Seronegative Rheumatoid Arthritis: A Case Control Study

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Abstract: *Background*: Seronegative Rheumatoid Arthritis (RA) is a disorder associated with considerable diagnostic, prognostic and therapeutic uncertainty for many clinicians.

Objectives: The aim of this study is to elucidate clinical features at diagnosis, manifestations and treatment of patients with RA with negative serologies, as compared to a control group of patients presenting with similar polyarthralgias but diagnosed with alternative (non-RA) musculoskeletal disorders.

Methods: The study was a retrospective chart review of electronic medical records from January 2003 to December 2012. Patients were identified using ICD-9 code Rheumatoid Arthritis 714.0 and at least two rheumatology clinic visits during the specified time. Charts were reviewed individually by two investigators. The inclusion criteria were a diagnosis of RA confirmed by a rheumatologist and normal values for both rheumatoid factor (RF) and anti-cyclic citrullinated protein antibodies (ACPA, third generation assay). Charts were also reviewed for eventual final diagnosis, either seronegative RA or alternate diagnosis (control group). Data were collected on demographics (sex, race, smoking status), family history of RA, and laboratory values (presence of anemia, inflammatory markers) at the time of diagnosis. The presence of erosions and synovitis identified by imaging studies was assessed. In addition, the presence of extra-articular manifestations of RA including nodules, pleural or parenchymal lung disease, eye involvement and osteoporosis was recorded. The therapies (disease modifying anti-rheumatic drug (DMARD), biologic) used to treat the seronegative RA were also reviewed. The family & smoking history and laboratory values of the seronegative RA patients were compared to the control group and analysis was done using Fisher's exact test.

Results: Charts from 107 patients were reviewed. Forty-four patients were eventually classified as having an alternate diagnosis and were considered the control group. Sixty-three patients were considered to have an established clinical diagnosis of seronegative RA. Among all patients at the time of diagnosis, 25% were smokers, 13% had a family history of RA, 54% were anemic, and 76% had abnormal ESR or CRP. The RA patients had statistically higher proportion with anemia compared to controls at presentation, and statistically lower proportion with ESR elevation compared to controls (p=0.033 and p=0.013, respectively). Seven of the 59 (11%) patients who had hand/wrist films during their care had erosions on radiography, and 6 of 13 (46%) patients who had an MRI of an extremity had findings of synovitis. Extra-articular manifestations were infrequent in this group. Forty-eight of the 62 were initiated on a DMARD, most commonly hydroxychloroquine (16% patients) or methotrexate (29% patients) or a combination of methotrexate and hydroxychloroquine therapy (35%). Of the 63 patients, 17 (27%) patients required a biologic therapy during treatment course.

Conclusions: This study supports the hypothesis that clinical history and physical examination can be important determinants in helping to diagnose seronegative RA and distinguish it from other polyarthopathies. In addition to characteristic symptoms, factors which might contribute to diagnosis of RA in a patient without seropositivity include presence of anemia, and results of imaging studies.

Keywords: Rheumatoid Arthritis, serology, diagnosis, anemia.

INTRODUCTION

Seronegative rheumatoid arthritis (RA) is a disorder associated with considerable diagnostic, prognostic and therapeutic uncertainty for many clinicians. Approximately one-third of patients with RA have negative serologies including rheumatoid factor (RF) and anti-citrullinated protein/peptide antibodies (ACPA) [1]. The 2010 American College of Rheumatology/ European League Against Rheumatism (ACR/EULAR) Classification Criteria, designed to identify RA patients early in disease, include serology in the diagnosis [2]. Although these criteria were designed to be applied to research populations, and not used as diagnostic criteria, physicians may apply them in their clinical practice. Using these criteria, a patient with symmetric polyarthritis for greater than 6 weeks and negative serologies may easily satisfy the criteria for RA. However, this diagnosis should only be made when the clinical presentations and data are not better explained by another disease.

