Research paper

Cohort profile of a US military population for evaluating pre-disease and disease serological biomarkers in rheumatoid and reactive arthritis: Rationale, organization, design, and baseline characteristics

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ABSTRACT

Purpose: The etiology of several autoimmune disorders, including rheumatoid arthritis, remains unknown. While there are clear phases of disease progression, the mechanisms of transition between these phases are poorly understood. Additionally, treatment focuses on an alteration of the biological processes to prevent joint damage and functional decline. A goal is to potentially treat the disease during the preclinical phase to mitigate the disease process. Reactive arthritis is another rheumatologic condition known to be secondary to a distal infection. While prevention of infection would mitigate risk, serologic profiling patients with the disease may assist in the elucidation of potential disease risk factors. This study was initiated to enable an assessment of pre-disease biomarkers in patients newly diagnosed with rheumatoid arthritis and reactive arthritis.

Participants: A retrospective cohort of 500 rheumatoid and 500 reactive arthritis cases with 500 matched controls was drawn from a population of active component US military personnel. Appropriate inclusion criteria limited subject selection. Additionally, 4 serum samples (3 pre-disease and 1 disease-associated) were obtained for each case and control.

Findings to date: The established cohort provides the framework for novel exploration of the host response through serum profiling and seroepidemiology prior to disease onset.

Future plans: This study establishes the framework for the evaluation of novel serum biomarkers enabling the identification of signals prior to clinical disease that may enable disease prediction, elucidate disease pathogenesis and identify novel exposures leading to increased disease risk and/or disease severity.

1. Introduction

Rheumatoid Arthritis (RA) is a relatively common, chronic, systemic inflammatory disease of unknown aetiology that affects approximately 1% of the population worldwide [1–3]. RA is three times more common in women than men [4]. There is regional variation in the prevalence of RA. The incidence appears to be highest in Pima Indians (5.3%) and Chippewa Indians (6.8%), and lowest in people from China and Japan (0.2%–0.3%), suggesting the possibility that genetic factors contribute to RA [5,6]. These differences in regional RA prevalence also may suggest a role for environmental factors. It predominantly targets joints and can lead to significant long-term disability, secondary to cartilage and bone erosion. These long term effects are reflected in the work disability secondary to RA, which can be as high as 59%, with 90% of patients with RA retiring from work prematurely [7].

Reactive arthritis (ReA) is a well-known sequelae following gastrointestinal (Salmonella, Yersinia, Shigella, and Campylobacter spp) and urogenital (Chlamydia trachomatis, neisseria gonorrhoeae) infections. The maximum interval between the preceding infection and the arthritis is generally accepted as 4 weeks. Typical features include asymmetric oligoarthritis, most often in the large joints of the lower extremities. Up to half of patients also have arthritis in their upper limbs. A recent study

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has estimated that the annual incidence of enteric-infection-related ReA within a population may approach one per 1000 [8]. The frequency of ReA or new-onset joint pain following a gastrointestinal infection varies widely among studies likely confounded by variable study design and outcome definitions [9–23].

2. Study rationale

Initial development of RA autoimmunity likely occurs in the pre-clinical/asymptomatic period of inflammatory arthritis and it is important to study the biology in this early period to understand the progression of the disease from asymptomatic to symptomatic phase and ultimately to clinical RA. RA patients show antibodies reactive against several citrullinated peptides or proteins, e.g., anti-citrullinated protein antibodies (ACPAs) against enolase, vimentin, fibrinogen, and type II collagen. Since these autoantibodies develop in asymptomatic individuals much before the onset of inflammatory arthritis and RA, ACPAs are excellent serological tools to understand disease progression and to explore the biology associated with pre-RA stage. ACPAs develop 5–10 years before the clinical onset of the disease and their sub-specificities may help us identify appropriate diagnostic, prognostic and patient stratification markers. However, there is a paucity and limited understanding of biomarkers of disease progression, specifically those which are altered in patients 6–24 months before the clinical diagnosis of RA.

