Rheumatoid Arthritis Diagnosis
Avoiding CCP False Positives Through Test Selection

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Dr. Tarrant is a Clinical Immunologist, board certified in Allergy, Immunology and Rheumatology. She specializes in diseases of and related to Rheumatoid arthritis, Sjögrens syndrome, Inflammatory Eye disease, CVID, and Immunodeficiency in Aging.

After graduating from the University of Florida College of Medicine, she performed her fellowship and residency at Duke University Hospital in North Carolina. In addition to her active medical practice, over the last 10 years, Dr. Tarrant has held two other major roles in her daily work; first as a medical liaison, where she assists in the evaluation and selection of immunoassays, including authoring or co-authoring peer-reviewed scientific articles of their evaluations, and secondly as an Associate Professor, Medicine.

Her work began within the hospital system and school of medicine at the University of North Carolina (UNC), and recently she became Associate Professor of Medicine at Duke University and Vice Chief of Translational Research, Rheumatology.
Dr. Tarrant has received consulting fees as well as an honorarium for today’s presentation. In addition, presentations are by their very nature, very brief overviews of complicated subject matter. No medical decision should be made solely based upon the information presented.
After participating in this educational activity, participants will be able to:

• Understand evidence-based approaches described in the American College of Rheumatology Guidelines for the diagnosis and management of Rheumatoid Arthritis (RA)

• Identify the importance of specificity in test selection, and the optimal usage of two recommended serologic markers for rheumatoid arthritis — anti-CCP and rheumatoid factor IgM

• Recognize how test efficacy and disease prevalence impact the accuracy of results, and when to consult with or refer the patient to a specialist
RA is the most common form of autoimmune arthritis

RA can start at any age

~1.5M in 2005

Adults age ≥ 18 have RA

Average age has increased steadily over time

Rheumatoid Arthritis (RA) – An Autoimmune Disease Primarily in Women

Affects women 2–3x more than men¹

1–3 % of women may get rheumatoid arthritis in their lifetime.¹

Rheumatoid Arthritis Disease Characteristics
Characteristics of Joint Damage

Rheumatoid Arthritis (RA)

• Early-stage: Very early RA showing swollen and painful PIP joints
• Late-stage: Long-standing RA with typical signs including swollen MCP joints, ulnar deviation of fingers, atrophy of musculi interossei and rheumatoid nodules.
• Affected joints are swollen, tender and warm, and stiffness limits their movement
Key Features of Rheumatoid Arthritis (RA)

- Chronicity
- Inflammatory symptoms
- Joint distribution
• Marginal erosions and joint space narrowing on x-ray

• Nodules occur in about 30 – 40% of patients
  • Positive RF and/or HLA-DR4 positive
  • Males
  • Severe and active disease
• Can occur at any age after onset
• Occasionally systemic manifestations include vasculitis, visceral nodules, Sjögren’s syndrome, or pulmonary fibrosis
Burden of Disease
## Burden of Rheumatoid Arthritis (RA)

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual estimated direct health care costs in the US</td>
<td>$19B</td>
</tr>
<tr>
<td>Hospitalizations in 2012</td>
<td>9,100</td>
</tr>
<tr>
<td>Annual direct purchase cost of biologic medications before insurance</td>
<td>~$30,000</td>
</tr>
<tr>
<td>Total hospital charges in 2012</td>
<td>$374M</td>
</tr>
<tr>
<td>Ambulatory care visits in 2007</td>
<td>2.9M</td>
</tr>
</tbody>
</table>

The most common comorbidities among people with arthritis in order of prevalence:

1. Cardiovascular Disease\(^1,2\)
2. Infections\(^1,2\)
3. Mental Health Condition\(^1\)
   - Anxiety and depression
4. Malignancies\(^1\)
   - i.e. Lymphoma and Multiple Myeloma
5. Others\(^2\)
   - Osteoporosis
   - Rheumatoid nodules
   - Abnormal body composition (BMI)
   - Lung disease
   - Dry eyes and mouth. (Sjögren's syndrome)
   - Carpal tunnel syndrome

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Prognosis: A historical perspective

Mortality

- Increased mortality compared to general population
- Lymphoma, atherosclerosis / myocardial Infarction

