data to identify potential biomarkers will be performed. Validation of those targets will then be necessary.

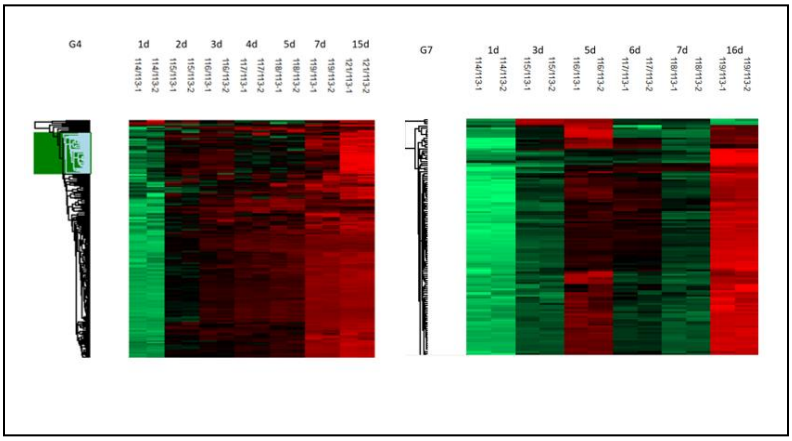
Major Task 6: *Maintain accurate and responsible budget.*

- STATUS: completed

Major Task 7: *Publish research data.*

- STATUS: yet to start. To be considered once the sample and data analyses are complete.

Samples from patients taken over an approximate 2-week period were analyzed via mass spectrometry to identify all proteins present in the samples, in comparison to a reference sample. The two heat maps show preliminary results for protein detection from 2 subjects at various times. There was strong representation of the following functional groups in the individual patient samples: Complement and coagulation cascade, response to bacterial infections, systemic inflammation, platelet activation, and cell surface binding. Whether these proteins are present as a result of the burn or due to infections remain to be seen.



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

Not applicable

d. How were the results disseminated to communities of interest?

Nothing to report

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

This is the final technical report; work is not completed but will continue with other funding.

4. IMPACT

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

Once the analyses are completed, the results should include proteins that may serve as biomarkers for infection. The results will need to be validated in a larger cohort of burn patients, or in other patient populations as these results may pertain to them as well. The ability to predict who may develop infections would allow earlier monitoring or preventative measures to be taken before the infection actually develops.

b. What was the impact on other disciplines?

Nothing to report; once the analyses are complete, the findings may apply to other clinical situations where patients have infections or sepsis.

c. What was the impact on technology transfer?

Nothing to report

d. What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS

a. Changes in approach and reasons for change

Nothing to report

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

Low enrollment was addressed by adding an additional site, Shriners Hospitals for Children Galveston, that had high enrollment. Unanticipated temporary suspension of research protocols at UT Southwestern further impacted enrollment.

Enrollment was terminated early due to temporary suspension of the burn research program at UTMB.

Files, both electronic and paper, have been lost due to unexpected staff turnover / loss coupled with inability to regain access to the files from Shriners. Negotiations are ongoing between the two institutions. In the meantime, several clinicians have volunteered to extract the information from the medical charts and this effort is underway. Until this information is complete in our database, we are unable to move ahead with sample analysis, which relies on being able to classify patients according to their clinical trajectories.

Missing samples due to temporary suspension of the burn research program at the University of Texas Medical Branch, subsequent loss of research staff, and inability of PIs to access freezers. Additionally, due to PI turnover at UT Southwestern, samples were not relocated to UTMB. Samples collected from patients admitted to the Shrine were inadvertently left out of the freezer. Samples from UT Health Sciences Center Houston were

presumed destroyed when freezer outage due to construction was discovered after all samples in the freezer were thawed. These samples will be tested to determine whether any viable proteins can be detected.

