Inflammatory Arthritis:

What can be learned from rheumatoid arthritis as a prototype autoimmune disease?

Leiden, The Netherlands

- The term disease broadly refers to any condition that impairs normal function.
- Classification criteria enable the stratification of groups of individuals in order to standardize recruitment into clinical trials and related studies, and provide the basis for a common approach to disease definition that can be used to compare across studies and centers

How to arrive at groups of patients with a smilar pathogenetic mechanism?

In RA recently new criteria: Why?

- ***To minimise continued joint damage and subsequent disability in RA, several studies have demonstrated the importance of:***
  - *Early diagnosis*
  - *Early, aggressive intervention*
- ***One-third of future patients cannot be classified using the established 1987 criteria at presentation***
- ***PROMPT-study shows that MTX-treatment of UA is beneficial[1]***

1. van Dongen H, et al. Arthritis Rheum 2007;56:1424–32.

Probability is Implemented in the 2010 Criteria[1]

ACR 1987 criteria[2]

1. Morning stiffness
2. Arthritis of 3 or more joint areas
3. Arthritis of hand joints
4. Symmetric arthritis
5. Rheumatoid nodules
6. Serum rheumatoid factor
7. Radiographic changes

ACR/EULAR 2010 criteria[3]

1. Joint involvement
   - 1 medium-large joint (0)
   - 2–10 medium-large joints
   - 1–3 small joints (large joints not counted) (2)
   - 4–10 small joints (large joints not counted (3)
   - >10 joints (at least one small joint) (5)
2. Serology
   - Negative RF and negative ACPA (0)
   - Low positive RF or now positive ACPA (2)
   - High positive RF or high positive ACPA (3)
3. Acute phase reactants
   - Normal CRP and normal ESR (0)
   - Abnormal CRP or abnormal ESR (1)
4. Duration of symptoms
   - <6 weeks (0)
   - ≥6 weeks (1)

2010 criteria for RA

External Validation

	2010+	2010-
1987 +	644	82
1987 -	455	1077

- *68% of the patients that fulfilled 1987 criteria during the first year did already fulfill 2010 at baseline*
- *18% of pts with clear other diagnoses also fulfilled 2010 criteria*

Van der Linden et al. Arthritis Rheum 2011;63:37–42.

Performance in early arthritis

	Outcome Measure								
	MTX-initiation			***DMARD-initiation***			***5-year Persistency***		
Criteria Set	***Sens.***	***Spec.***	***AUC***	***Sens.***	***Spec.***	***AUC***	***Sens.***	***Spec.***	***AUC***
1987 ACR Classification Criteria	*0.61*	*0.74*	***0.67***	*0.54*	*0.87*	***0.71***	*0.53*	*0.75*	***0.61***
2010 ACR/EULAR Classification Criteria	*0.84*	*0.60*	***0.72***	*0.74*	*0.74*	***0.74***	*0.71*	*0.65*	***0.65***

Van der Linden et al. Arthritis Rheum 2011;63:37–42.

What can be learned from RA as a prototype autoimmune disease?

- Classification criteria classify a mix of patients
- Does that matters in intervention trials?

IMPROVED-study n=610

- Multicenter single blind randomized clinical trial
- Inclusion: recent onset RA and UA
- Treatment steered at remission: DAS <1.6
- Tapering to drugfree if remission
- First four months all MTX 25 mg/wk + Prednisone 60 mg/day tapered with 10 mg per week to 7.5 mg/day

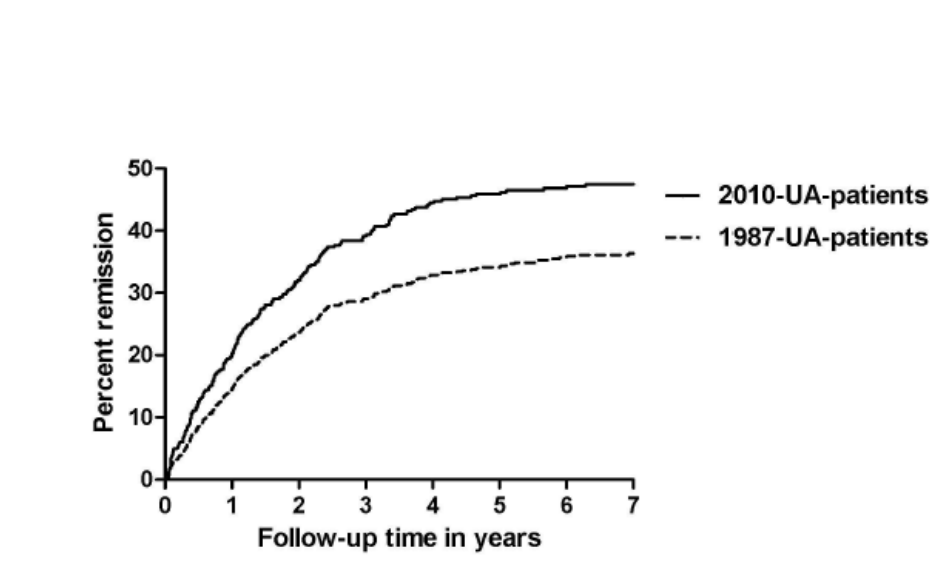
Number of patients that fullfill respective criteria

	RA (%DAS<1.6)		UA (%DAS<1.6)	
1987	364	(58%)	246	(66%)
2010	479	(61%)	122	(65%)

- *Remission rates with same treatment similar in RA 87 & RA 2010*
- *Remission rates in RA & UA differ*
- *RA & UA differ in risk factors for spontaneous remission (more ACPA-negative patients in UA group)*
- *What is the evaluation of the UA group 2010 versus 87?*

Evaluation undifferentiated arthritis

- 2010-UA-patients (n=776) had milder baseline characteristics than 1987-UApatients (n=1,166).
- 10% of 2010-UA-patients had ACPA versus 25% of the 1987-UA
- During follow-up, 24% of the 2010-UA-patients fulfilled the 1987 RA-criteria compared to 32% of the 1987-UA-patients.
- The 2010-UA-patients started less frequent DMARD-therapy and reached more frequent sustained DMARD free remission



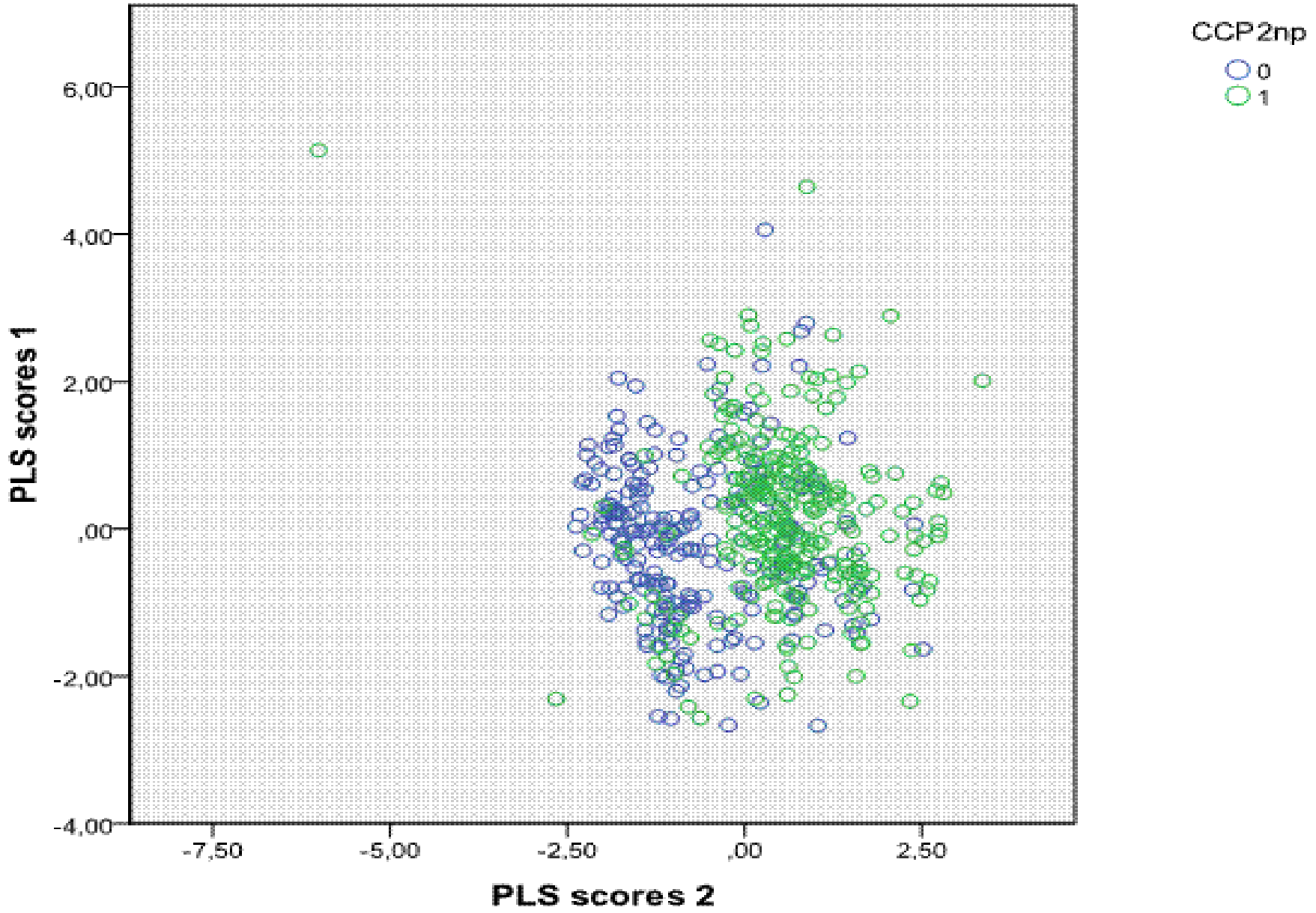
What can be learned from RA as a prototype autoimmune disease?