Men lose 4 years

Women lose 10 years

People with Rheumatoid Arthritis (RA) have lower functional status than those with osteoarthritis, and those without arthritis

One quality of life study compared those with RA (self-reported) and those without RA, and people with RA were:

- 40% more likely to report fair or poor general health
- 30% more likely to need help with personal care
- 2x as likely to have a health-related activity limitation

Pathogenesis / Causal Factors
Growing evidence suggests RA initiates outside the joint

• Smoking is the primary environmental risk factor

• Association between RA and mucosal sites (lung, oral cavity and gut)

• Increases in gut bacteria *Prevotella copri*, a gram-negative anaerobe

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2 Brusca SB, Abramson SB, Scher JU. Emerging data implicates the microbiome in RA pathogenesis. Mucosal sites exposed to a high load of bacterial antigens - such as the periodontium, lung, and gut – may represent the initial site of autoimmune generation. *Curr Opin Rheumatol*. 2014 January;26(1):101–107.
RA Susceptibility Loci
• Expression of two HLA-DRB1*04 alleles – causes an elevated risk for nodular disease, major organ involvement and surgery related to joint destruction\textsuperscript{2}

50% of the risk for development of RA is attributable to genetic factors\textsuperscript{1}

>80% of patients carry the epitope of the HLA-DRB1*04 cluster\textsuperscript{2}

• Arthritic synovial fibroblasts
  • Main source of destructive proteinases (e.g. matrix metalloproteinase and cathepsins)
  • Mediate pannus invasion of bone and articular cartilage
• Pannus-infiltrating macrophages contribute to joint degradation after their activation by increased cytokine and protease expression
• Complex interaction of immune modulators
  • Cytokines and effector cells) and signaling pathways
  • Responsible for joint damage that begins at the synovial membrane and covers most IA structures

• Synovitis
  • T cells, B cells, plasma cells, dendritic cells, macrophages and mast cells) influx and/or local activation of mononuclear cells; and by angiogenesis

• Synovial lining becomes hyperplastic, and the synovial membrane expands and forms villi.

• Osteoclast-rich portion of the synovial membrane, or pannus, destroys bone, whereas enzymes secreted by neutrophils, synoviocytes and chondrocytes degrade cartilage

Guidance Criteria for Diagnosis
2010 Rheumatoid Arthritis Classification Criteria

An American College of Rheumatology/European League Against Rheumatism Collaborative Initiative


American College of Rheumatology (ACR)

European League Against Rheumatism (EULAR)
Classification Criteria ≠ Diagnostic Criteria in Rheumatic Diseases

- Criteria are labeled as “classification” criteria NOT diagnostic criteria
- Influenced by age, gender, population, etc.
- Includes many more aspects than can be included in formal criteria
- May help clinical diagnosis by a rheumatologist
- For the purpose of classification, radiographs should only be performed
- Need to precisely define erosions (size, site, number)
- No exhaustive list of exclusions is defined
- Limits false positive classification

### 2010 ACR / EULAR Classification Criteria for Rheumatoid Arthritis (RA)

#### Joint Distribution (0 – 5 points)

<table>
<thead>
<tr>
<th>Points</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1 large joint</td>
</tr>
<tr>
<td>1</td>
<td>2 – 10 large joints</td>
</tr>
<tr>
<td>2</td>
<td>1 – 3 small joints (large joints not counted)</td>
</tr>
<tr>
<td>3</td>
<td>4 – 10 small joints (large joints not counted)</td>
</tr>
<tr>
<td>5</td>
<td>&gt;10 joints (at least one small joint)</td>
</tr>
</tbody>
</table>

#### Serology (0 – 3 points)

<table>
<thead>
<tr>
<th>Points</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>RF IgM (–) AND ACPA (–)</td>
</tr>
<tr>
<td>2</td>
<td>RF IgM (low positive) OR ACPA (low positive)</td>
</tr>
<tr>
<td>3</td>
<td>RF IgM (high positive) OR ACPA (high positive)</td>
</tr>
</tbody>
</table>

#### Symptom Duration (0 – 1 points)

<table>
<thead>
<tr>
<th>Points</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt; 6 weeks</td>
</tr>
<tr>
<td>1</td>
<td>≥ 6 weeks</td>
</tr>
</tbody>
</table>