Impeded ability to measure the analytes that we initially proposed. The equipment that was previously in the PIs lab was retained by the Shrine when the lab relocated to UTMB, and we no longer have access to it. Additionally, some equipment that was moved was not brought back online following the move; we are working with other facilities within the UTMB campus to measure analytes once the initial analyses are completed.

c. Changes that had a significant impact on expenditures

As the clinical data is not complete, we were unable to order all of the kits and reagents needed to validate the initial proteomic analysis as we cannot predict which proteins will be significantly different between patients with infections versus those without. As a result, we are returning a large sum of money that would have paid for these studies.

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

Following the early study closure, a new protocol was developed and submitted to the UTMB Institutional Review Board to allow extraction of clinical data and protein analysis. This approval was obtained in late 2020, allowing resumption of study closeout activities.

6. PRODUCTS

a. Journal publications

Nothing to Report

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

Nothing to Report

Other publications, conference papers, and presentations

Nothing to Report

c. Website(s) or other Internet site(s)

Nothing to Report

d. Technologies or techniques

Nothing to Report

e. Inventions, patent applications, and/or licenses

Nothing to Report

f. Other Products

This project resulted in the creation of a database consisting of study data, and a small biospecimen collection, both of which are being analyzed.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project?

Name: Suman, Oscar
Project Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0003-2184-0204
Nearest person month worked: 0.24
Contribution to Project: Submits reports .

Name: Celeste Finnerty
Project Role: PI
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-1524-5110
Nearest person month worked: 1.2
Contribution to Project: regulatory, lab work, ordering, communicate with clinical partners regarding clinical data.

Name: Kudlicki, Andrzej
Project Role: Senior Staff
Researcher Identifier (e.g. ORCID ID): Andrzej Kudlicki https://orcid.org/0000-0001-8158-9600
Nearest person month worked: 0.60
Contribution to Project: Develop and implement algorithms and automated pipelines for data extraction, processing and formatting. Statistical analysis and filtering of data.

Name: Yingxin Zhao
Role: Co-investigator
Researcher Identifier (e.g. ORCID ID): 0000-0002-8872-5860
Nearest person month worked: 1.2
Contribution to Project: Dr. Zhao is a member of the biomarker discovery team. Has ordered reagents, inventoried samples, performed mass spec analysis and prepared for additional analysis.

Name: Heidi Spratt
Project Role: Biostatistician
Researcher Identifier (e.g. ORCID ID): https://orcid.org/0000-0002-9420-5028
Nearest person month worked: 1.2
Contribution to Project: I will provide statistical assistance guidance

Name: Ye Wang
Project Role: Research associate
Researcher Identifier (e.g. ORCID ID): I don't have one
Nearest person month worked: 6.0
Contribution to Project: I will analyze samples

Name: Steve Wolf
Project Role: Collaborator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked:
Contribution to Project: extracting clinical data

Name: Jong Lee
Project Role: Collaborator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked:
Contribution to Project: extracting clinical data

b. 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

c. What other organizations were involved as partners?

Shriners Hospitals for Children – Galveston
Galveston, TX
Contribution to the Project: 1) in-kind support (provided access to software, computers, and equipment to project staff); 2) facilities (project staff offices and labs were housed in their facility); 3) collaboration (Shriners was a recruitment site for this study)

University of Texas Health Science Center Houston
Houston, TX
Contribution to the Project: 11) in-kind support (provided access to software, computers, and equipment to project staff); 2) facilities (project staff offices and labs were housed in their facility); 3) collaboration (was a recruitment site for this study)

University of Texas, Southwestern (Parkland)
Dallas, Texas
Contribution to the Project: 11) in-kind support (provided access to software, computers, and equipment to project staff); 2) facilities (project staff offices and labs were housed in their facility); 3) collaboration (was a recruitment site for this study)

Army Institute for Surgical Research
San Antonio, Texas
Contribution to the Project: 11) in-kind support (provided access to software, computers, and equipment to project staff); 2) facilities (project staff offices and labs were housed in their facility); 3) collaboration (was a recruitment site for this study)
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8. SPECIAL REPORTING REQUIREMENTS

QUAD CHART
Convert this report to a PDF file and append updated quarterly Quad Chart in PDF as an appendix.

9. APPENDICES

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.