- Classification criteria classify a mix of patients
- Matters in intervention trials given different clinical course of different subsets

Statistical methodology to find subsets

- Principal Component Analysis
  PCA makes use of overlap of variables and combines variables which frequently occur together into components. Reduces number of variables explaining data. The components resulting from the PCA are based on the observed variance and not on pre-defined hypotheses, making that this technique is suitable for exploring unknown relationships between variables
- Partial Least Squares Regression
  PLS does an analysis which is comparable to PCA, but which has the advantage that it makes also use of outcome measures
- Clusteranalysis
  Evaluates grouping of patients: given all characteristics available of the patients, it evaluated which patients resemble each other.

PLS with clinical variables and outcome: two major factors (no ACPA included)



Same analysis for CCP-negative patients

PCA Resulted in 8 components

Two main components plotted against each other

- No indication for clustering

PLS identified 2 factors

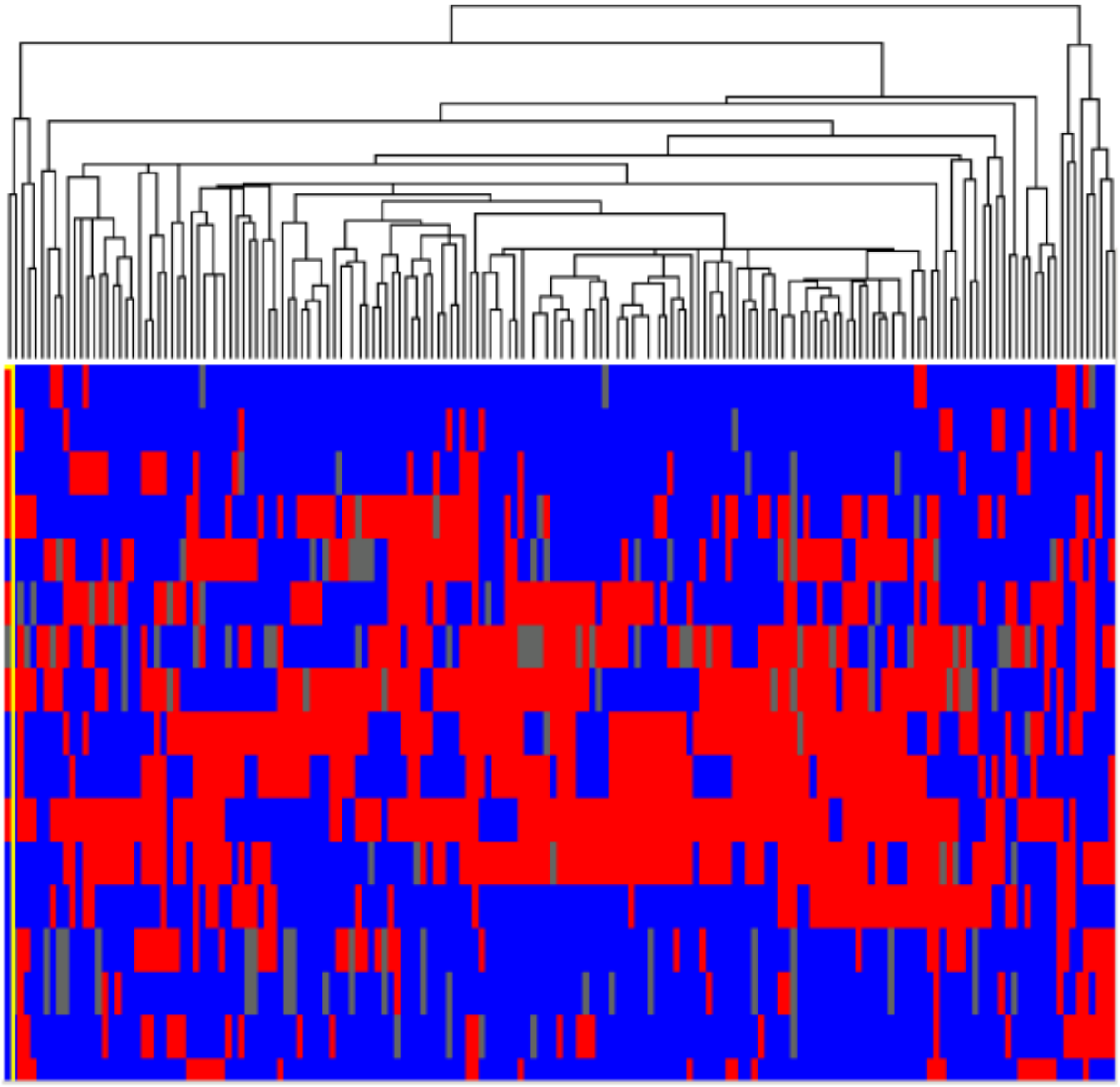
After including baseline data and the radiographic progression rate

- Explained 30% of the observed variance
- Plotting factors showed no clustering

Cluster Analysis showed no clustering

No clustering visible on heatplot or dendogram

No clear clusters in CCP-negative patients



What can be learned from RA as a prototype autoimmune disease?

- Classification criteria classify a mix of patients
- Matters in intervention trials given different clinical course of different subsets
- Statistical methodology: CCP+ versus CCP- groups different subgroups

What can be learned from RA as a prototype autoimmune disease?

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- riskfactors/histology/course/treatment response ?
- CCP2 is an artificial peptide, indications for real antigen?

Genetic & environmental risk factors differ: ACPA+ versus ACPA- RA

ACPA+ versus ACPA-

HLA-SE

PTPN22

rs- C5-TRAF1

rs- TNFAIP3-OLIG3

rs- CTLA4

rs- STAT4

rs- CCL21

rs-MMEL1-TNFRSF14

rs-CDK6, PRKCQ, KIF5A

CD40, IL2RA, IL2RB

HLA-DR3

rs- IRF5

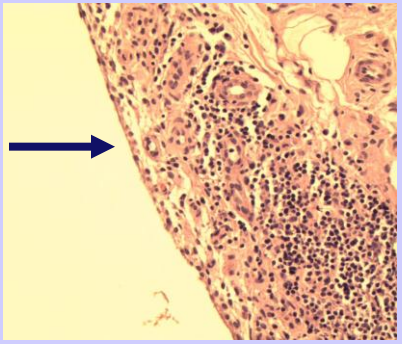
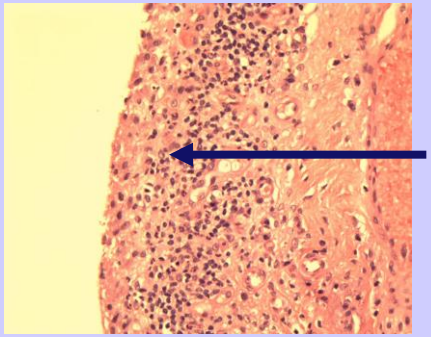
rs- STAT4

Raychaudhuri S et al. **Nat Genet**. 2008 Oct;40(10):1216-23
van der Helm A & Huizinga T. **Arthr Res Ther**. 2008;10(2):205.
Huizinga et al. A&R, **Arthritis Rheum**. 2005 Nov;52(11):3433-8.