#### Acute Phase Reactants (0 – 1 points)

<table>
<thead>
<tr>
<th>Points</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal CRP AND normal ESR</td>
</tr>
<tr>
<td>1</td>
<td>Abnormal CRP OR abnormal ESR</td>
</tr>
</tbody>
</table>

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**Interpretation of “SEROLOGY”**

- **Negative:** ≤ULN (for the respective lab)
- **Low positive:** >ULN but ≤3xULN
- **High positive:** >3xULN

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www.rheumatology.org/Portals/0/Files/ra_class_slides.pdf
Biomarkers for Assessing Rheumatoid Arthritis
Rheumatoid Factor (RF), the original Rheumatoid Arthritis Biomarker

- Introduced in the 1940’s¹
- Sensitivity 50 - 90%¹
- Low specificity¹
  - Present in other inflammatory diseases¹
  - Present in up to 25% of healthy individuals¹
- RF activity can be found in IgM, IgA, IgG, IgD, & IgE

### Rheumatoid Factor (RF) Assays Differ in Performance

<table>
<thead>
<tr>
<th>Method</th>
<th>Manufacturer</th>
<th>RF Isotype Detected</th>
<th>Average Sensitivity(^1)</th>
<th>Average Specificity(^1)</th>
<th>Average Positive Likelihood Ratio(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex Agglutination</td>
<td>various</td>
<td>IgM</td>
<td>61.7%</td>
<td>84.0%</td>
<td>9.9</td>
</tr>
<tr>
<td>Nephelometry</td>
<td>Beckman Immage 800, Siemens Vista</td>
<td>IgM, IgG, IgA*</td>
<td>72.9%</td>
<td>78.8%</td>
<td>6.7</td>
</tr>
<tr>
<td>EliA RF IgM</td>
<td>Thermo Fisher Scientific</td>
<td>IgM</td>
<td>63%</td>
<td>88.6%</td>
<td>10.3</td>
</tr>
<tr>
<td>Turbidimetric</td>
<td>Roche, Abbott, Siemens, and Beckman automated platforms</td>
<td>IgM, IgG, IgA*</td>
<td>86%</td>
<td>82%</td>
<td>High false positive rate because RF IgG is common in healthy individuals and other diseases</td>
</tr>
</tbody>
</table>

* Nephelometric and turbidimetric assays cannot differentiate the individual RF isotypes\(^2\)

• 2010 Criteria includes both RF IgM and CCP as equal options for serologic workup

• In contrast to a combined elevation of IgM and IgA RF, elevation of only one RF isotype may not be a significant risk factor for the development of RA

• Positivity of RF IgM and CCP correlates with a higher risk of RA

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- CCP antibodies appear in early stage rheumatoid disease
- Early diagnosis allows earlier treatment – early therapy slows disease progression\(^1\)
- Anti-CCP antibody and RFs of all isotypes predated the onset of RA by several years\(^1\)

Adapted from Figure 2. Rantapää-Dahlqvist et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum.*, 2003;48:2741–2749.
Sebbag, et al. demonstrate both autoantibodies are directed against citrullinated filaggrin.

First commercial ACPA test (1st generation cyclic citrullinated peptide/CCP test) introduced by Eurodiagnostica.

~12 million synthetic peptides screened for better antibodies - introduction of CCP2.

First fully automated CCP2 test introduced (ELIA CCP).

CCP3 / 3.1 prepared from limited set of peptides.

Young, et al. later detect anti-keratin antibodies.

Schellekens, et al. produce synthetic linear citrullinated peptides derived from human filaggrin.

Nienhuis, et al. identify anti-perinuclear factor autoantibody.

Anti-CCP2 assays have been the subject of investigations in more than 160 peer-reviewed articles, including comparisons against CCP3 and CCP3.1.