Unlike the serological markers of RA, there is a significant gap in our understanding of the markers of ReA. The presence and identity of citrullinated antigens, carbamylated antigens or other autoantigens and their autoantibodies is an open question and worthy of study. Londono et al. described the association between several potential serum biomarkers and disease prognosis in patients with ReA and other spondyloarthritidies [24]. Specifically, an increase in several cytokines, including IL-6, IL-1β as well as C-reactive protein was associated with poorer disease prognosis. It is unclear if these changes are temporally associated with disease onset or if this variability is present prior to initiating infection.

The goal of this effort was to establish a cohort that could provide insights into the pathophysiological mechanisms that operate before the clinical diagnosis of RA/ReA. Insights and predictions gleaned from such a cohort has the potential to change the treatment paradigm focused on the remedial treatment of established and essentially incurable disease to a future treatment paradigm of prevention or early intervention. This early pre-diagnosis phase may be the ideal opportunity to restore immune tolerance and homeostasis, something that is difficult to achieve in established RA/ReA. Additionally, identification of novel immune biomarkers may point to diagnostic and prognostic assays and new drug targets worthy of subsequent preclinical and clinical development.

Another goal of this cohort is to enable the comparison of serologic and serum proteome associated with ReA to those in RA. ReA is an acute disease, whereas RA is a chronic disease. We hypothesize that initiating triggers and disease pathways might be shared between the two forms of arthritis. This has important ramifications not only in understanding disease processes but also in the identification of novel targets for treatment, prevention and cure of RA. In this project, we propose to identify the acute pathways or networks that are modulated in reactive arthritis compared to healthy controls. We will then interrogate the status of these pathways or networks in RA serial samples. Therefore, using reactive and rheumatoid arthritis serum samples, we hope to identify pathways involved in the manifestation of ReA disease as well as pathways or networks that may be the initial triggers of autoimmunity in RA.

In brief, this study enables a unique opportunity to compare reactive and rheumatoid arthritis prior to diagnosis. Data resulting from the analysis of samples from this cohort have the potential to not only identify the earliest triggering pathways and networks leading to disease, but may also facilitate the identification of novel drug and therapeutic targets as well as diagnostic and prognostic markers associated with these two forms of arthritis.

3. Cohort description

3.1. Study design and participants

This study was designed as a nested case-control study that incorporates non-diseased subjects as a comparator population using age and gender frequency matching. Subjects with RA/ReA between 1998 and 2012 were identified from the Defense Medical Surveillance System (DMSS), the main data repository for all US armed forces and contains relevant data from more than 7 million members having served in the armed forces since 1990, documenting their military and medical experiences throughout their career [25].

3.2. Case and control selection

Cases were defined as subjects with at least 2 medical encounters with any of the relevant International Classification of Diseases 9 - Clinical Modification (ICD9-CM) codes for reactive arthritis (099.3, 711.1, 711.3) or rheumatoid arthritis (714.0). The date of onset was defined as the first medical encounter with a relevant ICD9-CM code, confirmed by ≥ 1 additional encounter. A matched control population based on similar age-group and gender distributions of the cases subjects was obtained from a random sample of subjects in the DMSS with no medical encounters for reactive or rheumatoid arthritis. Additionally, all subjects with documented history of non-specific arthropathies and arthralgias were excluded.

3.3. Serum sampling

For each case and control subject, up to four serum samples were obtained from the US Department of Defense (DoD) Serum Repository as per Fig. 1. For RA subjects, sample A was the first sample prior to the first RA diagnosis, unless a sample was available within 30 days of the first diagnosis. In this case, the sample 30 days subsequent to diagnosis was obtained. Sample D for RA cases was the first sample available in the repository, and samples B and C were interim samples approximately evenly distributed across the service member’s duty time. For ReA, samples A and B represent the first available samples after and before an initial ReA diagnosis, respectively. Sample D was the first sample available in the repository, and sample C was an interim sample approximately evenly distributed between samples B and D. For control subjects, sample A was matched by year (± 1 year) to ReA and RA cases (50% of controls matched to RA cases and 50% matched to ReA cases) such that an equal proportion of Sample A from the controls were collected at approximately the same time (± 1 year) as Sample A from the cases. Samples B and C for control subjects represented the two preceding serum samples available from the repository and sample D was the earliest serum sample available.

