

## DIAGNOSTIC PERFORMANCES OF ANTIBODIES AGAINST CARBAMYLATED PROTEINS IN U.S. RHEUMATOID ARTHRITIS AND OTHER AUTOIMMUNE RHEUMATIC DISEASES

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# ABSTRACT

## PURPOSE

Recently, antibodies directed against carbamylated antigens (anti-CarP antibodies) were identified in rheumatoid arthritis (RA). Studies have established the predictive and prognostic value of this antibody system. As most of the previous reports described the performance characteristics of anti-CarP assay in European populations using a two-step research assay, we analyzed the performances characteristics of a single step anti-CarP assay in US based population of patients with autoimmune rheumatic diseases.

## METHODS

Anti-CarP antibodies were measured using fetal calf serum based single step assay (research use only, Inova Diagnostics). The analytical and diagnostic performances of the anti-CarP assay were established using a biobank of specimens collected from consented subjects and controls (640 RA patients fulfilling the 1987 or 2010 criteria, 197 normal healthy volunteers and 636 other autoimmune disease patients). The median age of RA subjects at diagnosis was 60 years, and it ranged from 41 to 55 years in disease controls. Mean disease duration in RA was 12 years, versus 3 to 12 years in controls. Anti-citrullinated protein antibodies (ACPA) and rheumatoid Factor (RF-IgM) status were tested using EliA CCP and RF IgM assays, respectively (ThermoFisher, Upsala Sweden). Diagnostic performances in distinguishing RA from other control groups were established using sensitiv-

## RESULTS

The intra-assay and inter-assay variations of the Anti-CarP ELISA were 7.0% and 11%, respectively. Anti-CarP antibodies, anti-CCP antibodies and RF-IgM were present in 34%, 66% and 67% of RA subjects, respectively. The overall specificity of anti-CarP antibodies was 78%. When stratified by anti-CCP status, the prevalence of anti-CarP antibodies was 42% and 16% among anti-CCP positive and negative anti-CCP RA subjects, respectively, a finding consistent with previous studies (Table 1). The prevalence of anti-CarP antibodies in various disease conditions ranged from 5.9% to 30%, thereby yielding specificities ranging from 70% (systemic lupus erythematosus, SLE) to 94% (primary fibromyalgia). Diagnostic odds ratios (DOR) for RA versus the different control groups ranged from 1.2 to 8.2.

## CONCLUSION

These data replicate previous studies and support the notion that anti-CarP antibodies are helpful in the setting of ACPA negative RA subjects. However, clinicians should be aware of the frequent elevation of the marker in other autoimmune rheumatic diseases, particularly SLE.

## OBJECTIVE AND METHODS

- To analyze the performance characteristics of a single step anti-CarP assay in US based population of patients with autoimmune rheumatic diseases.
- Anti-CarP antibodies were measured using fetal calf serum based single step assay (Inova Diagnostics).
- The analytical and diagnostic performances of the anti-CarP assay were established using a biobank of specimens collected from consented subjects (640 RA subjects fulfilling the 1987 or 2010 criteria, 197 normal healthy volunteers and 636 other disease subjects).

- Anti-citrullinated peptide antibodies (ACPA) and rheumatoid factor IgM (RF-IgM) status were tested using EliA CCP and RF-IgM assays, respectively (ThermoFisher).
- Analytical characteristics were analyzed using coefficient of variation or Deming linear regression plus correlation analysis.
- Diagnostic performances in distinguishing RA from other control groups were analyzed using sensitivity, specificity, likelihood ratio and diagnostic odds ratio.