CCP2 offers the highest sensitivity when stratifying at 98% specificity.
Comparing Sensitivity & Predictive Value of Rheumatoid Arthritis Serology Tests

Average Sensitivity\(^1\)

- **EliA CCP2**: 69.2%
- **CCP 3.0**: 66.1%
- **CCP 3.1**: 57.4%
- **RF**: 29.9%

Stratified 98% Specificity

Predictive Value\(^2\)

- **CCP2 versus CCP3 Test Using Stratified 98% Clinical Specificity from 10 Studies**
  - **CCP2**: 91.1%
  - **CCP3**: 84.9%

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2 Wiik AS, et al. All you wanted to know about anti-CCP but were afraid to ask. *Autoimmun Rev* (2010), doi:10.1016/j.autrev.2010.08.009.
• CCP2 across studies continues to be the better performing APCA test¹

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¹ Grenmyr E, Sommarin Y. Anti-CCP2 is the anti-citrullinated protein antibody (ACPA) test with highest diagnostic value in rheumatoid arthritis. Poster no. 32, 11th Dresden Symposium on Autoantibodies, September 2013
### Primary Care Source

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>1.0%</th>
<th>1.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCP2 (EliA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCP3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity¹</td>
<td>74.0%</td>
<td>74.0%</td>
</tr>
<tr>
<td>Specificity¹</td>
<td>98.6%</td>
<td>89.6%</td>
</tr>
<tr>
<td>Population Size</td>
<td>500,000</td>
<td>500,000</td>
</tr>
</tbody>
</table>

### Evaluation Summary

<table>
<thead>
<tr>
<th></th>
<th>CCP2 (EliA)</th>
<th>CCP3.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPV</td>
<td>33.3%</td>
<td>6.7%</td>
</tr>
<tr>
<td>NPV</td>
<td>99.7%</td>
<td>99.7%</td>
</tr>
<tr>
<td>False +</td>
<td>7,425</td>
<td>51,480</td>
</tr>
<tr>
<td>False –</td>
<td>1,300</td>
<td>1,300</td>
</tr>
</tbody>
</table>

**CCP2 = 44,055 fewer false positives**

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** Rheumatology Advisor to Thermo Fisher Scientific.
## Specialty Practice Source

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>50.0%</th>
<th>50.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCP2 (EliA)</td>
<td>CCP3.1</td>
<td></td>
</tr>
<tr>
<td>Sensitivity(^1)</td>
<td>74.0%</td>
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### Evaluation Summary

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</thead>
<tbody>
<tr>
<td>PPV</td>
<td>98.0%</td>
<td>87.7%</td>
</tr>
<tr>
<td>NPV</td>
<td>79.1%</td>
<td>77.5%</td>
</tr>
<tr>
<td>False +</td>
<td>3,750</td>
<td>26,000</td>
</tr>
<tr>
<td>False –</td>
<td>65,000</td>
<td>65,000</td>
</tr>
</tbody>
</table>

**CCP2 = 22,750 fewer false positives**

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** Rheumatology Advisor to Thermo Fisher Scientific.
Effect of Prevalence and Specificity on Clinical Utility: Rheumatoid Arthritis

1% Prevalence Primary Care Population

- 7x Fewer False Positives
- Improved Clinical Utility
- 98% Stratified Specificity

Why Does Clinical Accuracy Matter?

- Missed diagnosis
- Untreated disease
- Deterioration

- Unnecessary referrals
- Additional lab testing
- Higher healthcare utilization and costs

- Over and misdiagnosis
- Emotional trauma
- Inappropriate therapy
### Example / Simple Referral Guide – Serologic Algorithm for Rheumatoid Arthritis (RA)

<table>
<thead>
<tr>
<th>1. Clinical Suspicion</th>
<th>Symptoms of early Arthritis (one or more Joints) (assess joint distribution and assign points)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>↓</td>
</tr>
</tbody>
</table>
| 2. Lab Diagnostics    | **SEROLOGY**
|                       | CCP + RF IgM + RF IgA  | **ACUTE PHASE REACTANTS**
|                       | Inflammatory Markers (ESR, CRP)                                                             |
|                       | ↓                                                                                             |
| 3. Differential Diagnosis | CCP (+) RF IgM (+) RF IgA (+)   | CCP (-) RF IgM (+) RF IgA (+)  |
|                       | CCP (-) RF IgM (+) RF IgA (+)  | CCP (-) RF IgM (-) RF IgA (-) |
|                       | Normal CRP + Normal ESR  | Abnormal CRP or Abnormal ESR |
|                       | ↓                                                                                             |
|                       | RA Very Likely   | RA Very Likely  | Possible RA  | RA Less Likely | Active RA | Active RA Possible |
|                       | Less Likely  | Less Likely |
|                       | (non-specific) |                                                     |
|                       | ↓                                                                                             |
| 4. Re-evaluation / Treatment | Referral or appropriate treatment                                                             | Re-evaluate clinical symptoms, imaging and other serologic markers |

