AWARD NUMBER: W81XWH-20-1-0204

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

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CONTRACTING ORGANIZATION: University of Colorado Denver Anschutz Medical Campus

REPORT DATE: MAY 2023

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
MAY 2023	Annual Technical Report	15APR2022-14APR2023

4. TITLE AND SUBTITLE Key Autoantibody and Inflammatory Factors in the Initiation, Propagation, and Transition to Clinically Apparent Rheumatoid Arthritis

5a. CONTRACT NUMBER W81XWH-20-1-0204

5b. GRANT NUMBER PR191079

5c. PROGRAM ELEMENT NUMBER

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

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

16. SECURITY CLASSIFICATION OF: Unclassified			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE	UU	13	USAMRDC
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Technical Report

(revised due to feedback from Science Officer Jessica Smith, resubmitted 25-Aug-2023)

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-2022 to 14-Apr-2023
Period of Overall Project: 15-Apr-2020 to 14-Apr-2024

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 – completed 2023
Anti-PAD	Inova Research Laboratories	Completed 2023
Anti-CarP	Inova Research Laboratories	Completed 2023
Autoantibody glycosylation	Inova Research Laboratories	Completed 2023
Anti-nuclear antibody and anti-thyroid immunity	Oklahoma Medical Research Foundation	Completed 2021
Anti-malondialdehyde-acetaldehyde	University of Nebraska Medical Center	Completed 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	Completed 2023
Serum Calprotectin	Inova Research Laboratories	Complete 2021 (paper published)

- Goal/Objective 4 (Aim 3): Complete analyses and manuscript/abstract submission Nov 2023-April 2024.

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. As presented in the Year 2 report, we thought we had resolved these issues in Year 2; however, after that report, there were additional delays in procuring supplies and that delayed completion

of testing – and in particular there were delays in testing by Inova Research Laboratories (see Tables 1 and 2). Fortunately, all testing is complete as of June 2023. Therefore, while the project has had to ask for a no-cost extension (that was approved until April 2024), we are on-track for completing analyses and publications by April 2024.

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 but is now complete.**
- Goal/Objective 3 (Aim 2): Complete non-autoantibody biomarker testing as outlined in Table 2. **Some testing delayed but is now complete.**
- Goal/Objective 4 (Aim 3): Complete analyses and manuscript/abstract submission Nov 2022-April 2023. **Estimated completion for by Apr 2024 (and no-cost extension for the project has been approved).**

Significant results

For this period, we have published a paper that used data from this project to validate a prediction model for future RA that incorporated multiple biomarkers. Specifically, as demonstrated in Figure 3 below (which was taken from the publication and therefore the Figure 3 designation refers to the paper's figure numbering, not number of figures within this report), we used the DoDSR sample set and biomarker testing obtained in this project to demonstrate that combinations of biomarkers (anti-cyclic citrullinated peptide-3 [anti-CCP3] and multiple rheumatoid factor [RF] isotypes) are more likely to be present closer to diagnosis of RA in both men and women. These results helped to validate findings in other cohorts. The implications of this are that in prospective studies, combinations of these antibodies are likely indicative of imminent onset of RA.

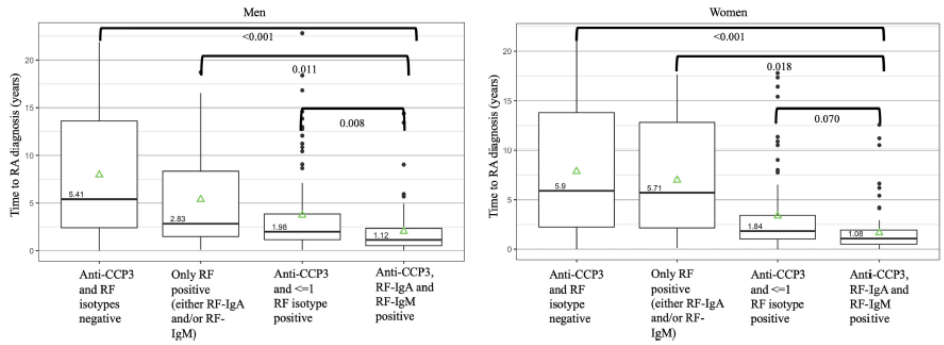

FIGURE 3 | Autoantibody positive states and median time to a future diagnosis of rheumatoid arthritis in the Department of Defense Serum Repository cohort. The times to diagnosis are stratified by men (n=113) and women (N=103) as women had a higher overall prevalence of rheumatoid factor (RF) positivity than men. Overall, positivity for anti-CCP3, RF-IgA and RF-IgM in a sample was seen closest to diagnosis. Of note, while not in the figure, in men, anti-CCP3 positivity at >60 units (with or without positivity for ≤1 RF isotype) was present a median of 1.93 years prior to diagnosis; in women, anti-CCP3 positivity at >60 units (with or without positivity for ≤1 RF isotype) was present a median of 1.64 years prior to diagnosis. P-values represent comparisons between autoantibody positive states using pairwise contrasts and age-adjusted Cox regression model as well as adjusting using the false-discovery method of Benjamini-Hochberg. The green triangles represent the mean time of autoantibody positivity prior to RA diagnosis. DoDSR, Department of Defense Serum Repository; RA, rheumatoid arthritis; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptide antibody; Ig, immunoglobulin.

