

The humoral immune response to citrullinated proteins in patients with rheumatoid arthritis (RA):

Genetic, clinical, technical, and epidemiologic aspects

Allan Wiik, Department of Autoimmunology, Statens Serum Institut, Copenhagen, Denmark

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Early synovitis in RA



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Clinical aspects

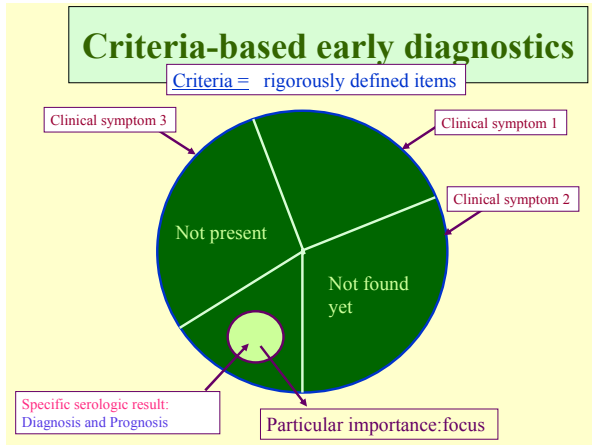
- The disease progresses quickly from a predominantly immunoinflammatory to a destructive phase where established pannus erodes bone, tendons and joint capsule.
- The "therapeutic window" to get control of the early phase is very short (few months), and later therapy has little or no effect on the destructive phase.

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Early diagnosis

<ul style="list-style-type: none">-Clinical history-Manifestations-Physical examination<ul style="list-style-type: none">-Radiology-Specialist evaluations-Histopathology-Immunopathology	<ul style="list-style-type: none">-Laboratory tests to look for inflammation-Immunoglobulin levels-Complement activation-Autoantibodies
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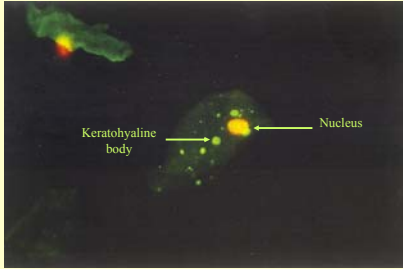
Serological markers in RA

- Rheumatoid factors (Waler's sheep red blood cell agglutination test) (1939)*
- Rheumatoid factors (Wager's Streptococcus agglutination test) (1950)*
- Anti-RA 33 antibodies (1995)*
- Anti-calpastatin antibodies (1995)
- (Anti-p68/-BIP) (1998)

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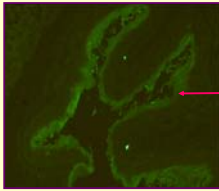
* Rather non-specific tests for RA

Anti-perinuclear factor



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Rheumatoid arthritis



Esophagus tissue

Antibodies to the dying epithelium

An autoimmune response to modified (citrullinated) proteins

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Related serological markers for RA

- Anti-perinuclear factor (1964) □
- Anti-keratin antibodies (1979) □
- Anti-filaggrin antibodies (1993) □
- Anti-Sa (1995) □
- Anti-citrullinated peptides (1998) □

□=citrullinated antigens

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Autoantibodies in RA which are related to anti-citrullinated protein antibodies

Antibody system:

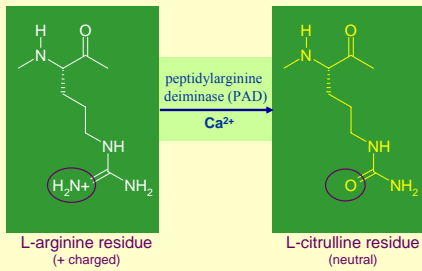
Citr.antigens:

- Anti-perinuclear factor
- Anti-keratin
- Anti-Sa
- Anti-(pro)filaggrin
- Anti-citrullinated peptides (a-CCP1) (a-CCP2)
- Anti-citrullinated proteins (human fibrinogen peptides)

