

# Anti-Cyclic Citrullinated Peptide in Preclinical Rheumatoid Arthritis. Food for Thought



Citrullinated (cit-) epitope detection is an evolving science with different substrates being proposed continuously<sup>1-4</sup>. Given all the published enthusiasm with anti-cyclic citrullinated peptide (CCP), why does the test need to be improved? It is assumed that the cit-epitopes targeted in cit-peptides/proteins are all detected by anti-CCP and are invariable in the various individuals during the phases of disease. That premise may be incorrect.

Recent objective critical evaluation of anti-CCP is severe. Its added value in rheumatoid arthritis (RA) diagnostics, over and above previously existing clinical and laboratory tools, is deemed marginal<sup>5</sup>. The huge anti-CCP literature contains recurring inconsistencies suggesting that authors are using the same test to measure different things in a very heterogeneous disease. Moreover, it is difficult to understand how a test can be associated with more severe evolution in early RA<sup>6</sup> and also be positive without arthritis for 10 to 15 years before people get sick<sup>7-10</sup>. How does one reconcile that?

The solution of Chibnik, *et al* in this issue of *The Journal*<sup>7</sup> is to pay more attention to titers of anti-CCP to explain the transition from pre-RA to RA. Just having anti-CCP is not sufficient; also important is how much one has. The higher the titer, the shorter the interval to disease onset! Titers rise steadily until disease onset and then stabilize, as is the current experience in established disease. That the titers rise near RA onset has already been suggested<sup>8</sup> and is convincingly confirmed here<sup>7</sup>. What does that mean exactly? Either the disease manifests itself only when a sufficient level of autoantibody is reached (quantitative change) or when a given autoantibody emerges whose specificity is associated with disease onset (qualitative change). The 2 explanations are not mutually exclusive, as maturation of an immune response is accompanied by rising titers and epitope dominance.

If the quantitative interpretation is correct, setting a cut-off becomes a double statistical task. Two cutoffs are needed to distinguish normal or non-RA versus RA predisposition versus RA disease. If such fine distinctions are ever possible, the clinician will be more comfortable in reassuring the patients with low titers, as they may never develop

RA or will do so only in 10 to 15 years. On the other hand, he will observe more carefully and maybe treat more aggressively those with rising (high) titers, as these patients are going to get the disease sooner rather than later. Given the prevalence of the HLA shared-epitope alleles and their link to the immune response to citrullinated epitopes<sup>11</sup>, there may be a significant number of people with low to mid-range levels of these autoantibodies. Most will never get sick. How many and who will go on to develop disease cannot be answered by the retrospective study of pre-RA cohorts<sup>7-10</sup>. That question can be answered only prospectively, as in the ongoing study in North American Natives (NAN), which involves RA patients, their healthy first-degree relatives, and healthy unrelated controls<sup>12</sup>. The risk of developing RA in NAN is very high because of the concentration of the permissive gene pool and the harsh environmental challenges. The rate of anti-CCP-positive findings in the 3 groups mentioned is also high at 79%, 20%, and 9%, respectively. Anti-CCP titer differences between the 3 groups were not obviously skewed but will have to be reassessed. Two sets of qualitative differences were observed (see below). Similarly, in early undifferentiated arthritis, anti-CCP titers did not also seem to be predictive of who would develop RA a year later<sup>13</sup>.

If a qualitative autoantibody change occurs near disease onset, that would be easier to resolve with a complementary test to identify the most likely culprit hiding in the proprietary CCP2 mixture<sup>14</sup>. That general need was recognized independently after a recent overview of the anti-CCP literature<sup>5</sup>. Such a test already exists. In the NAN cohort<sup>12</sup>, the rate of anti-Sa (cit-vimentin) was 51%, 0%, and 0% in the groups mentioned. Strictly disease related! Only one anti-CCP-positive healthy person (a relative) developed a RA-like arthritis during the first 3 years of the study. That patient seroconverted to anti-Sa-positive just before disease onset. Nobody else seroconverted or developed disease! Another qualitative aspect was measured in that cohort. RA patients used significantly more anti-CCP immunoglobulin isotypes than the anti-CCP-positive healthy relatives and unrelated controls. The anti-Sa-positive RA patients were

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largely responsible for the extra anti-CCP isotype usage. Thus, RA patients constituted at least 2 different groups of patients who were anti-CCP-positive: those behaving like healthy people (very little isotype usage) were anti-Sa-negative and those behaving like true RA (more usage) were anti-Sa-positive. No similar data exist for the other cit-protein autoantigens. That extra usage of anti-CCP2 isotypes was also seen in patients with early undifferentiated arthritis, but only in those who developed RA after 1 year<sup>13</sup>. Anti-Sa was not tested in that cohort.

As space does not allow discussion of other legitimate candidates here, I will only summarize why anti-Sa should be the logical complement to anti-CCP<sup>15</sup>. Citrullinated vimentin<sup>2,14</sup> is generated during apoptosis, like most autoantigens<sup>16</sup>. Cit-vimentin peptides have preferential interaction with shared-epitope HLA alleles<sup>11</sup>. RA pannus is loaded with natural and altered cleavage products of cit-vimentin isoforms<sup>2,17</sup>, some of which may be mutated neoantigens produced during inflammation<sup>3</sup>. The qualitative explanation reflects an ongoing immune response and recoups the quantitative one, as anti-Sa-positive means higher anti-CCP titer<sup>15</sup>.

The overall serological situation can be compared to that of systemic lupus erythematosus (SLE). I submit that anti-CCP is to RA what antinuclear antibody is to SLE. Both are screening tests almost always present but not *ipso facto* diagnostic. Indeed, both are mostly useful for their negative predictive value. When they are positive and disease-associated, one or more dominant specificities is identified. I posit that anti-Sa is to RA what anti-dsDNA is to SLE. Indeed, anti-Sa has emerged as strictly linked to disease or disease onset<sup>2,12</sup>. It is a better tool in early arthritis to predict persistent and severe disease than anti-CCP and rheumatoid factor put together<sup>18,19</sup>. Finally, anti-Sa titers vary closely and reliably in individual patients with disease activity and adequate response to some treatments<sup>19,20</sup>, like a pathogenic antibody should, and unlike anti-CCP. My prediction is that anti-Sa would best separate patients at high and low risk in the 2 anti-CCP-positive preclinical groups<sup>7</sup>. Unfortunately, none of the sera used in military or American female cohorts could be made available for anti-Sa testing. That would have either challenged or confirmed, and in any case clarified, the conclusions of the authors on how to interpret and deal clinically with people with low versus high titers.

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