Refernce for Figure 3 above: Bergstedt DT, Tarter WJ, Peterson RA, Feser ML, Parish MC, Striebich CC, Demoruelle MK, Moss L, Bemis EA, Norris JM, Holers VM, Edison JD, Thiele GM, Mikuls TR, Deane

KD. Antibodies to Citrullinated Protein Antigens, Rheumatoid Factor Isotypes and the Shared Epitope and the Near-Term Development of Clinically-Apparent Rheumatoid Arthritis 2022;13:1-10. doi: https://doi.org/10.3389/fimmu.2022.916277.

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 delays in laboratory testing due to COVID19-related shut-downs and supply chain issues. We have now seen resolution of these issues and have completed testing, and will meet final deliverables by April 2024.

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.**

This period

Bergstedt DT, Tarter WJ, Peterson RA, Feser ML, Parish MC, Striebich CC, Demoruelle MK, Moss L, Bemis EA, Norris JM, Holers VM, Edison JD, Thiele GM, Mikuls TR, Deane KD. Antibodies to Citrullinated Protein Antigens, Rheumatoid Factor Isotypes and the Shared Epitope and the Near-Term Development of Clinically-Apparent Rheumatoid Arthritis 2022;13:1-10. doi: https://doi.org/10.3389/fimmu.2022.916277.

Prior periods

Bettner LF, Peterson RA, Bergstedt DT, et al. 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 Rheumatol 2021;3(10):684-689. (In eng). DOI: 10.1002/acr2.11309.

Mikuls TR, Edison J, Meeshaw E, et al. Autoantibodies to Malondialdehyde-Acetaldehyde Are Detected Prior to Rheumatoid Arthritis Diagnosis and After Other Disease Specific Autoantibodies. Arthritis Rheumatol 2020;72(12):2025-2029. (In eng). DOI: 10.1002/art.41424.

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

This period

Nothing to report.

Prior periods

Nothing to report.

- **Other publications, conference papers, and presentations.**

This period

Nothing to report

Prior periods

Buscema PM, Massini G, Della Torre F, Asadi-Zeydabadi M, O'Donnell C, Newman F, Tagg R, Lodwick W, Collora C, Feser M, Moss L, Robinson W, Thiele G, Mikuls T, Edison J, Holers VM, Deane K. Abstract 1650 Identifying Trajectories and Endotypes in the Evolution of Pre-Rheumatoid Arthritis with Autoantibody Testing and Artificial Adaptive System Analysis. American College of Rheumatology Annual Meeting 2021

Bergstedt D, Peterson R, Feser M, Moss L, Thiele G, Mikuls T, Edison J, Holers VM, Deane K. ABSTRACT 1648 Increasing Rates of Positivity of Autoantibodies Indicates a Shorter Time-to-Diagnosis of Future Rheumatoid Arthritis. American College of Rheumatology Annual Meeting 2021.

Greenblatt HK, Mikuls TR, Edison JD, Feser ML, Parish MC, Moss LK, Mewshaw E, Deane KD. ABSTRACT 1721 Increasing Autoantibody Positivity During Pre-RA Is Associated with the Imminent Development of Clinical RA: A Retrospective Cohort Study. American College of Rheumatology Annual Meeting 2020.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

The following individuals are participating in the project as trainees, and have not changed since the prior report:

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 during this period.

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:	Semeion Research Center
Location of organization	*Rome, Italy*
Partner's contribution to the project	*Analyses (as consultant)*

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.*

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** Not applicable.
- **QUAD CHARTS:** Not applicable.
- **AWARD CHART:** See Appendix

9. APPENDICES

See Award Chart

APPENDIX: AWARD CHART (SEE NEXT PAGE)

Award Log Number: Award Title: GW81XWH-20-1-0204, PR191079: Key Autoantibody and Inflammatory Factors in the Initiation, Propagation, and Transition to Clinically Apparent Rheumatoid Arthritis

PI: Deane, Kevin D. University of Colorado, CO, USA **Budget (total):** $1,637,481

Topic Area: Rheumatoid arthritis **Mechanism:** CDMRP PRMRP Expansion Award

Research Area(s): 0505, 0700 **Award Status:** 15-Apr-2020 to 14-Apr-2024 (reporting period 15-Apr-2022 to 14-Apr-2023)

Study Goals:

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. With this as background, the primary goal 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.

Specific Aims:

Specific Aim 1. Evaluate a set of autoantibody-related biomarkers across the development of RA in cases and controls.
Specific Aim 2. Evaluate broad set of inflammatory, immunologic and cell related biomarkers across the development of RA in cases and controls.
Specific Aim 3. Application of artificial intelligence (AI) and other statistical approaches to enhance understanding of prediction and pathways in RA development.

Key Accomplishments and Outcomes:

Publications:

1. Bergstedt DT, Tarter WJ, Peterson RA, et al. Antibodies to Citrullinated Protein Antigens, Rheumatoid Factor Isotypes and the Shared Epitope and the Near-Term Development of Clinically-Apparent Rheumatoid Arthritis. 2022;13:1-10. DOI: https://doi.org/10.3389/fimmu.2022.916277.
2. Bettner LF, Peterson RA, Bergstedt DT, et al. 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 Rheumatol 2021;3(10):684-689. (In eng). DOI: 10.1002/acr2.11309.
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Patents: none to date

Funding Obtained: none to date