- Trichohyalin in mouth mucosal cells
- Keratin in oesophagus mucosal cells
- Filaggrin in epithelium
- Vimentin in MΦs
- Fibrin in RA joints
- Alpha-enolase
- Collagen I and II

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Citrullination of arginines in proteins



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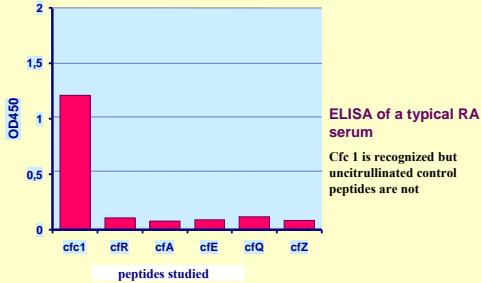
Peptide cfc1 and control peptides

cfC1	SHQESTXGRSRGRSGRSGS	X=Citrullin, aa 306-324 in filaggrin
cfR	SHQESTRGRSRGRSGRSGS	R=arginine
cfA	SHQESTAAGRSRGRSGRSGS	A=alanine
cfE	SHQESTEGRSRGRSGRSGS	E=glutamic acid
cfQ	SHQESTQGRSRGRSGRSGS	Q=glutamine
cfZ	SHQESTZGRSRGRSGRSGS	Z=ornithine

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Schellekens et al., J Clin Invest 1998, 101:273-281

Binding of RA antibodies to cfc1 and control peptides in ELISA



ELISA of a typical RA serum
Cfc 1 is recognized but uncitrullinated control peptides are not

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Citrullinated peptides of filaggrin
Amino acids 306-324

cf0	SHQESTRGRSRGRSGSGS
cfc1	SHQESTXGRSRGRSGSGS
cfc2	SHQESTRGXSRGRSGSGS
cfc3	SHQESTRGRSXGRSGSGS
cfc4	SHQESTRGRSRGXSGSGS
cfc5	SHQESTRGRSRGSGXSGS
cfc6	SHQESTXGXSRGRSGSGS
cfc7	SHQESTXGRSXGRSGSGS
cfc8	SHQESTXGRSRGXSGSGS
cfc9	SHQESTXGRSRGSGXSGS

Schellekens et al., J Clin Invest 1998, 101:273-281

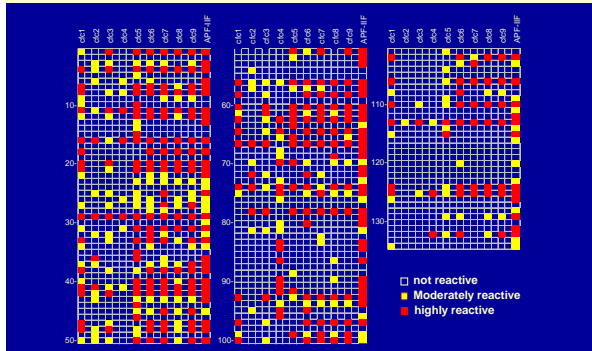
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Reactivity of RA sera with citrullinated peptides

- Practically every patient serum was found to react differently with the various citrullinated peptides, and thus no particular peptide was found to carry a dominant epitope.
- Very often several citrullinated peptides were recognized by each RA serum.
- Hence, the first assays used several citrullinated peptides as antigen source.

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Each RA serum has a unique pattern of recognition of citrullinated peptides



Cyclic Citrullinated Peptide: CCP

cfc1-cyc **HQCHQESTXGRSRGRRCGRSGS**

Cyclisation of the peptide enhances its recognition by RA autoantibodies

Schellekens et al. Arthritis Rheum 2000, 43:155-163

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How do synovial antigens become modified: arginine to citrullin?