3.4. Biorepository description

Similar to our previously described repository [26], we have established a centralized data and sample storage at the Naval Medical Research Center (NMRC). All data are linked by a unique identifier to subject samples. Samples were accessioned upon arrival and are maintained at NMRC at ~80 °C. For testing, the parent samples were thawed, sub- aliquoted and multiple daughter aliquots made to meet the volume requirements of the planned testing as well as anticipated future testing. Aliquots were refrozen and sent to partner laboratories. All aliquots are maintained in bar-code labeled cryovials with a desiccation proof seal without additives. All aliquots are linked to the parent sample through the bar coding and a laboratory information management system.
marker in cases and controls. For example, several studies to date have studied groups. For these algorithms, models will be developed on a subset (approximately 1/3) and validated on a third subset (approximately 50%) [27]. Presuming comparable estimates of sensitivity, the specificity (approximately 90%) yet relatively lower sensitivity (approximately 1/3). Thus, the study population was active component US military personnel, which constitute a large cohort of generally young people without severe co-morbidities. These characteristics are particularly appropriate for studies of RA/ReA, as these diseases mainly affect young adults. Moreover, regarding studies on impact of GI infections on further development of chronic conditions (such as ReA), cohorts of military members are of great interest as these subjects are exposed to high rates of GI infections due to deployment to endemic regions where exposure risk is high [28,29]. It is important to note there are some differences between this population and the general population that may be important in interpreting the findings from this cohort.

Demographic data were obtained from personnel data and deployment data were derived from deployment rosters and deployment health assessments. All medical encounters with a RA/ReA diagnosis at any diagnostic position were obtained from AFHSB. Additionally, any medical encounters associated with a procedural code associated with RA/ReA diagnosis were also obtained. Data on infectious gastroenteritis episodes were obtained based on ICD-9 codes for specific bacterial and viral pathogens as were events with no pathogens identified. Medical encounters for Chlamydia and gonorrhea were also obtained.

This study was reviewed and approved by the Institutional Review Board at the NMRC in compliance with all regulations.

4. Baseline description of study population

The demographics of the study population are shown in Table 1. In brief, the population was broadly reflective of active duty US military personnel in that it is predominately young, male, with a high school (or equivalent) education. Subjects with RA were more commonly female than were those with ReA (p < 0.001). Additionally, subjects with RA represented an older subset of the active component population compared to those with ReA with means of 37 and 30, respectively (p < 0.001). This corresponded to RA subjects that were of more advanced rank (p < 0.001) and more commonly married (p < 0.001) than the ReA subjects. Subjects with reactive arthritis were more commonly white (70.4%) compared to those with RA (56.4%) (p < 0.001). The majority of subjects (65.9%) had at least one operational deployment prior to being included as a case or control. Control subject demographics were comparable to the cases. An approximately equal proportion of subjects was selected from each year of study from 1998 through 2012 (Fig. 2).

4.1. Findings to date

Serum samples were identified for all subjects and represented the planned sampling scheme (Fig. 3). Samples after ReA (sample A) were collected a mean of 293 days (std dev: 308) post-diagnosis, while the last sample prior to diagnosis was collected a mean of 295 days pre-diagnosis (std dev: 288). Antecedent samples were a mean of 4.6 years and 7.9 years prior to diagnosis. In contrast, the disease associated sample for RA cases were collected a mean of 289 days (std dev: 286) prior to diagnosis while the antecedent samples were a mean of 4.7, 8.7 and 12.7 years pre-diagnosis. Samples from control subjects were from a comparable distribution relative to their matched case sample. It should be noted that future analyses should assess for the influence of sample age which may yield a lower overall statistical power. Furthermore, sero-prevalence of ACPAs in 50% of the RA cases would enable an estimation of sero-prevalence in the general RA population ± 4%.