# RESULTS

Table 1 Analytical performances of anti-CarP assay

| Analytical Characteristics                        | Estimate    |
|---------------------------------------------------|-------------|
| Intra assay precision [CV%]                       | 7           |
| Inter assay precision [CV%]                       | 11          |
| Inter-lab concordance [slope, R <sup>2</sup> ]    | 0.96 [0.92] |
| Serum/plasma concordance [slope, R <sup>2</sup> ] | 1.15 [0.96] |
| Inter-lot variability [R <sup>2</sup> ]           | 0.97        |

Figure 1: Anti-CarP levels in RA versus other ARDs

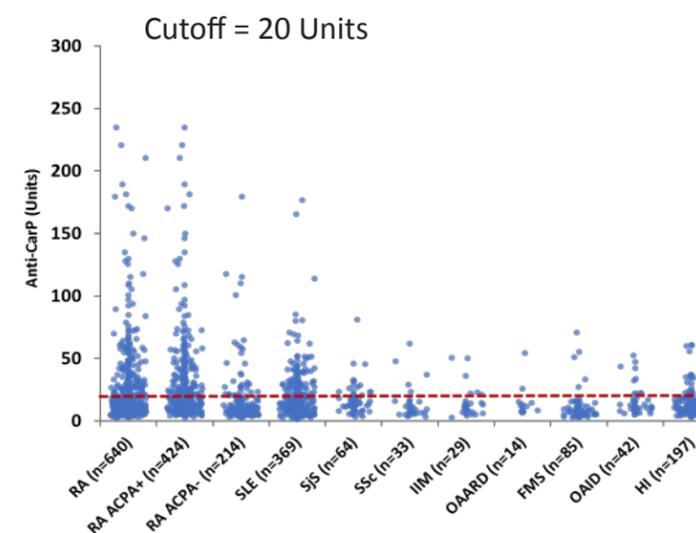


Table2: Anti-CarP sensitivity in RA by ACPA status.

|               | Sensitivity |
|---------------|-------------|
| Overall       | 34%         |
| ACPA negative | 42%         |
| ACPA positive | 16%         |

Table3: Anti-CarP Specificity and performances by control group

| Control groups                     | Anti-CCP+ % | RF-IgM+ % | Anti-CarP+ % | Specificity % | LR+ [95% CI]   | LR- [95% CI]     | DOR [95% CI]   |
|------------------------------------|-------------|-----------|--------------|---------------|----------------|------------------|----------------|
| Systemic lupus erythematosus       | 4.1%        | 13%       | 30%          | 70%           | 1.1 [0.94,1.4] | 0.94 [0.87,1.0]  | 1.2 [0.91,1.6] |
| Sjogren's syndrome                 | 4.7%        | 41%       | 27%          | 73%           | 1.3 [0.83,1.9] | 0.90 [0.77,1.1]  | 1.4 [0.79,2.5] |
| Autoimmune thyroid/hepatitis       | 2.4%        | 7.1%      | 26%          | 74%           | 1.3 [0.77,2.2] | 0.90 [0.74,1.1]  | 1.4 [0.71,2.9] |
| Idiopathic inflammatory myopathies | 3.5%        | 6.9%      | 21%          | 79%           | 1.6 [0.79,3.4] | 0.84 [0.69,1.0]  | 2.0 [0.78,4.9] |
| Systemic sclerosis                 | 6.1%        | 27%       | 15%          | 85%           | 2.2 [0.99,5.0] | 0.78 [0.67,0.91] | 2.9 [1.1,7.5]  |
| Other autoimmune diseases          | 0%          | 14%       | 14%          | 86%           | 2.4 [0.65,8.6] | 0.77 [0.62,0.96] | 3.1 [0.68,14]  |
| Primary fibromyalgia               | 1.2%        | 1.2%      | 5.9%         | 94%           | 5.7 [2.4,14]   | 0.70 [0.65,0.76] | 8.2 [3.3,20]   |
| Normal healthy individuals         | 0.50%       | 6.2%      | 14%          | 86%           | 2.4 [1.7,3.4]  | 0.77 [0.71,0.84] | 3.1 [2.0,4.7]  |

## CONCLUSION

- Anti-CarP is helpful in the setting of ACPA negative RA.
- Clinicians should be aware of the frequent elevation of the marker in other autoimmune rheumatic diseases.