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TITLE: Identifying Key Autoantibody and Inflammatory Factors in the Initiation, Propagation, and Transition to Clinically Apparent Rheumatoid Arthritis

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Identifying Key Autoantibody and Inflammatory Factors in the Initiation, Propagation, and Transition to Clinically Apparent Rheumatoid Arthritis

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8. PERFORMING ORGANIZATION REPORT NUMBER

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14. ABSTRACT

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease with the hallmark clinical finding of inflammatory arthritis (IA). RA affects ~1% of the population leading to substantial morbidity, increased mortality and high financial costs. The current paradigm for management of RA is to identify a patient with disease and treated once clinical signs of disease (e.g. joint pain and swelling) have been identified. However, there is now known to be a 'Pre-RA' period of RA during which circulating biomarkers including autoantibodies are present on average 3-5 years prior to the first appearance of clinically-apparent IA. Importantly, elevations of serum autoantibodies (e.g. antibodies to citrullinated protein antibodies [ACPA] and rheumatoid factor [RF]) can be used to accurately predict future RA in individuals without *current IA.* Indeed, the predictive ability of these autoantibodies has underpinned the development of several clinical prevention trials for RA. However, there are still substantial limits in prediction models for future RA; furthermore, specific biologic pathways that could be targeted in Pre-RA for prevention need additional exploration. As such, the primary objective for this project is to build on our initial findings from a prior CDMRP project and utilize a unique sample set of individuals from pre- and post-RA diagnosis obtained from the Department of Defense Serum Repository (DoDSR) to expand our knowledge about the development of RA and in particular improve prediction of future RA as well as identify potential pathways/targets for prevention by utilizing state-of-the-art biomarker testing.

15. SUBJECT TERMS

Pre-rheumatoid arthritis, prediction, pathogenesis, prevention

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Project Title: Key Autoantibody and Inflammatory Factors in the Initiation, Propagation, and Transition to Clinically Apparent Rheumatoid Arthritis
Contract/Grant #: W81XWH-20-1-0204/PR191079
PI: Kevin D. Deane, MD/PhD
Period of Report: 15-Apr-2021 to 14-APR-2022

1. INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease with the hallmark clinical finding of inflammatory arthritis (IA). RA affects ~1% of the population leading to substantial morbidity, increased mortality and high financial costs. The current paradigm for management of RA is to identify a patient with disease and treated once clinical signs of disease (e.g. joint pain and swelling) have been identified. However, there is now known to be a 'Pre-RA' period of RA during which circulating biomarkers including autoantibodies are present on average 3-5 years prior to the first appearance of clinically-apparent IA. Importantly, elevations of serum autoantibodies (e.g. antibodies to citrullinated protein antibodies [ACPA] and rheumatoid factor [RF]) can be used to accurately predict future RA in individuals without *current IA.* Indeed, the predictive ability of these autoantibodies has underpinned the development of several clinical prevention trials for RA. However, there are still substantial limits in prediction models for future RA; furthermore, specific biologic pathways that could be targeted in Pre-RA for prevention need additional exploration. As such, the primary objective for this Expansion Project is to build on our initial findings from a prior CDMRP project and utilize a unique sample set of individuals from pre- and post-RA diagnosis obtained from the Department of Defense Serum Repository (DoDSR) to expand our knowledge about the development of RA and in particular improve prediction of future RA as well as identify potential pathways/targets for prevention by utilizing state-of-the-art biomarker testing.

2. KEYWORDS:

Antibodies to carbamylated proteins (anti-CarP)
Antibodies to citrullinated protein antigens (ACPA)
Antibodies to peptidyl arginine deiminase (anti-PAD)
Pre-rheumatoid arthritis (Pre-RA)
Prediction
Prevention
Rheumatoid arthritis (RA)
Rheumatoid factor (RF)

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- Goal/Objective 1 – Obtain final Human Research Protection Office (HRPO) approval
- Goal/Objective 2 (Aim 1): Complete autoantibody testing on sample set (Table 1):

Table 1. Aim 1 Autoantibody testing		
Biomarker Testing	**Collaborator**	**Status**
ACPA	Stanford, Inova	Inova – complete 2021 Stanford – pending completion 2022
Anti-PAD	Inova Research Laboratories	Pending 2022
Anti-CarP	Inova Research Laboratories	Pending 2022
Autoantibody glycosylation	Inova Research Laboratories	Pending 2022
Anti-nuclear antibody and anti-thyroid immunity	Oklahoma Medical Research Foundation	Complete 2021
Anti-malondialdehyde-acetaldehyde	University of Nebraska Medical Center	Complete 2020 (paper published)

- Goal/Objective 3 (Aim 2): Complete non-autoantibody biomarker testing (Table 2).

Table 2. Aim 2 Non-autoantibody testing		
Biomarker Testing	**Collaborator**	**Status**
Proteomic measures	Olink Proteomics, Inc.	Complete 2021
C-reactive protein	University of Colorado	Pending 2022
Serum Calprotectin	Inova Research Laboratories	Complete 2021 (paper published)

- Goal/Objective 4 (Aim 3): Complete analyses and manuscript/abstract submission Nov 2022-April 2023.

What was accomplished under these goals?

Major activities

The contract for the project was fully executed on 15-April-2020 which was several weeks after the University of Colorado as well as multiple collaboratives sites were shut-down for research a shut-down around COVID-19. The shut-down including limits on personnel access to the laboratory and alterations of research laboratory testing which required high levels of sample processing safety (e.g. Biosafety Level 2+, including need for human tissue processing in a hood) at the University stuttered but was basically persistent until Spring of 2021. As such, while we competed HRPO approval 29-May-2020, we were delayed in sample disbursement and testing. Another issue that has arisen is limits in testing kits due to supply chain issues. Specifically, some materials for autoantibody testing (Table 1) and C-reactive protein (Table 2) have not been available. However, as of April 2022, supplies are present and we estimate to complete all planned testing in calendar year 2022. Therefore, overall, we will be on-track for completing analyses and publications as planned.