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respectively) with a high median number of related medical encounter rheumatoid and reactive arthritis cases included diseases of the musculoskeletal system and connective tissue (73.6% and 74.0%, respectively) with a high median number of related medical encounter visits (32 and 24, respectively). In contrast, only 5.6% (p < 0.0001) of the healthy controls had any medical encounters associated with diseases of the musculoskeletal system and connective tissue with 3–4 fold fewer encounters per subject. The next most prevalent ICD9-CM code among ReA cases was infectious and parasitic diseases (31.0%), higher (p = 0.01) than in RA cases (23.8%) and almost 3-times more common than the healthy controls (p < 0.0001). Similarly, symptoms, signs and ill-defined conditions were more common among RA cases (41.0%) than among those with ReA (25.4%) or the healthy controls (2.2%). The next most prevalent conditions were endocrine, nutritional, and metabolic diseases and immunity disorders occurring in 25.8% of the RA cases and 13.4% of the ReA cases (p < 0.0001) with also a higher number of encounters per diagnosis.

5. Discussion

There is now increasing evidence that RA develops in phases and its underlying pathogenesis begins many years before clinical diagnosis [30]. The natural course of RA evolution involves genotype-environment interaction in the susceptible individuals resulting in the development of asymptomatic autoimmunity (first phase), where subjects are healthy but for the presence of autoantibodies, namely ACPA and/or rheumatoid factors (RFs). Over time these autoantibodies gain access to joints and asymptomatic subjects transition to an arthralgia phase (second phase), where they start experiencing non-specific joint symptoms without any clinical signs of synovitis. As autoantibodies and immune cells enter joints, arthralgia patients progress to an undifferentiated/inflammatory arthritis (UA/IA) phase, characterized by clinical signs of synovitis (third phase). Finally, with increased systemic and joint inflammation and ACPA epitope spreading, UA/IA patients convert to clinically identifiable RA (fourth phase). Since UA/IA is characterized by tenderness and swelling of joints, this stage may indicate an imminent RA diagnosis, and thus it may be difficult to clearly identify a population of these patients for a prevention/interception clinical trial. Therefore, asymptomatic autoimmunity and arthralgia are referred to as two pre-RA phases [30], wherein basic pathogenic processes of inflammation, namely T-B cell interaction, production of autoantibodies and infiltration of a small number of autoimmune cells into joints, have begun but the inflammatory burden is low and subjects have no (or minimal) joint inflammation, synovial hyperplasia, tissue damage or joint destruction, hallmarks of RA. The current therapeutic paradigm limits patient treatment until after diagnosis with full-fledged RA. However, due to heterogeneity of the disease and the irreversible tissue damage that has occurred by the time of RA diagnosis, the remission rates with down-stream sequela of irreversible pathogenic tissue changes that lead to inadequate drug responses and therapy-resistant RA in patient populations with established disease.

In order to develop a successful paradigm of disease prevention or disease interception, one needs to identify biomarkers of disease progression from pre-RA to RA that can classify subjects at risk of

### Table 1

Demographics of study population.