It is unclear whether seropositive and seronegative RA are part of a disease spectrum or represent two different disorders with similar clinical presentations. Aside from serologies, little study has been done regarding baseline features differentiating seronegative RA from other diseases. Given the limited data available for seronegative RA, the aim of this study is to elucidate clinical features at diagnosis, manifestations and treatment of RA with negative serologies.

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MATERIALS AND METHODS

The study was a retrospective review of electronic medical records from January 1, 2003 to December 31, 2012. Charts were reviewed individually by two investigators (IC & MA). Patients were identified using the ICD-9 code Rheumatoid Arthritis 714.0 on at least two rheumatology clinic visits during the specified period. The inclusion criteria were a diagnosis of RA confirmed by a rheumatologist, and normal values for both rheumatoid factor (RF) and ACPA (third generation assay). Patients were excluded if they were seen by a rheumatologist more than once and eventually determined to have a diagnosis other than RA. This group was considered as the control group for comparison of diagnostic features. Eventual alternative diagnoses for this control group were collected if present. Data were collected on demographics: sex, age, and race. In addition, family history of RA was noted if documented at any time throughout the chart. Tobacco and alcohol use and BMI were noted at the time of diagnosis as were laboratory values (presence of anemia as defined by reference range for hemoglobin and hematocrit for each sex; inflammatory markers of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) as defined by cut offs for normal reference range for our laboratory). The presence of erosions and synovitis identified by imaging studies was assessed, using both radiographs as well as MRI of any joint in our system. In addition, the presence of extra-articular manifestations of RA during the course of care was noted, including suspected rheumatoid nodules, pleural or parenchymal lung disease, eye involvement and osteoporosis. The therapies used to treat the seronegative RA patients were also reviewed; any therapy prescribed for each individual patient was included. Therapies of interest included traditional disease modifying anti-rheumatic drugs (DMARD) (methotrexate, hydroxychloroquine, sulfasalazine, and leflunomide), any biologic (TNF-inhibitors, rituximab, abatacept, and tocilizumab), or any combination of such therapies. Fisher exact test was used to compare parameters at diagnosis (anemia, smoking, family history and presence of erosions) between the seronegative group and the control group with a significant *p*-value of 0.05. The study was approved by the institutional review board. As this was a retrospective chart review study, no informed consent was required as no more than minimal risk was involved.

RESULTS

Charts from 107 patients were reviewed. Forty-four patients were excluded based on low clinical suspicion for seronegative RA by the rheumatologist or eventual alternate final diagnosis; these 44 patients were the control group used in comparison of features considered for diagnosis. The most common final diagnoses for patients in the control group in the study were: spondyloarthritis (6), polyarticular juvenile RA (6), erosive or inflammatory osteoarthritis (5), inflammatory bowel-related arthropathy (3), polymyalgia rheumatic (2) and fibromyalgia (1); the remainder of the patients did not have a conclusive diagnosis or did not follow up for a long enough duration for a specific diagnosis to be made.

The study group of seronegative RA patients included 63 patients with a confirmed clinical diagnosis; confirmation by ACR classification criteria could not be made with available documentation. Age and gender distributions were similar to the general demographics known for seropositive RA patients: more commonly diagnosed in females and with peak incidence between fourth and fifth decade [3]. For the study group, the mean age at first visit was 54 years old, the age range was 19-90 years old. Patients are predominantly female. Table **1** depicts the comparison of age, race and BMI between the seronegative RA group and the control group.

Patients were followed during the time described above for 2 to 49 visits with the rheumatology department, the mean was 12.8 visits/patient over the study period and a mean of 4.5 years between first and last visit.