- Peptidyl-arginine deiminase enzymes (PAD2 and PAD4) are richly represented in monocytes, macrophages and neutrophils
- When these cells undergo **apoptosis** Ca⁺⁺ ions are transferred into the cells and activate PADs
- Ca⁺⁺ concentration in normal cells ~10⁻⁷M
- Threshold for PAD enzyme activity ~10⁻⁵M
- PAD enzymes most likely act on the enzyme-containing cells themselves (Monos, MΦs, PMNs)

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Citrullinated proteins are richly represented in human inflamed synovia

- Citrullinated proteins are present both intra- and extracellularly in synovial membranes from patients with various forms of arthritis. Vossenaar E et al. Arthritis Rheum 2004
- Only RA patients produce significant amounts of anti-citrullin peptide antibodies.
- Citrullinated peptides fit perfectly into the P4 pocket of shared epitope on APC, but the homologous arginine peptides do not. Hill JA et al. J Immunol 2003

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Candidate citrullinated proteins in Non-RA and RA tissues.

- **Filaggrin** in senescent human epithelial cells Schellekens GA et al. J Clin Invest 1998, Girbal-Neuhauser E et al. J Immunol 1999.
- **Vimentin** in macrophages (= Sa antigen) Vossenaar E et al. Arthritis Res Ther 2004.
- **Fibrin in the joint** Masson-Bessiere C et al. J Immunol 2001, Chapuy-Regaud S. et al. J Immunol 2005, Vossenaar E et al. Arthritis Rheum 2004.
- **Type I and II collagens** Koivula MK et al. Ann Rheum Dis 2005.
- **Alpha-enolase** Kinloch A et al. Arthritis Res Ther 2005

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Association between anti-CCP production and shared epitope

HLA DR typing and anti-CCP2 antibodies were studied in 268 RA patients from an early arthritis clinic cohort in Leiden. Radiographic disease progression was measured over 4 years. Carriers of shared epitope DRB1 alleles were more commonly anti-CCP positive than non-carriers (OR 13.3) and also showed the most pronounced radiographic progression. (van Gaalen FA et al.: Arthritis Rheum 50:2113-2121, 2004).

Shared epitope-encoding alleles are associated with anti-CCP production, not with RA. (Huizinga T et al.: Arthritis Rheum 52:3433-38, 2005).

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Local production of anti-filaggrin antibodies in the rheumatoid pannus.

- Anti-filaggrin (AFA) antibodies were studied at equal IgG concentrations in paired syn. membrane extracts, syn.fluids and sera from 31 RA patients.
- No difference was found between syn.fluid and serum levels of AFA, whereas extracts contained 7.5 fold higher AFA levels.
- Long-term cultures of syn. membrane explants showed *de novo* synthesis of AFA for several weeks. Masson-Bessiere C et al. Clin Exp Immunol,2000.

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Studies done on anti-citrulline antibodies are difficult to compare

- The citr. antigens are very different
- The cut-offs used are different
- The RA populations studied are different
- The differential diagnostic populations studied for comparison with RA patients are different
- Some studies include undifferentiated arthritis, palindromic syndrome, RF+JRA, RF+psoriatic arthritis etc. all of which may actually become RA.

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Commercially available assays for anti-CCP (now CCP2)

The most recent version of anti-CCP2 ELISAs from Euro-Diagnostika, Axis-Shields and INOVA give identical results (identical coating of peptide)

Beads, multiarrays, and ELIA solid phase assays have been coated in basically the same way and are thus expected to give identical results.

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Nosographic sensitivity (sensitivity in RA patients)

- Anti-CCP2, anti-filaggrin and APF show very similar sensitivities:
 - at diagnosis < 6 months: around 50%
 - at diagnosis 1 year: around 60%
 - at diagnosis >2 years: around 70%
- AKA and anti-Sa: usually show lower sensitivity than the above methods

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Diagnostic specificity

- When compared with healthy controls the specificity is around 99% for anti-CCP2, anti-filaggrin, anti-citrullinated human fibrin peptide, and anti-Sa, while the specificity is somewhat lower for APF and AKA
- When compared with different immuno-inflammatory CTDs the specificity is around 95 to 98%
- 1-3% of acute and chronic infectious disease sera are anti-CCP2 positive (at low levels)