Pathology:

Synovitis of ACPA-positive RA differs from ACPA-negative:

- More infiltrating lymphocytes in anti-CCP positive RA
- More fibrosis and increased synovial lining layer in anti-CCP negative

ACPA-positive   ACPA-negative

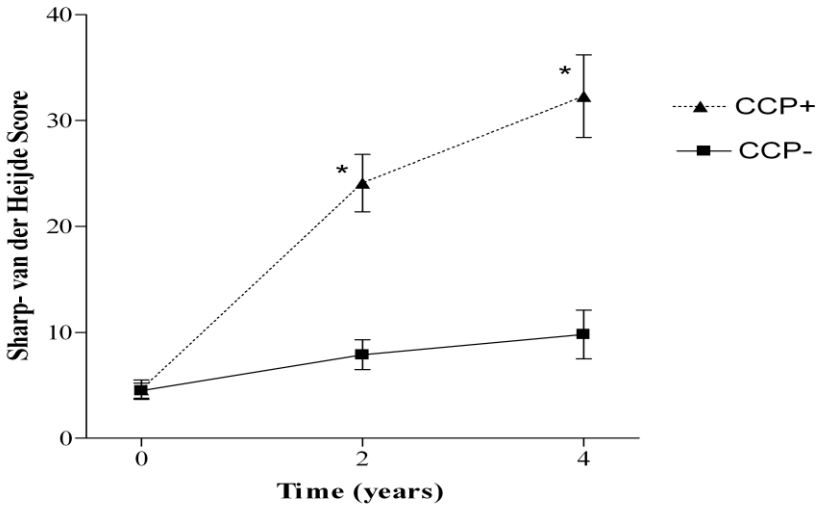
- Difference is already present early in the disease

van Oosterhout M, Bajema I, Levarht EW, Toes RE, Huizinga TW, van Laar JM.

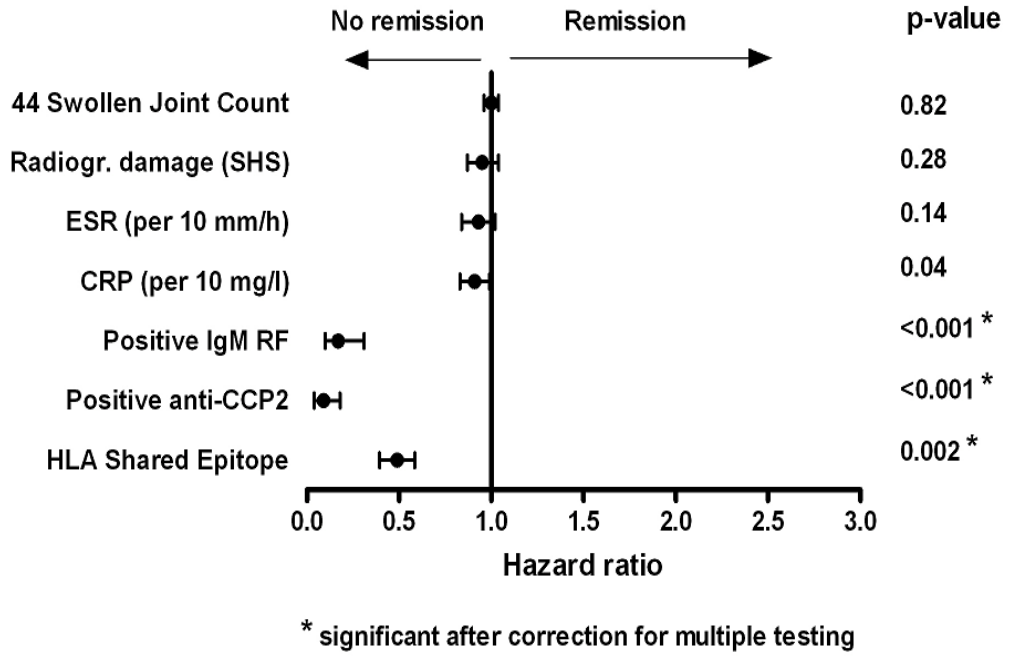
Arthritis Rheum. 2008 Jan;58(1):53-60

Clinical course different

Joint destruction over time



Drug free remission rate

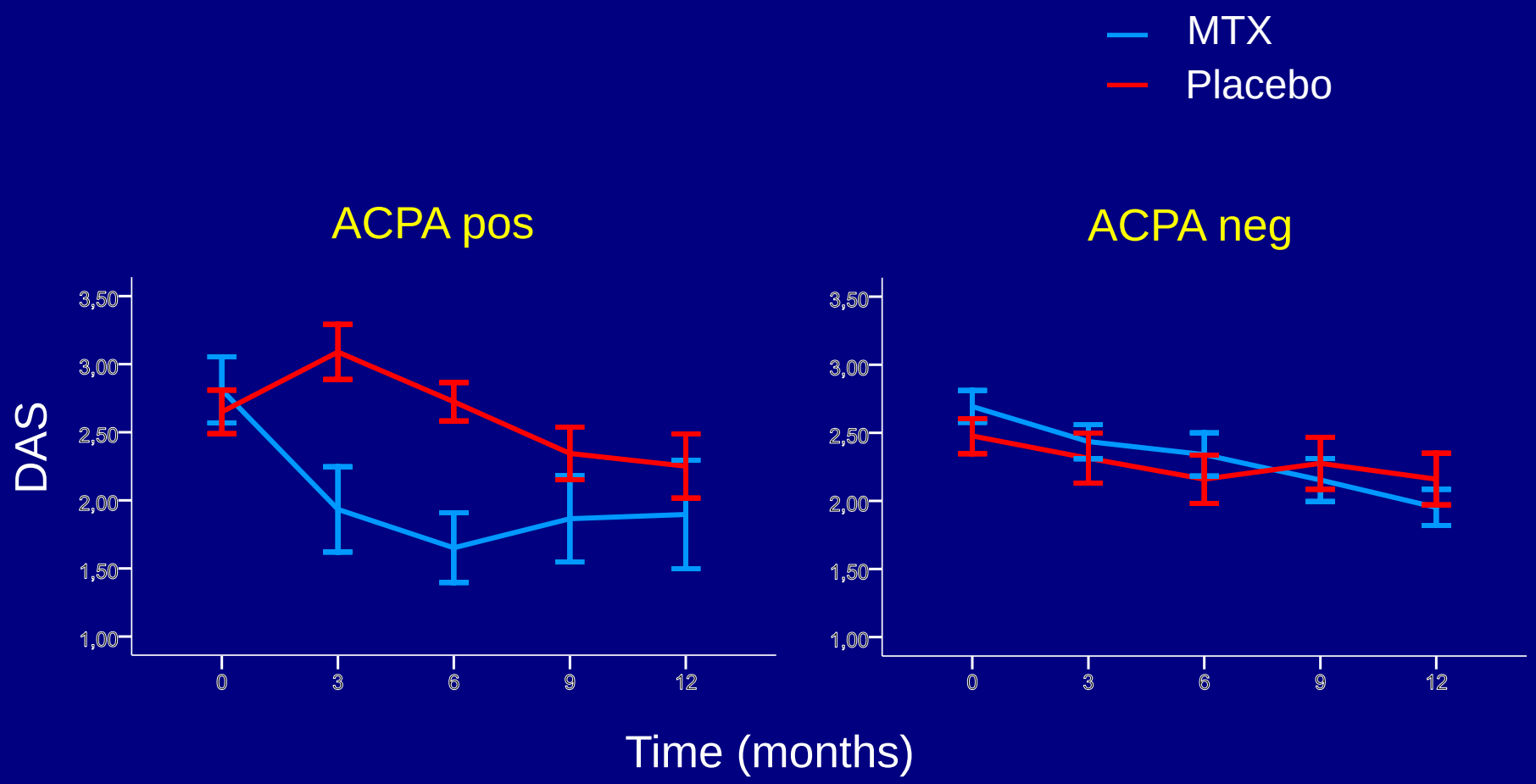


Fulfillment of the criteria for RA after

	1 Year	2 Years	3 Years
69 CCP+ Pts	83%	90%	93%
249 CCP- Pts	18%	24%	25%
318 Pts	32%	38%	40%

#

DAS in time MTX versus placebo in RA-2010 criteria



What can be learned from RA as a prototype autoimmune disease?