Table **2** depicts the comparison of features at time of diagnosis between seronegative RA patients and controls. Among study patients at the time of diagnosis, 25% of the patients were smokers, 13% had a family history of RA, and 54% were anemic. Seventy-seven percent had abnormal ESR or CRP (34/44 with either measure available) and 53% (20/38 with both measures available) had elevations in both ESR and CRP (not shown in Table **1**). Seven of the 59 (12%) patients who had hand/wrist films had erosions on radiography at some point during their care. Nearly all patients (59/63) had hand/wrist films near the diagnosis or initial visits in their care in the practice. Foot/ankle films were done in few patients, only when clinical symptoms indicated. Six of 13 (46%) patients who had

Table 1:

Demographic	Seronegative RA	Control
Sex (N (%))	52/63 (82.5) female 11/63 (17.5) male	40/44 (90.1) female 4/44 (9.9) male
Race (N (%))	41/63 (65) white 13/63 (20.6) black 4/63 (6.3) other 5/63 (7.9) declined/unavailable	29/44 (65.9) white 10/44 (22.7) black 5/44 (11.4) other
Average BMI	31.8	32.3

Table 2:

Disease Characteristic	Seronegative RA Number/total with available data (%)	Control Number/total with available data (%)	*p*-value
Tobacco use	12/48 (25)	8/25 (32)	0.585
Family History	6/48 (12.5)	4/31 (12.9)	1
Anemia	25/46 (54)	5/21 (23.8)	0.033
CRP elevation	16/44 (36)	13/21 (61.9)	0.065
ESR elevation	14/40 (35)	10/15 (67)	0.013
Erosions	7/59 (11.8)	5/37 (13.5)	1
Treatment			
DMARD		-	-
MTX	18/63 (29)	-	-
HCQ	10/63 (16)	-	-
Leflunomide	3/63 (5)	-	-
MTX + HCQ	22/63 (35)	-	-
Other combination DMARDs	10/63 (16)	-	-
None	8/63 (13)	-	-
Biologics			
Anti-TNF agent	11/63 (17)	-	-
Other	6/63 (10)	-	-
None	46/63 (73)	-	-

MRI done of extremity during work up had MRI findings of synovitis. Four of these six MRIs were done in search for corroborative evidence in order to make the diagnosis of RA (lack of adequate clinical and laboratory data); two were performed by non-rheumatologists for workup of symptoms (chronic progressive hand swelling and chronic knee pain), which resulted in referral to rheumatology and aided in the diagnosis of RA. Four of the six were small joint imaging (hand, and/or wrist, foot and/or ankle); the other two MRIs were of the knee and hip. The majority were done with and without contrast (one done without contrast for unclear reason). A total of 17% (10 patients of 59 who had films and/or MRI) had either erosions or demonstrated imaging findings of synovitis. The extra-articular manifestations we analyzed were found infrequently; 5 patients with osteoporosis (no data collected on prednisone use), 1 with lung fibrosis (only treated with hydroxychloroquine, never on methotrexate), 1 with rheumatoid nodule (who was treated with methotrexate at the time of diagnosis of the nodule) and 1 with pleural thickening (who was on treatment with methotrexate at the time of diagnosis).

Forty-eight of the 63 were initiated on a DMARD, most commonly hydroxychloroquine (16% patients) or

methotrexate (29% patients). A large percentage of patients (35%) were treated with a combination of methotrexate and hydroxychloroquine therapy. Fifty-one percent (32/63) of patients were treated with combination therapy of two or more DMARDs at some point in their disease course. Of the 63 patients, 17 (27%) patients required a biologic therapy during treatment course.

DISCUSSION

This study supports the hypothesis that clinical history and physical examination are important determinants in diagnosing seronegative RA. Without diagnostic criteria for RA, physicians heavily rely on clinical suspicion and supporting history, exam, laboratory and imaging evidence of disease. In addition to characteristic symptoms, factors which might contribute to diagnosis of RA in a patient without seropositivity include presence of anemia and imaging studies.

A clear pattern of the prototypical seronegative RA patient did not emerge. This in part may have been due to the limited number of patients available for the study or to the limited number of characteristics examined during the study. However, it seems that several important findings may assist the clinician in making the diagnosis of seronegative RA. Of the available data, 76% of patients with seronegative RA had abnormal inflammatory markers. However, these individual inflammatory markers do not appear to be of much value for differential diagnosis; such markers were often elevated in the control group, many of whom were eventually diagnosed with other inflammatory disorders. Finally, although elevated inflammatory factors may be diagnostically supportive in the early clinical presentation of seronegative RA (though not with respect to non-RA inflammatory disorders), normal inflammatory markers (present in 24 % of our study population) should not prevent the clinician from making the diagnosis. The presence of anemia was significantly more frequent at the time of diagnosis in the seronegative RA patients compared to controls (p=0.033).