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Prediction of erosive disease

- Kroot EJ et al.: Arthritis Rheum 43:1831-1835,2000
- Meyer O et al.: Ann Rheum Dis 62:120-126,2003
- Vencovsky J et al.: Ann Rheum Dis 62:427-430,2003
- Jansen et al.: J Rheumatol 30:1691-1695,2003
- Van Gaalen et al.: Arthritis Rheum 50:2113-2121,2004
- Rönnelid J et al.: Ann Rheum Dis. 65:453-8,2005
- Hueber W et al.: Arthritis Rheum 52:2645-2655,2005.
- Berglin E et al: Ann Rheum Dis 65:453,2006.
- and many others!!

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Summary of data on anti-CCP2 in adult rheumatoid arthritis 2006

	No:	Pos:	%:
Rheumatoid arthritis	7769	5465	70%
-early	2936	1752	60%
-established	4833	3713	77%
Healthy donors	2855	26	1%
Non-RA controls	7978	420	1%

vanVenrooij W.J.et al, Autoimm Rev 2006

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Prediction of RA in patients with undifferentiated arthritis (UA)

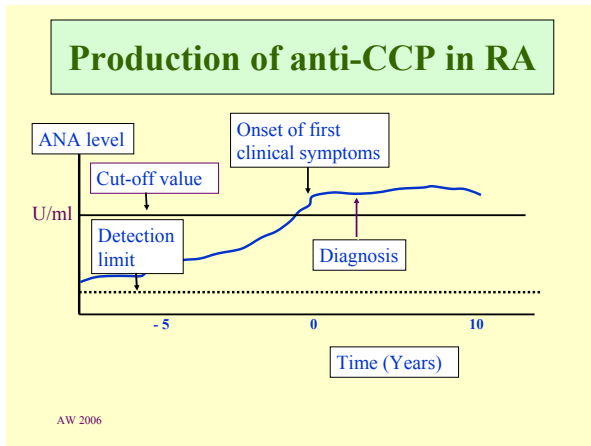
- RF and anti-CCP1 in patients with undifferentiated arthritis predicted later onset of RA with 55% sensitivity and 97% specificity. (Jansen A. et al., 2002: J Rheumatol 29:2074-2076,2002)
- 318 patients with undifferentiated arthritis followed for 3 years. 40% of these developed RA. 93% of anti-CCP2 positive and 25% of anti-CCP2 negative patients developed RA. (Van Gaalen F et al.: Arthritis Rheum 50:709-715,2004)

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Production of anti-citrullin antibodies before onset of clinical RA symptoms.

- Anti-CCP1 were found in 39% of donors at a median of 5.3 years before onset of RA symptoms. Nielen M et al., Arthritis Rheum 50:380-386,2004.
 - Anti-CCP2 were found in 25% of donors 1.5 to 9 years before onset of the first RA symptoms. Rantapää-Dahlqvist S et al. Arthritis Rheum 48:2741-2749,2003.
- Anti-CCP were developed together with increased CRP and sPLA2 and IgM RF in blood donors developing RA. Nielen MM et al. Ann Rheum Dis 2005.

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Decrease of anti-CCP after therapy?

- Decrease was seen after 6 months infliximab/ MTX in parallel with IgM RF and clinical improvement.
Alessandri C et al.: Ann Rheum Dis 63:1218-21,2004.
- 17 RA patients followed over many years of therapy showed decrease of anti-CCP during remission and increases with disease activity in parallel with CRP and ESR.
Aotsuka S et al.: Clin Exp Rheum 23:475-81,2005.

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Decrease of anti-CCP after therapy?