- Classification criteria classify a mix of patients
- Matters in intervention trials given different clinical course of different subsets
- Statistical methodology identified CCP+ versus CCP- RA patients as two different groups
- This was confirmed by different risk factors, histology, and treatment responses

But CCP is an artificial peptide, so an immune response towards an artificial peptide is intuitively a strange way to arrive at a pathogenetically defined grouping of patients

Anti-Citrullinated Protein Antibodies (ACPA) recognize various citrullinated substrates

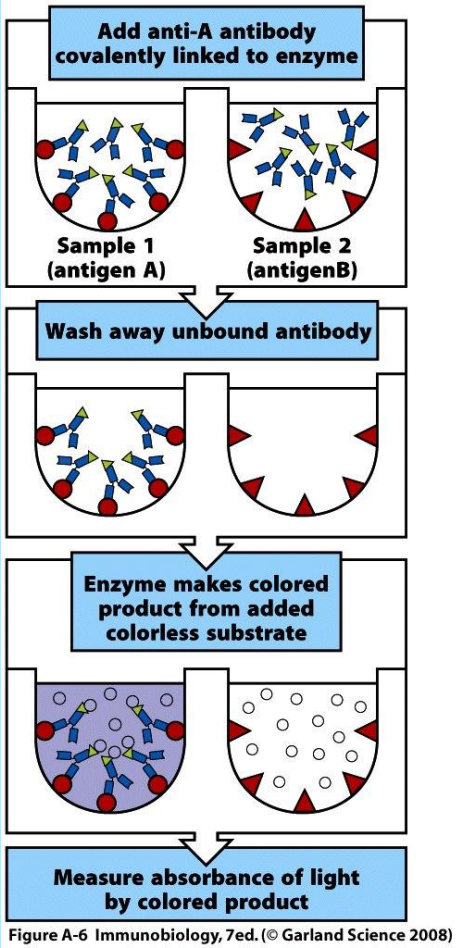
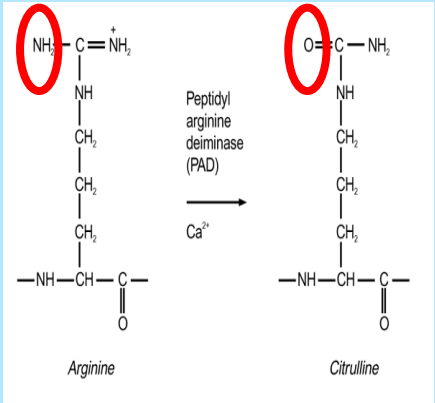
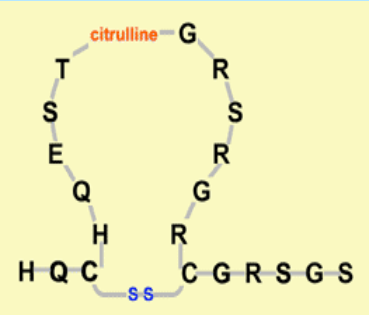


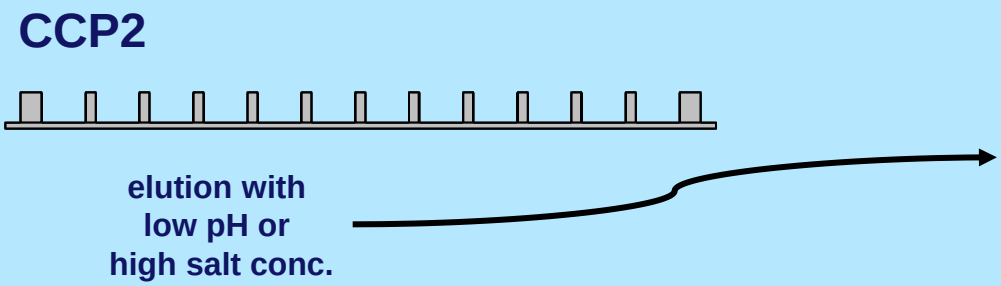
Figure A-6 Immunobiology, 7ed. (© Garland Science 2008)

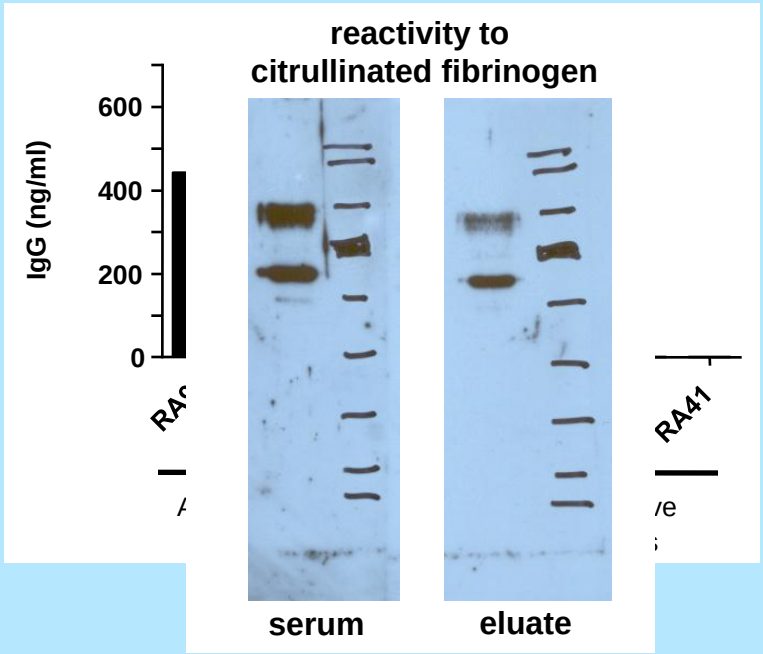




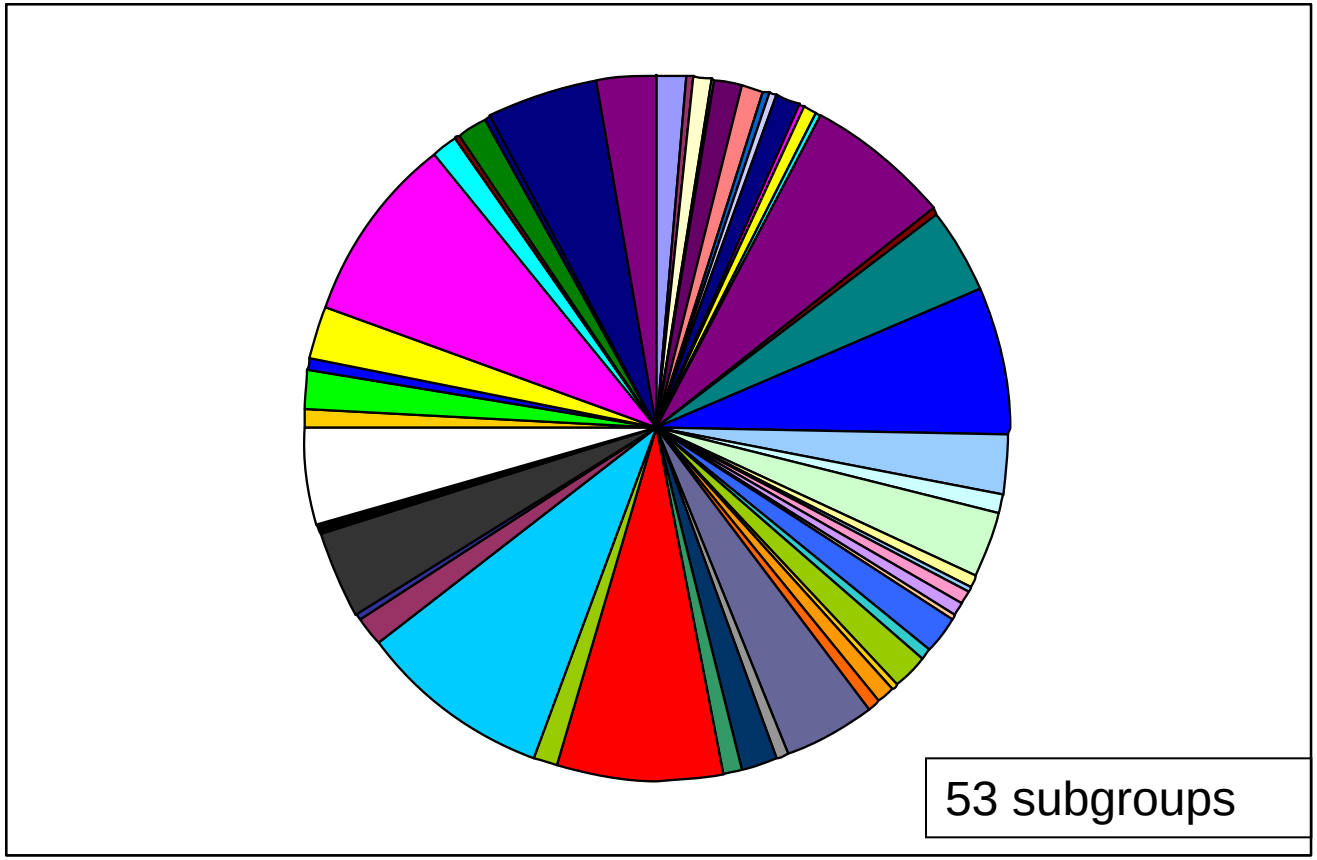
Anti-CCP:
Used to subgroup:
do they
really recognize
citrullinated
substrates?