Imaging studies were especially helpful, with 17% of the study subjects showing evidence of synovitis or erosive disease on radiography. Nearly all study patients (both RA with negative serologies and controls) had hand/wrist films, which is standard in our clinic practice, especially near the time of diagnosis; it is not standard within our practice to obtain foot/ankle films. Clinical disease activity assessment varies among clinicians and although some clinicians obtain radiography periodically to ascertain treatment utility, others do not. The vast majority of the radiographs included in this study were done at or near the time of diagnosis. This might explain the low prevalence of erosions.

In contrast, MRIs are not done frequently in practice. With the changes in clinical knowledge and general rheumatology practice, it is not surprising that only 24 MRIs were performed on 107 patients in the span of 10 years. Many patients had clinical synovitis and did not require imaging. However, insufficient clinical and laboratory data were the primary reason for MRI imaging. Nearly half of the RA patients with negative serologies were found to have synovitis on MRI, suggesting that this more sensitive imaging procedure may have an important role in establishing the diagnosis of seronegative RA. Without these imaging studies, the patients may have had limited follow up or delayed treatment, indicating that imaging studies can be an important diagnostic tool to provide further evidence of RA. Though not addressed in our study, patients with seronegative RA may benefit from ultrasound examination for evidence of synovitis in the joint.

As this cohort was selected from a clinical population, incomplete data were available regarding extra-articular manifestations. Five patients (8%) of the RA patients with negative serologies were noted to have osteoporosis. This may be disease or treatment (prednisone) related; data were not collected on prednisone use. Similarly, one patient was found to have lung fibrosis; this patient was only ever treated with hydroxychloroquine.

Only 25% of the patients with available data were smokers, which is consistent with prior research which substantiates that smoking is risk factor for development of RA patients with positive serologies and has a minimal effect on patients with negative serologies [4]. Treatment regimens varied, with the majority of the patients being treated with either methotrexate or hydroxychloroquine, or both. Previous studies indicate that the inflammatory mechanism(s) is similar in RA patients with negative serologies, therefore DMARDs should be effective [4]. Early treatment of RA is necessary to prevent joint destruction, therefore it is important to diagnose and treat patients early in their clinical course. A recent commentary [5] corroborates our findings that RA

without positive serologies cannot be considered a "generally mild form of the disease."

This study is a retrospective study, which lends itself to certain limitations. Although the presence of a control group with similar initial symptoms lends itself some strength to the observations made in the study, the control group consisted of patients with a variety of rheumatologic and non-rheumatologic pathologies. It is acknowledged that this is inherently not optimal. However, evaluation of a patient with atypical presentation and negative serologies can be challenging. Clinicians often consider numerous alternative diagnoses in these patients, as seen in our control group.

Recall bias was minimized due to the objective data that was collected at the time of diagnosis. The number of swollen and tender joints at presentation are variably noted within patient charts; as this was a retrospective review of patients' charts in general clinical practice, there was no standardization in documentation of physical exam. Thus, it cannot be stated that all patients adequately fit into any classification criteria. Thus, the results are most applicable to general practice but have the limitation of possibly being a less well-defined group than other RA study populations. The physician's clinical examination of synovitis was excluded given the lack of consistency between examiners. The routine incorporation of 28 swollen/tender joint counts into general rheumatologic practice could contribute additional diagnostic information relative to the diagnosis of seronegative RA. Though this is a small study of seronegative RA patients, it provides more clinical insight into characterizing these difficult patients.

CONFLICT OF INTEREST

There are no conflicts of interest or financial relationships to disclose from any of the authors.

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