Rheumatoid Arthritis (RA) Treatment
Paradigm shift: DMARD + biologic therapy for Rheumatoid Arthritis

2010 Treat to Target Recommendations
• Based on systematic literature review (19 full papers, 5 abstracts)

Early diagnosis
Early treatment
Damage prevention
Maintain structural integrity
Preserve function
AND
Quality of life
Rheumatoid Arthritis Goal: Gain Time for Treatments to Mitigate or Minimize Irreversible Destruction

Joint damage and functional disability

- Earlier diagnosis
  - Opportunity window for early and efficient treatment
  - Earlier conventional treatment
  - Earlier treatment with biologics

- Delayed diagnosis
  - Delayed conventional treatment
  - Delayed treatment with biologics
  - No treatment

The patient is a 32 year old female school teacher

Chief complaint: hand pain, neck stiffness, and worsening fatigue over the last 3 months

History
- No recent infections, trauma, or travel
- Pain is localized to knuckles of hand and pads of feet
- Mother has unknown form of crippling arthritis
- Ibuprofen helps some, and acetaminophen does not
- No rash, nodules, oral ulcers, alopecia, or chest pain
- Monogamous, no IV drug use

Differential Diagnosis
- Inflammatory arthritis (seronegative, psoriatic, rheumatoid, lupus)
- Chronic infectious arthritis (Lyme, GC, hepatitis)
- Fibromyalgia
• Normal complete physical examination with the exception of the hands*, which were tender when palpated over the 2\textsuperscript{nd} and 3\textsuperscript{rd} proximal interphalangeal joints

* representative
Case Continued – Diagnostic work up

- Imaging* – Marginal erosions were detected on radiographs
- Labs
  - CBC, Chem7, LFTs, urinalysis normal
  - ANA – Positive
  - Anti-dsDNA – Negative
  - Anti-CCP2 – Positive
  - RF IgM – Positive

* representative
Case – Wrap up

- **Diagnosis**
  - Inflammatory arthritis, rheumatoid

- **Treatment**
  - Steroids and methotrexate initially
  - Biologics or triple therapy if inadequate response

- **Follow-up**
  - Every 8-12 weeks in the beginning to assess therapies and monitor methotrexate labs
  - Physician visits may extend to every 3-6 months if well controlled
Summary
Disease and Disease Management

- RA is the second most common autoimmune disease
- It is a chronic, systemic inflammatory disorder affecting approximately 1.3 to 2.6M adults, and 294,000 children in the US\(^1\)
- The cause of RA is unknown and there is no cure
- New criteria are geared for diagnosing RA early for aggressive intervention with a goal of remission
- Early treatment can help prevent irreversible joint damage, premature death, disability, and improve quality of life\(^2\)
- Treatment costs are a burden, supporting the need to correctly identify patients

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Serologic Markers and Test Selection

- In adults anti-CCP may be present 12-14 years prior to onset of overt clinical symptoms
- In children, detection occurs closer to disease onset
- In combination with other clinical measures, testing for anti-CCP and RF isotypes produces a positive predictive value (PPV) near 100%, greater than the PPV of each test
- Not all CCP tests perform the same.
  - At a stratified specificity of 98%, the sensitivity of anti-CCP2 tests is superior to all other CCP tests (anti-CCP1, anti-CCP3 and 3.1 assays)
  - The higher CCP2 test specificity produces fewer false positive results, which can reduce inappropriate referrals, inappropriate treatments, and the associated costs

2 Grenmyr E, Sommarin Y. Anti-CCP2 is the anti-citrullinated protein antibody (ACPA) test with highest diagnostic value in rheumatoid arthritis. Poster no 32, 11th Dresden Symposium on Autoantibodies, September 2013.
Q&A