|                      | Rheumatoid Arthritis (N = 250) | RA Controls (N = 250) | Reactive Arthritis (ReA) (N = 250) | ReA Controls (N = 250) |
|----------------------|--------------------------------|-----------------------|------------------------------------|------------------------|
| N                    | %                              | N                     | %                                 | N                      |
| Gender               |                                |                       |                                    |                        |
| Male                 | 306 (61.2)                     | 210 (84.0)            | 361 (92.2)                         | 244 (97.6)             |
| Female               | 194 (38.8)                     | 40 (16.0)             | 39 (7.8)                           | 6 (2.4)                |
| Age                  |                                |                       |                                    |                        |
| <20                  | 0                               | 0.0                   | 0(0)                              | 0(0)                   |
| 20-29                | 74 (14.8)                      | 55 (22.0)             | 66 (169)                           | 169 (67.6)             |
| 30-39                | 247 (49.4)                     | 145 (58.0)            | 167 (33.4)                         | 68 (27.20)             |
| 40-49                | 149 (29.8)                     | 49 (19.6)             | 56 (11.6)                          | 13 (5.2)               |
| ≥50                  | 30 (6.0)                       | 1 (0.4)               | 8 (1.6)                            | 0 (0.0)                |
| Education ≥ 12       |                                |                       |                                    |                        |
| Less Than High School | 1                              | 0.2                   | 0.8                                | 5 (1.0)                |
| High School (or equivalent) | 279 | 55.8 | 140 | 56.0 | 331 | 66.2 | 191 | 76.4 |
| Some college         | 90 (18.0)                      | 23 (9.2)              | 48 (9.6)                           | 15 (6.0)               |
| Bachelor's Degree    | 76 (15.2)                      | 4 (1.9)               | 61 (12.2)                          | 26 (10.4)              |
| Master's degree      | 42 (8.4)                       | 30 (12.4)             | 34 (6.8)                           | 11 (4.4)               |
| Doctorate Degree     | 4 (0.8)                        | 0.2                   | 0.8                                | 0.6 (1.0)              |
| Marital status ≥ 12  |                                |                       |                                    |                        |
| Married              | 389 (77.8)                     | 186 (74.4)            | 278 (55.6)                         | 139 (55.6)             |
| Single               | 67 (13.4)                      | 54 (21.6)             | 192 (38.4)                         | 106 (42.4)             |
| Others               | 43 (8.6)                       | 10 (4.0)              | 29 (5.8)                           | 5 (2.0)                |
| Military Rank        |                                |                       |                                    |                        |
| Jr. Enlisted         | 28 (5.6)                       | 4 (1.8)               | 24 (4.8)                           | 0.4 (0.8)              |
| Sr. Enlisted         | 368 (73.6)                     | 151 (60.4)            | 217 (43.4)                         | 105 (42.0)             |
| Jr. Officer          | 78 (15.6)                      | 29 (11.2)             | 82 (16.4)                          | 35 (14.0)              |
| Sr. Officer          | 11 (2.2)                       | 1 (0.4)               | 9 (1.8)                            | 0 (0.0)                |
| Warrant Officer      | 15 (3.0)                       | 5 (2.0)               | 8 (1.6)                            | 2 (0.8)                |
| Branch of Service    |                                |                       |                                    |                        |
| Army                 | 201 (40.2)                     | 50 (20.0)             | 168 (33.6)                         | 61 (24.4)              |
| Air Force            | 118 (23.6)                     | 54 (21.6)             | 116 (23.2)                         | 30 (12.0)              |
| Coast Guard          | 5 (1.0)                        | 2 (0.8)               | 6 (1.2)                            | 0 (0.0)                |
| Marines              | 30 (6.0)                       | 30 (12.0)             | 69 (13.8)                          | 48 (19.2)              |
| Navy                 | 146 (29.2)                     | 110 (44.0)            | 138 (27.6)                         | 108 (43.2)             |
| Race/Ethnicity ≥ 12  |                                |                       |                                    |                        |
| American Indian      | 7 (1.4)                        | 5 (2.0)               | 8 (1.6)                            | 5 (2.0)                |
| Alaska Native        | 17 (3.4)                       | 8 (3.2)               | 11 (2.2)                           | 7 (2.8)                |
| Asian/Pacific Islander | 282 (56.4)                  | 157 (62.8)            | 352 (70.4)                         | 163 (65.2)             |
| White                | 126 (25.2)                     | 43 (17.2)             | 57 (11.4)                          | 38 (15.2)              |
| Black                | 51 (10.2)                      | 29 (11.6)             | 48 (9.6)                           | 26 (10.4)              |
| Hispanic             | 6 (1.2)                        | 3 (1.2)               | 2 (0.4)                            | 0 (0.0)                |
| Other                | 317 (63.4)                     | 176 (70.4)            | 317 (63.4)                         | 179 (71.6)             |

a There are a total of 33 subjects (8 RA cases; 5 RA controls; 18 ReA cases; 2 ReA controls) where education information was not provided.

b There are 2 individuals (1 Rheumatoid Arthritis; 1 Reactive Arthritis) in which marital status information was not provided.