Anti-CCP-antibodies are ACPA





CCP2+ stratum and all peptides: Many different subgroups: no phenotypic correlations



cVim1-16

cVim59-74

cFib α 36-52

cFib β 27-43

cEno5-20

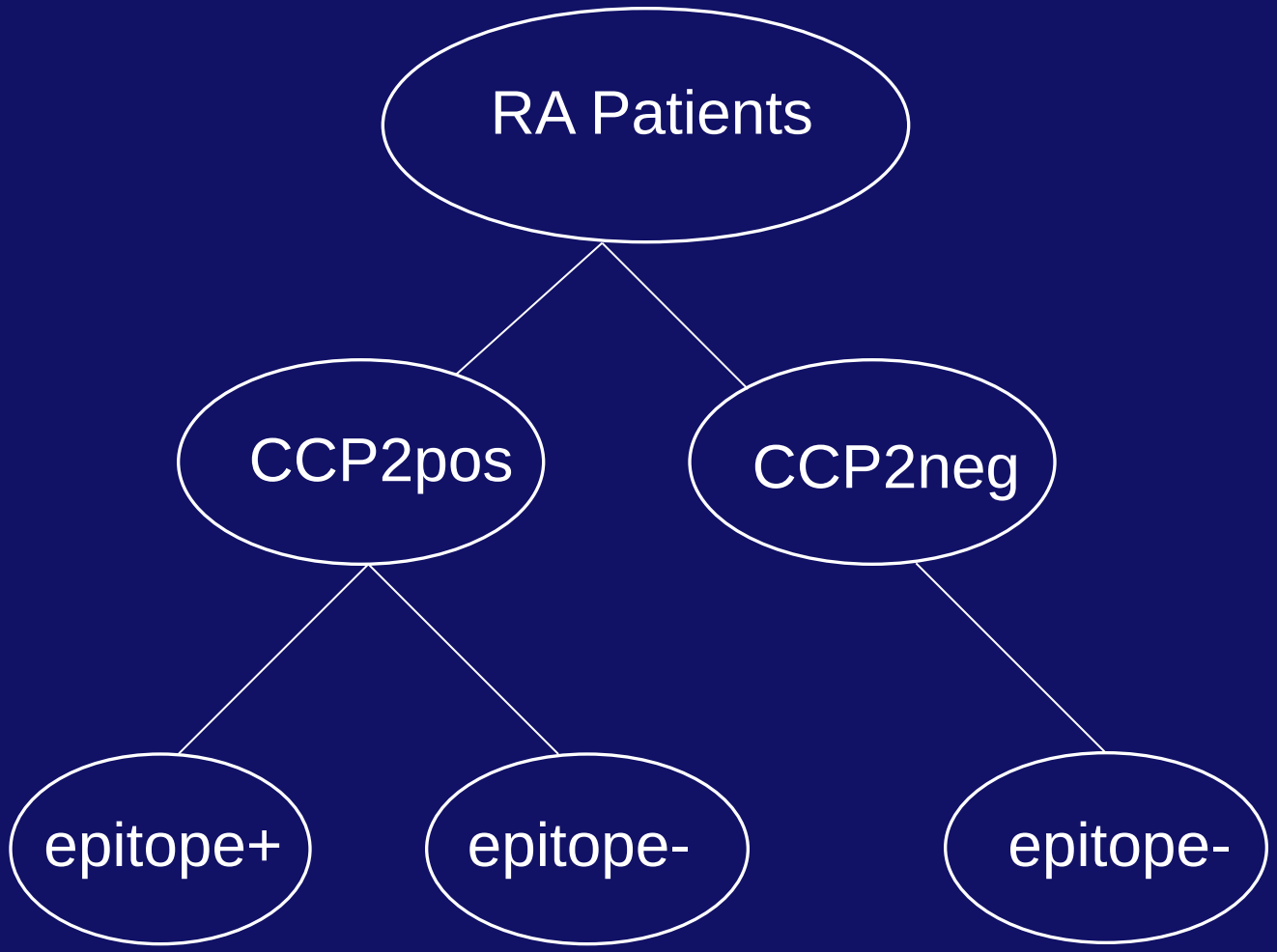
cMBP

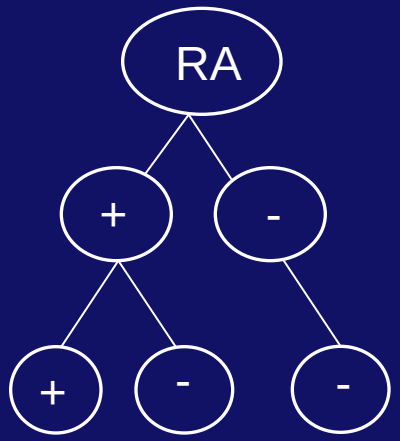
MCV

CCP3

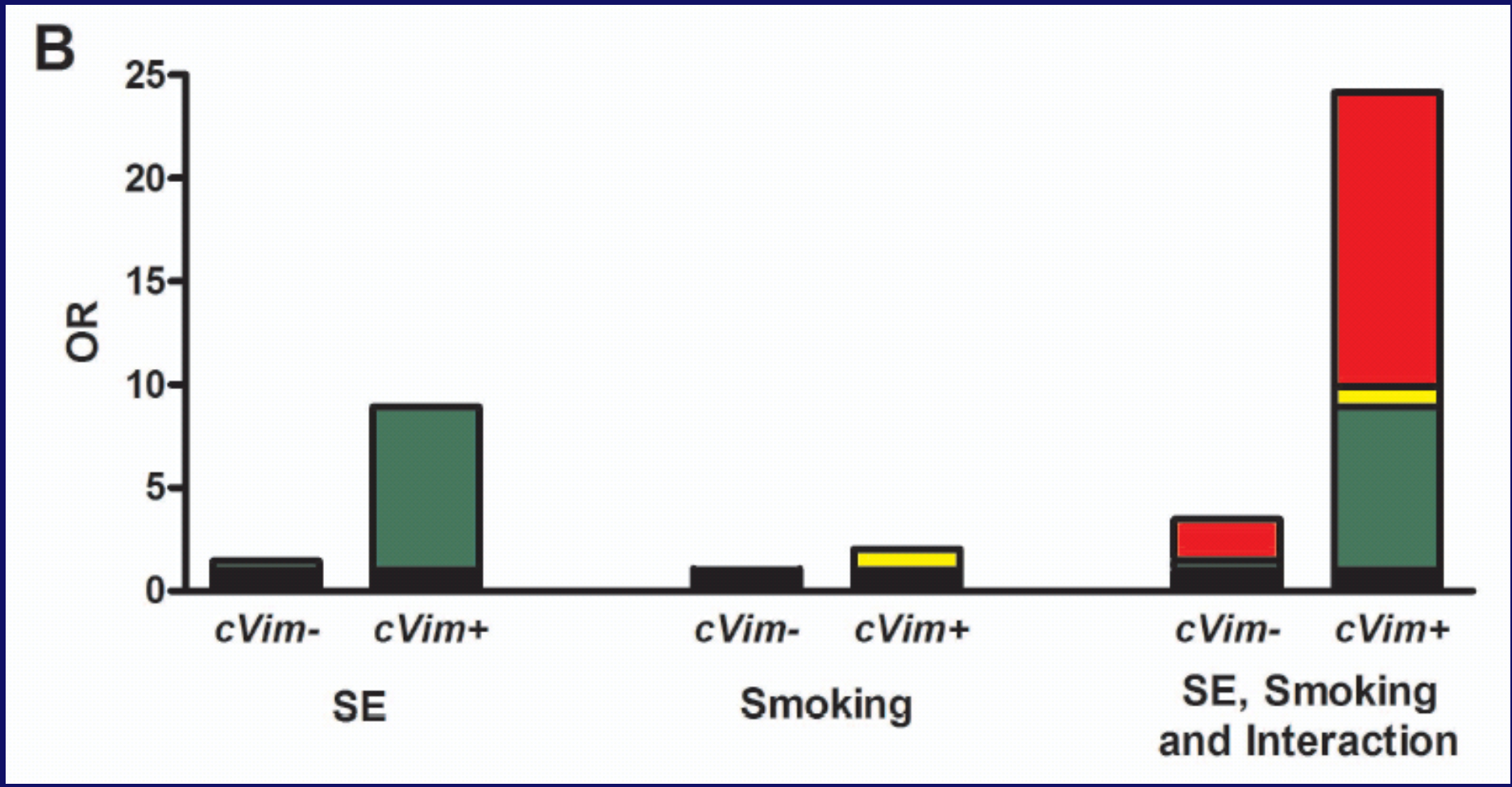
Thus, if not the specificity may risk factor interaction can give a clue?