- 5 year follow-up of early RA cohort showed that anti-CCP is a stable phenotype. The first year anti-CCP levels fell after sulfasalazine therapy, not other DMARDs. After that time little changes were seen.
Rönnelid J et al.: Ann Rheum Dis 64:1744-9,2005.
- 52/90 RA patients who failed DMARD treatment were given etanercept for 3 months. Mean anti-CCP levels fell 31% and IgM RF 36% during this time in parallel with CRP and disease activity.
Chen HA et al.: Ann Rheum Dis 65:35-9,2006.

Prediction of RA in palindromic rheumatism ??

- Salvador G et al. showed that 56% of **palindromic rheumatism** patients (pre-RA patients??) harboured anti-CCP. This compared with a frequency of 55% positive sera in early RA patients studied simultaneously. In contrast, only 2.5% of spondylarthropathy patients sera were positive. (Rheumatology (Oxford) 42:972-975,2003)

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Anti-CCP in juvenile chronic arthritis

- Anti-CCP shown in only 2 patients among 109 JCA patients from Slovenia and Italy, 1 polyarticular and 1 oligoarticular onset JCA. Avcin T et al.: Ann Rheum Dis 61:608-611,2002.
- Anti-CCP found in 5% of JCA patients from The Czech Republic. Hromadnikova I et al. Autoimmunity,2002.
- Anti-CCP shown in 6 of 59 JCA patients from Hong Kong, 4 of which were RF-positive with a polyarticular onset and 1 was oligo-articular onset JCA. All had erosive JCA. Kwok S et al.: Scand J Rheumatol 34:359-366,2005.

Anti-CCP in Psoriatic arthritis

- Anti-CCP found in 7% of 160 patients with PsA and 1 of 146 Swedish non-arthritis patients. Alenius GM et al. Ann Rheum Dis, 2005
- Anti-CCP found in 5.6% of 126 English patients with PsA. Korendowych E et al. Rheumatology,2005.
- Anti-CCP found in 16% of 102 PsA patients from Italy. Bogliolo L et al. J Rheumatol 2005.
- Anti-CCP found in 8% of 192 Belgian PsA patients, **using a chosen specificity level of 98.5% for RA.** Van der Cruyssen B et al. Ann Rheum Dis 2005.

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Use for differential diagnostics towards RA-mimicking conditions

Towards erosive polyarthritis in SLE:

-Mediawake R et al.: Ann Rheum Dis 60:67-68,2001

Towards hepatitis C-associated polyarthritis

-Zuckerman E et al.: BioDrugs 15:573-584,2001

-Leone N et al.: J Med Virol 66:200-2003,2002

-Olivieri et al.: Rheum Dis Clin North Am 29:111-122,2003

All studies showed a high differential diagnostic specificity for RA!

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Conclusions

- Anti-citrullinated protein/peptide antibodies (ACPA) are very specific markers for RA.
- Cyclic citrullinated peptide 2 (CCP2) acts as a sensitive artificial mimotope for ACPA antigens in solid phase assays.
- Anti-CCP antibodies are present very early in disease, sometimes before clinical onset.
- Anti-CCP levels decrease with remission induction and increase with disease exacerbation.

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Conclusions ctd.

- High levels of anti-CCP antibodies are prognostic for an erosive disease course, not only in adult RA.
- Anti-CCP antibodies prevail in RA patients carrying the HLA-DR4 shared epitope, most of which are RF-positive.
- Several environmental factors influence the onset of anti-CCP positive RA (tobacco smoking, coffee consumption, alcohol consumption, exercise).

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Some references

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- Girbal-Neuhauser E et al.: J Immunol 162:585,1999
- Després N et al.: J Rheumatol 21:1027,1994
- Schellekens GA et al.: Arthritis Rheum 43:155,2000
- Goldbach-Mansky R et al.: Arthritis Res 2:236,2000
- Bizzaro N et al.: Clin Chem 47:1089,2001
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- Van Gaalen FA et al.: Arthritis Rheum 50:709,2004
- Van Gaalen FA et al.: Arthritis Rheum 50:2113,2004
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- Kastbom A et al. Ann Rheum Dis 63:1085,2004.
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