Specific objectives (also see Tables 1 and 2 above)

- *Goal/Objective 1 – Obtain final Human Research Protection Office (HRPO) approval June 2020.* **Completed May 2020**
- *Goal/Objective 2 (Aim 1): Complete autoantibody testing on sample set as outlined in Table 1.* **Some testing delayed; estimated completion Nov 2022**
- Goal/Objective 3 (Aim 2): Complete non-autoantibody biomarker testing as outlined in Table 2. ***Some testing*** **delayed; estimated completion Nov 2022**
- Goal/Objective 4 (Aim 3): Complete analyses and manuscript/abstract submission Nov 2022-April 2023. **Estimated completion on-track for Nov 2022-Apr 2023**

Significant results

We have published a paper with proposed testing of anti-MAA antibodies. In this paper, we have demonstrated that anti-MAA antibodies are present prior to the onset of RA. We have also published a paper demonstrating that serum calprotectin is elevated in some individuals prior to an onset of RA, and that 'triple positivity' for ACPA, RF and serum calprotectin is indicative of near-term onset of future RA.

References/publications:

Mikuls TR, Edison J, Meeshaw E, Sayles H, England BR, Duryee MJ, Hunter CD, Kelmenson LB, Moss LK, Feser ML, Wagner B, Parish MC, **Deane KD**, Thiele GM. Autoantibodies to Malondialdehyde-Acetaldehyde Are Detected Prior to Rheumatoid Arthritis Diagnosis and After Other Disease Specific Autoantibodies. Arthritis Rheumatol. 2020. Epub 2020/07/06. doi: 10.1002/art.41424. PubMed PMID: 32621635.

Bettner LF, Peterson RA, Bergstedt DT, Kelmenson LB, Demoruelle MK, Mikuls TR, Edison JD, Parish MC, Feser ML, Frazer-Abel AA, Moss LK, Mahler M, Holers VM, **Deane KD.** Combinations of Anticyclic Citrullinated Protein Antibody, Rheumatoid Factor, and Serum Calprotectin Positivity Are Associated With the Diagnosis of Rheumatoid Arthritis Within 3 Years ACR Open

Other achievements

Not applicable.

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

- We have enlisted several trainees in analyses and publications including Leah F. Bettner (medical resident, now rheumatology fellow) and Dylan T. Bergstedt (medical student now medical resident).

How were the results disseminated to communities of interest?

- Abstract presentations at American College of Rheumatology annual meetings and published papers

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

- We will complete biomarker testing and analyses as listed above.

4. IMPACT:

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

- The findings of anti-MAA and serum calprotectin are being investigated further.

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:

Changes in approach and reasons for change

As described above in 'Major Activities', there have been some delays in laboratory testing due to COVID19-related shut-downs and supply chain issues. We have now seen resolution of these issues and plan to complete all testing by Nov 2022, and meet final deliverables by April 2023.

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

- Please see above.

Changes that had a significant impact on expenditures

- Please see above.

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

- There have been no changes in use of human subjects, etc.

Significant changes in use or care of human subjects

- No changes at this time.

Significant changes in use or care of vertebrate animals.

- Not applicable.

Significant changes in use of biohazards and/or select agents

- Not applicable.

6. PRODUCTS:

Publications, conference papers, and presentations

- **Journal publications.** See above for published papers.
- **Books or other non-periodical, one-time publications.** Nothing to report.
- **Other publications, conference papers, and presentations.** Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

The following individuals newly participated in the project as trainees:

Name:	*Leah Bettner*
Project Role:	Medical resident, analysis
Researcher Identifier (e.g. ORCID ID):	-
Nearest person month worked:	1
Contribution to Project:	*Analysis and paper*
Funding Support:	N/A

Name:	*Dylan Bergstedt*
Project Role:	*Medical student, analysis*
Researcher Identifier (e.g. ORCID ID):	-
Nearest person month worked:	*1*
Contribution to Project:	Analysis and paper
Funding Support:	N/A

The following individuals have not had any change to their roles on the project

Name:	*Kevin Deane*

Name:	*Marie Feser*

Name:	*Ted Mikuls*

Name:	*Geoff Thiele*

Name:	*William Robinson*

Name:	*Laurie Moss*

Name:	*Brandie Wagner*

Name:	*Colin O'Donnell*

Name:	*Masoud Asadi-Zeydabadi*

Name:	*Michael Holers*

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

- *There have been no changes in Other Support that have impacted this project for any personnel.*

What other organizations were involved as partners?

Organization name:	*Inova Research Laboratories*
Location of organization	*San Diego, California, USA*
Partner's contribution to the project	*Biomarker testing*

Organization name:	*Oklahoma Medical Research Foundation*
Location of organization	*Oklahoma City, Oklahoma, USA*
Partner's contribution to the project	*Biomarker testing*

Organization name:	*Olink Proteomics, Inc.*
Location of organization	*Boston, Massachusetts, USA*
Partner's contribution to the project	*Biomarker testing*

Organization name:	*Allen Institute for Immunology*
Location of organization	*Seattle, Washington, USA*
Partner's contribution to the project	*Analyses of biomarker data. Allen Institute for Immunology has a unique platform for analyses and we will share some data with them for analyses; no samples will be shared and no funding is required. The project will start June 2022 and as of this report, no data has been shared.*

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** Not applicable.
- **QUAD CHARTS:** Not applicable.

9. APPENDICES

Not appliable.