c There were 39 individuals (11 Rheumatoid Arthritis; 5 RA controls; 17 Reactive Arthritis; 6 ReA Controls) where race/ethnicity information was not provided.
developing RA. These biomarkers may also provide insight into the pathways involved in disease evolution and identification of potential targets for disease interception. A number of studies have reported that the presence of autoantibodies (ACPAs, RFs, anti-peptidyl arginine deiminase antibodies and anti-carbamylated protein antibodies) pre-date clinical RA by several years [30,33–41]. In a prospective study of
levels of serum cytokines were observed as seropositive subjects approached the time of disease diagnosis [43]. We have assembled a comparable but much larger cohort of RA patients. The current generation of biomarkers (ACPAs, RF and cytokines) that define pre-RA phases, develop 5–10 years before RA diagnosis. However, from a practical point of view, an interception clinical trial with a novel agent could be of maximum 2–3 year duration and therefore, one needs to identify at risk subjects 2–3 years before the development of the disease. The DoD cohort of pre-RA serum samples from RA patients described herein provides a unique opportunity to identify not only ACPA sub-specificities and cytokines but other protein analytes (using global proteomics profiling) as novel biomarkers for more precise classification of at-risk subjects. Since autoantibodies develop as a result of breakdown of tolerance and given the observations that therapeutic anti-PD-1 antagonistic antibodies have shown remarkable efficacy in human tumors along with the emergence of autoimmunity in responsive cancer patients due to loss of immune tolerance [44], we also plan to examine the levels of soluble PD-1 (sPD-1) protein in pre-RA samples [45].

Many autoimmune diseases, including RA, show gender differences in their prevalence. RA is three times more common in females than males with women having worse symptoms of disease [46]. However, the pre-RA and RA cohort assembled herein is more biased towards male subjects (61% males; Table 1). The large collection of male pre-RA and RA samples also provide an opportunity to understand pre-disease and disease differences between males and females in terms of their biomarkers, disease pathways and underlying mechanisms.

In contrast to RA, biomarkers of ReA arthritis disease pre-disposition and progression are not very well known. HLA-B27 has been shown to be linked with disease susceptibility in ReA patients with exposure to gastrointestinal or urinogenital bacterial infections (Campylobacter, Chlamydia, Salmonella, Shigella, and Yersinia) via mucosal surfaces [47]. HLA-B27 positive ReA patients exhibit more severe disease and the HLA positivity is more common in chronic or relapsing arthritis, uveitis, aortitis, sacroiliitis and spondylitis [47]. Since both RA and ReA show some level of HLA restriction (HLA Class II for RA and HLA class I ReA), and both types of arthritis show synovial inflammation, global proteomics, cytokine/chemokine and autoantibody profiling of the two cohorts will provide a unique opportunity to delineate inflammatory pathways and biomarkers that are common to both RA and ReA as well.

### Table 3

| ICD-9-CM Description | ICD-9-CM Code | (N (%) of subjects with Diagnosis) | Median (IQR) of separate medical encounters. |
|----------------------|---------------|------------------------------------|---------------------------------------------|
| RA (N=500) | ReA (N=500) | HC (N=500) | RA | ReA | HC |
| Infectious and parasitic diseases | 001-139 | 119 (23.8) | 155 (31.0) | 58 (11.6) | 8 (8, 16) | 8 (8, 16) | 8 (8, 14) |
| Neoplasms | 140-239 | 35 (7.0) | 11 (2.2) | 0 (0.0) | 8 (7, 8) | 8 (7, 8) | – |
| Endocrine, nutritional, and metabolic diseases and immunity disorders | 240-279 | 129 (25.8) | 67 (13.4) | 6 (1.2) | 12 (7, 24) | 8 (6, 14) | 7 (6, 7) |
| Diseases of the blood and blood forming organs | 280-289 | 57 (11.4) | 23 (4.6) | 1 (0.2) | 8 (7, 21) | 7 (6, 13) | 24 (24, 24) |
| Mental Disorders | 290-319 | 81 (16.2) | 55 (11.0) | 0 (0.0) | 11 (7, 18) | 7 (6, 13) | – |
| Diseases of the Nervous System | 320-359 | 97 (19.4) | 27 (5.4) | 1 (0.2) | 10 (7, 22) | 8 (7, 16) | 7 (7, 7) |
| Disease of the Sense Organs | 360-389 | 49 (9.8) | 81 (16.2) | 0 (0.0) | 13 (7, 21) | 14 (7, 24) | – |
| Diseases of the Circulatory System | 390-459 | 79 (15.8) | 46 (9.2) | 0 (0.0) | 18 (7, 29) | 8 (7, 19) | – |
| Diseases of the Respiratory System | 460-519 | 92 (18.4) | 38 (7.6) | 2 (0.4) | 8 (7, 20) | 7 (7, 13) | 7 (7, 7) |
| Diseases of the digestive system | 520-579 | 96 (19.2) | 48 (9.6) | 3 (0.6) | 8 (7, 15) | 8 (7, 15) | 7 (7, 8) |
| Diseases of the genitourinary system | 580-629 | 76 (15.2) | 35 (7.0) | 1 (0.2) | 8 (6, 15) | 7 (7, 10) | 65 (65, 65) |
| Complications of pregnancy, childbirth, and the puerperium | 630-679 | 7 (1.4) | 0 (0.0) | 0 (0.0) | 20 (7, 62) | – | – |
| Diseases of the skin and subcutaneous tissue | 680-709 | 70 (14.0) | 50 (10.0) | 2 (0.4) | 8 (7, 11) | 7 (6, 10) | 6.5 (5, 8) |
| Diseases Of The Musculoskeletal System And Connective Tissue | 710-739 | 368 (73.6) | 370 (74.0) | 28 (5.6) | 32 (15, 24) | 15, 8 | 8 (20) |
| Congenital Anomalies | 740-759 | 10 (2.0) | 5 (1.0) | 1 (0.2) | 7 (6, 8) | 7 (7, 14) | 6 (6, 6) |
| Certain Conditions Originating In The Perinatal Period | 760-779 | 0 (0.0) | 0 (0.0) | 0 (0.0) | – | – | – |
| Symptoms, Signs, And Ill-Defined Conditions | 780-799 | 205 (41.0) | 127 (25.4) | 11 (2.2) | 13 (7, 25) | 8 (7, 14) | 13 (8, 16) |
| Injury And Poisoning | 800-999 | 95 (19.0) | 65 (13.0) | 3 (0.6) | 8 (7, 16) | 7 (8, 12) | 8 (5, 16) |

RA, rheumatoid arthritis; ReA, reactive arthritis; HC, healthy control.
as those that are specific to these two diseases. To the best of our knowledge, the ReA cohort described herein will be the first systematic attempt to identify biomarkers associated with the development of ReA as well as those associated with disease susceptibility.

Herein we describe the establishment of a large cohort of adult subjects with RA or ReA as well as a matched cohort of control subjects. Serial serum samples starting up to 8–12 years prior to diagnosis have been obtained from subjects to enable an assessment of evolving proteomic markers of disease to potential elucidate novel mechanisms of disease and/or novel treatments that could be incorporated prior to full-fledged disease. Similar to the PREDICTS cohort for IBD [26], this first-of-its-kind cohort is well-suited to refine our understanding of two important rheumatological conditions with significant global disease burden.

Author contributions

Study concept and design: Chad K. Porter, Mark S. Riddle, Sunil Nagpal;
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The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

Human subjects research and data protections

The human subjects’ research (NMRC.2014.0012) under which the data and samples were obtained were approved as 'Exempt' research by the Naval Medical Research Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. In addition, this study is conducted under a support agreement with the AFHSB. All data are were de-identified by personnel at AFHSB who removed the list of 18 identifiers outlined in 45 CFR 164.514(b)(2) and DOD 6025.18-R (DoD Health Information Privacy Regulation) prior to providing the data to the investigators.

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RLG is a military service member and CKP is an employee of the U.S. Government. This work was prepared as part of their official duties. Title 17 U.S.C. §105 provides that “Copyright protection under this title is not available for any work of the United States Government.” Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person’s official duties.

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