Given Two Homogeneous groups of RA: Interaction studies within homogenous groups



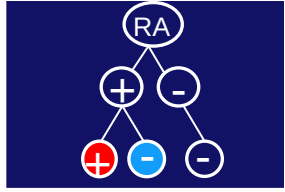


A gene-environment interaction affects the reactivity of autoantibodies to various citrullinated antigens in RA



Van der Woude et al. Nature genetics. 2010

Association Fine Specificity and Smoking within ACPA+ groups

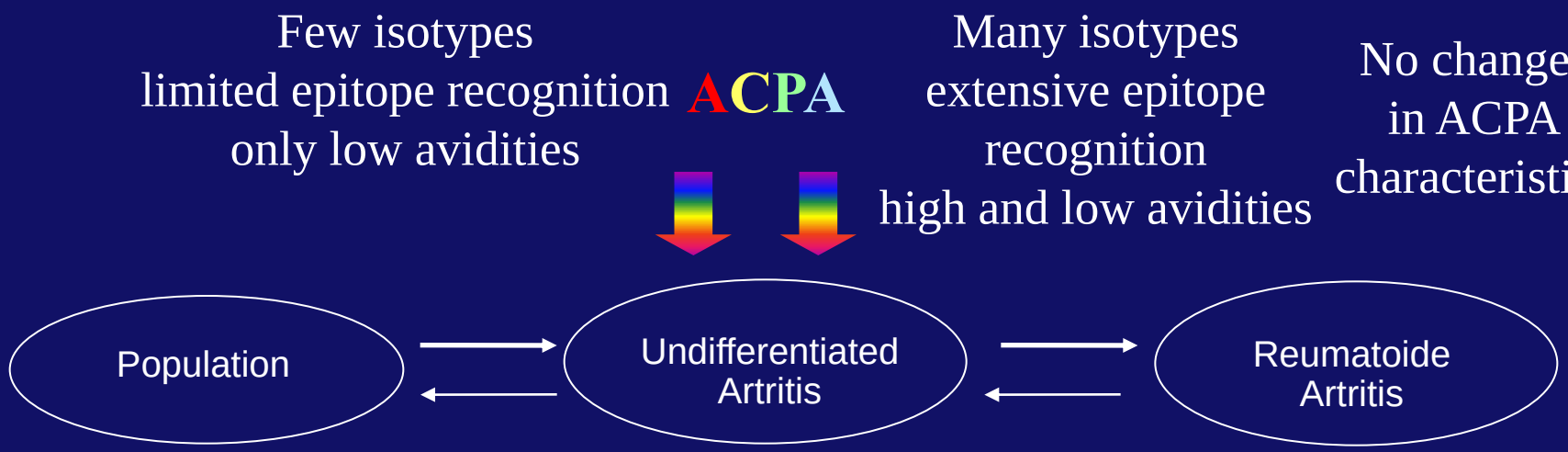


	Smoking	N total	%	OR	95% CI
cVim59-74+	86	150	57.1	1.20	0.76-1.88
cVim59-74-	83	157	52.9		
cEno+	64	100	64.0	1.73*	1.06-2.82
cEno-	105	207	50.7		
MBP+	116	199	58.3	1.45	0.91-2.32
MBP-	53	108	49.1		

What can be learned from RA as a prototype autoimmune disease?

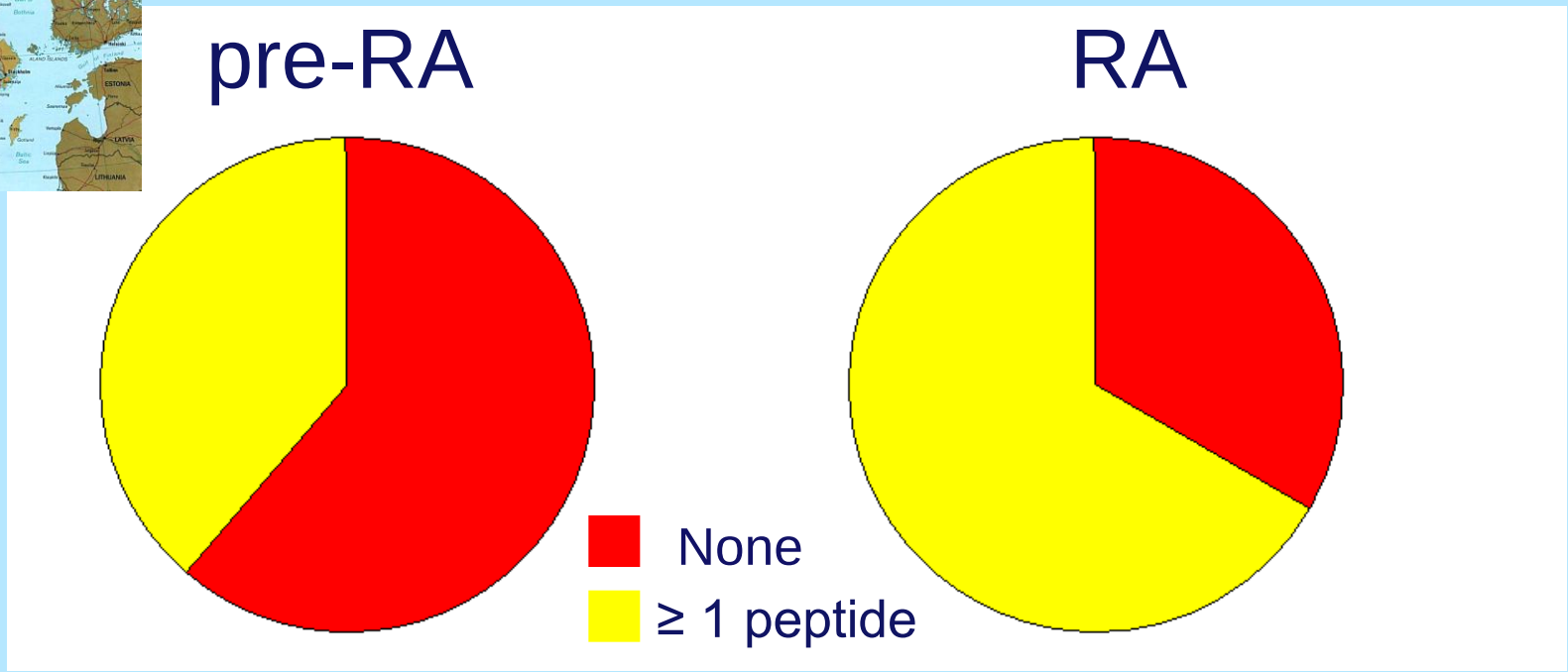
- Classification criteria classify a mix of patients
- Matters in intervention trials given different clinical course of different subsets
- Statistical methodology: CCP+ versus CCP- groups different subgroups
- Recognition of an arteficial peptide sofar the best way to group ACPA+ versus ACPA-negative RA

ACPA characteristics are marker of disease development

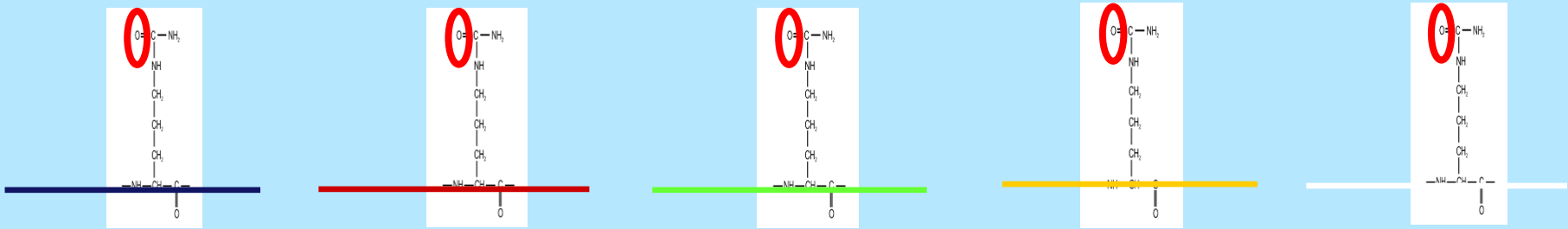


Results pre-RA versus RA 2

Number of epitopes recognized by sera from:



Recognition of ≥ 1 peptide: 38% 66% p=0.013



Vimentin peptide A

Vimentin peptide B

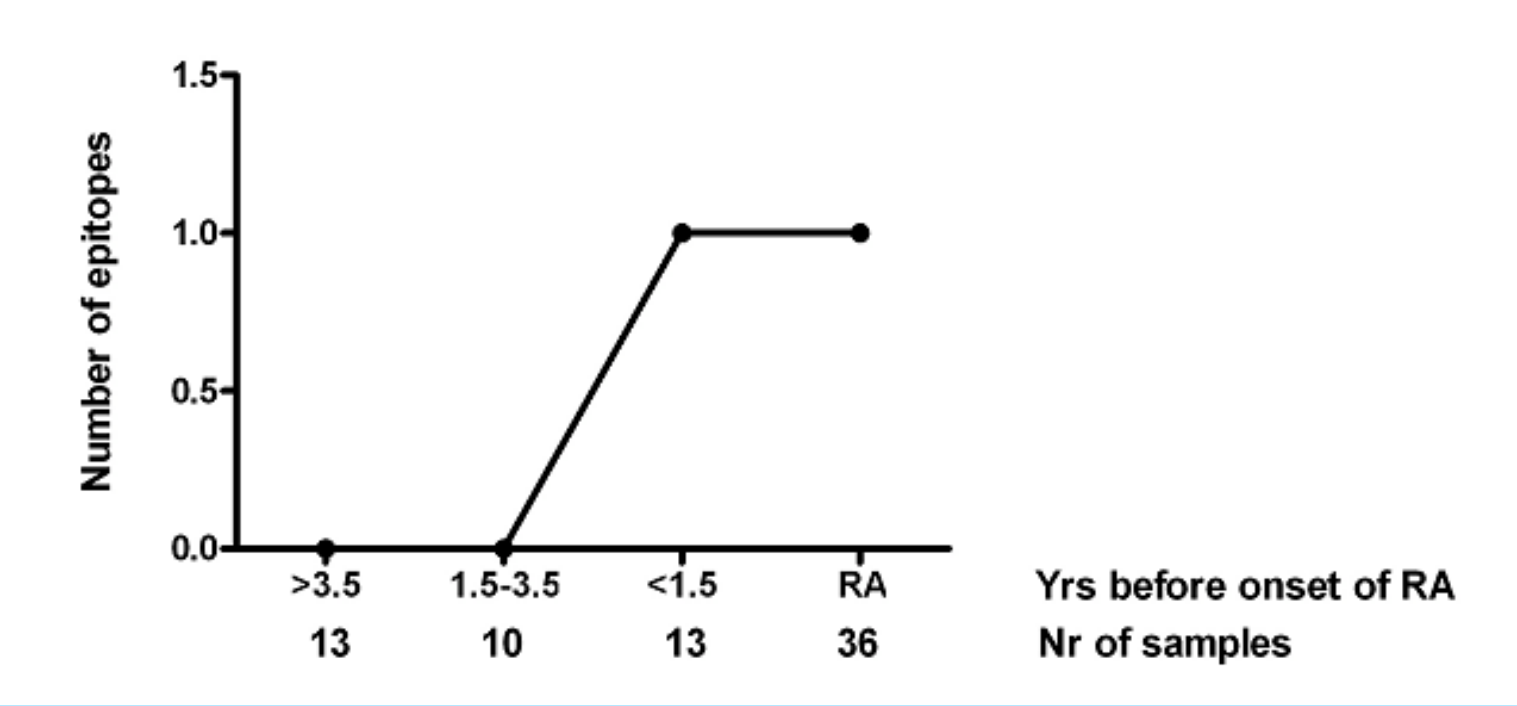
Fibrinogen peptide A

Fibrinogen peptide B

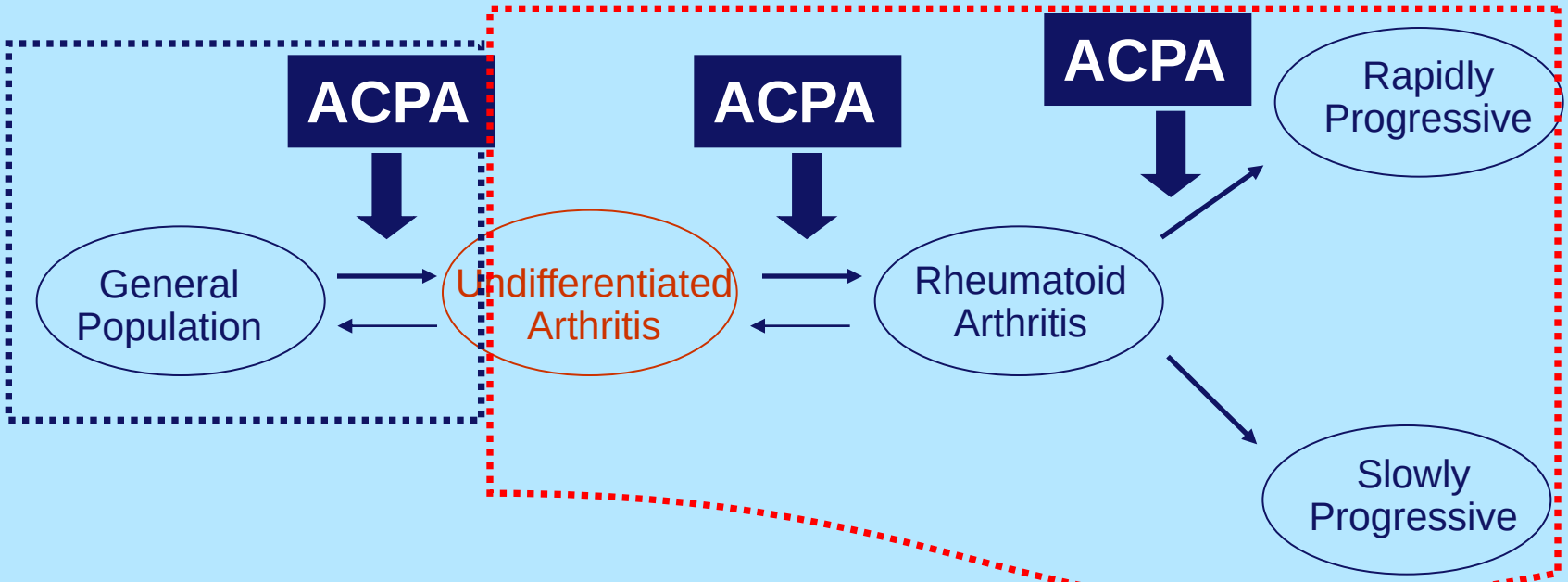
Enolase peptide

Number of epitopes recognized increase from pre-RA to RA

Median number of peptides recognized over time



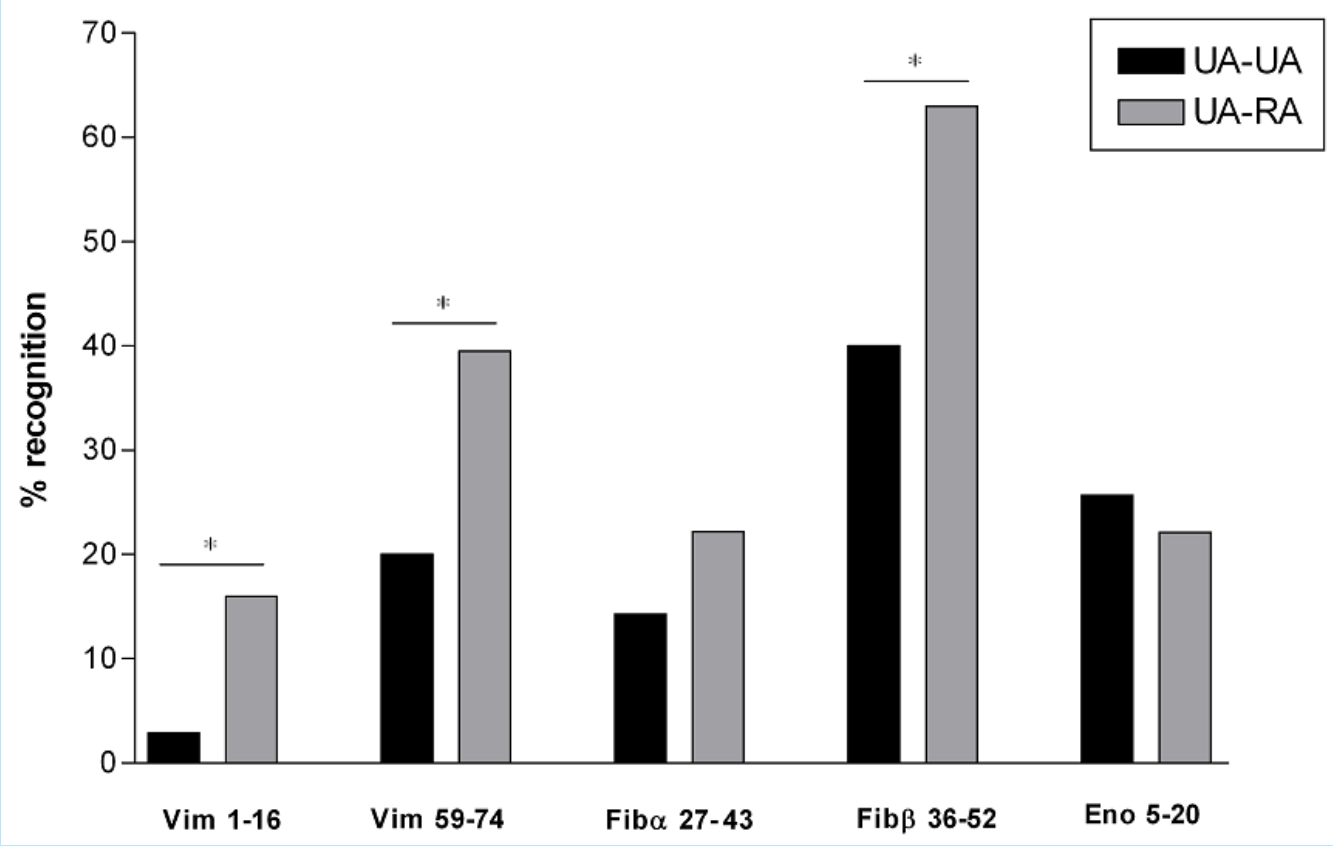
Study populations



Stages of disease development:

- Healthy ACPA-positive subjects: Canadian Ìndians & Swedish pre-patients
- Patients with Undifferentiated Arthritis (UA): Leiden EAC

Results – baseline UA-UA versus UA-RA

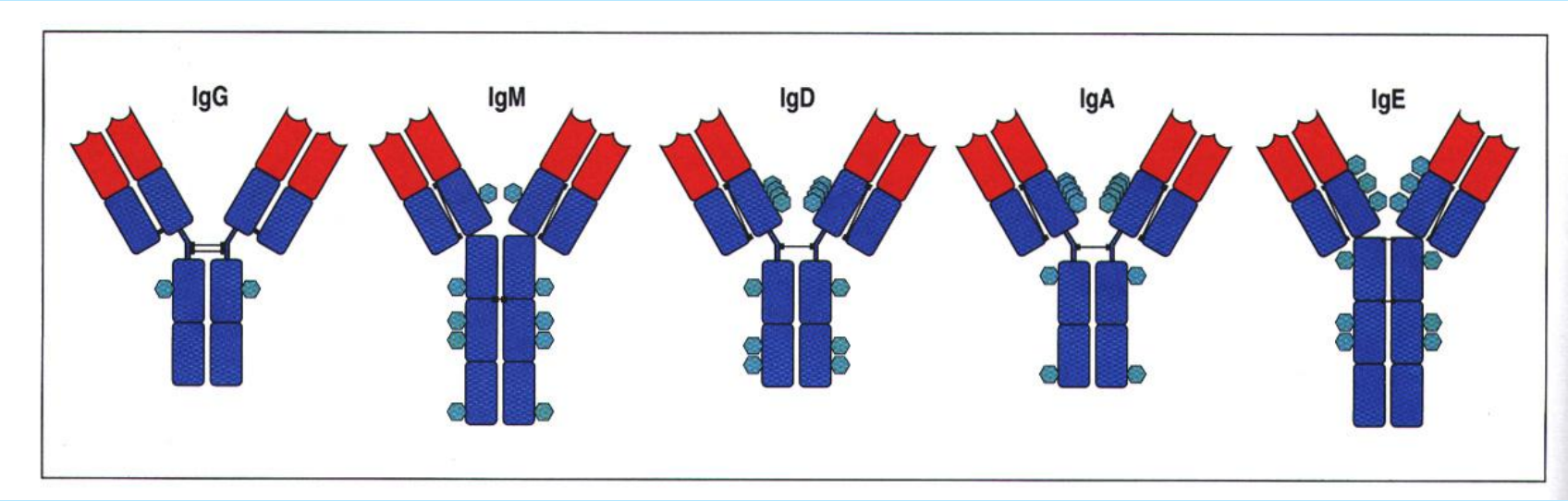


Median nr of peptides

UA-UA	1
UA-RA	2

P = 0.02

Antibody isotypes

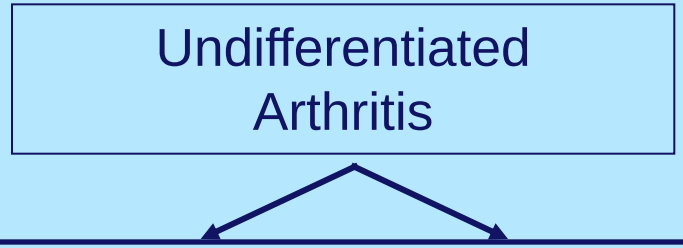


Antibody isotype is determined by the heavy chain constant region:

-Bound by cell-surface receptors (Fc receptors)
-Bound by complement
-Determines antibody function

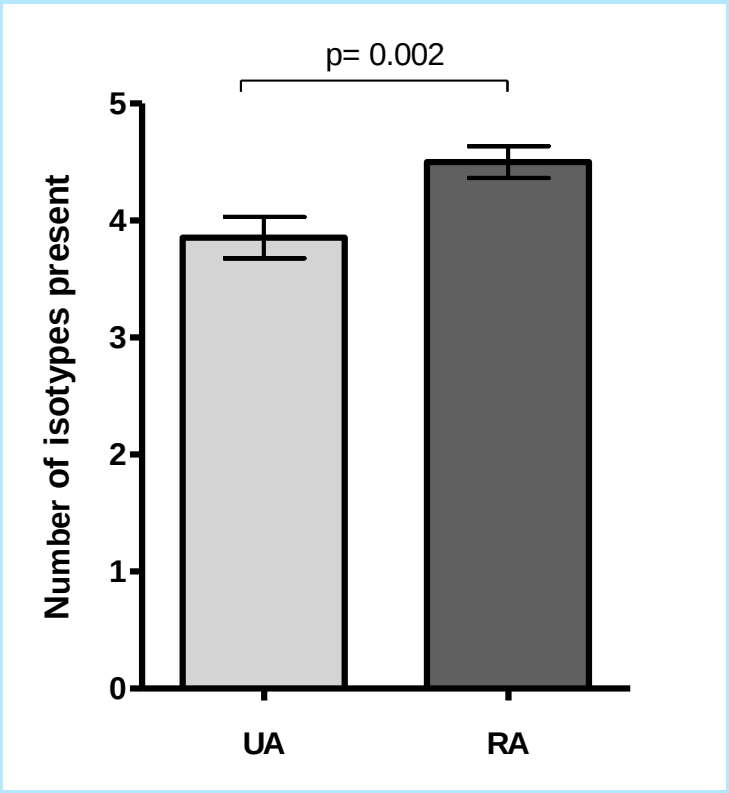
Does the usage of certain isotypes associate with disease severity?

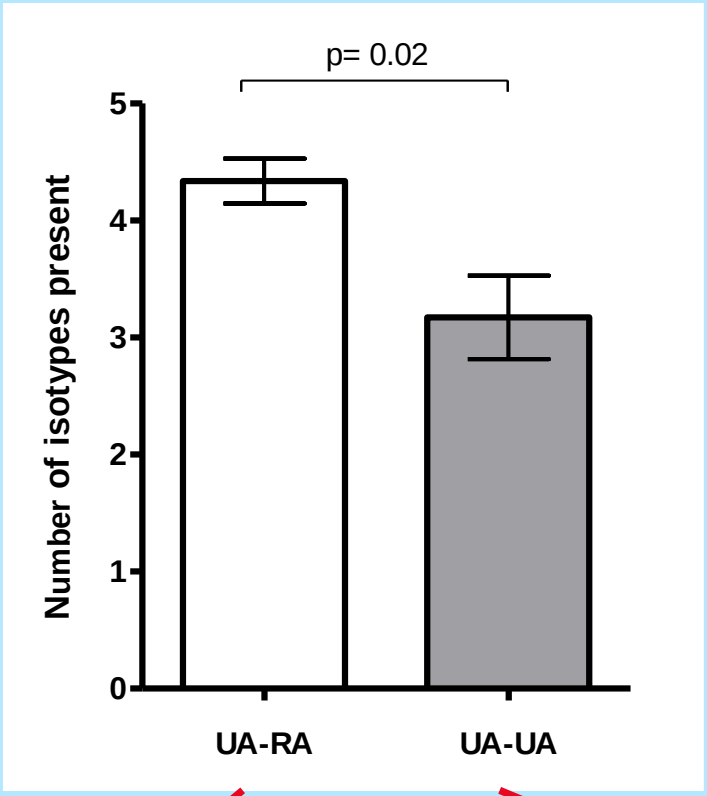
ACPA-positive Undifferentiated Arthritis patients that progress to RA display a broader isotype-usage

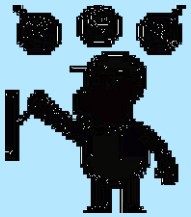


	Non RA (n=46)	RA (ACR criteria (n=91)	OR (CI)	P-value
# of ACPA-isotypes (median, IQR)	3 (2 - 5)	5 (3 - 6)	1.5 (1.2-1.9)	<0.001

The ACPA reaction in UA patients that progress to RA display a broader isotype usage

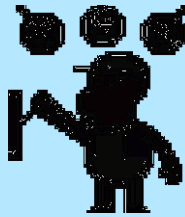




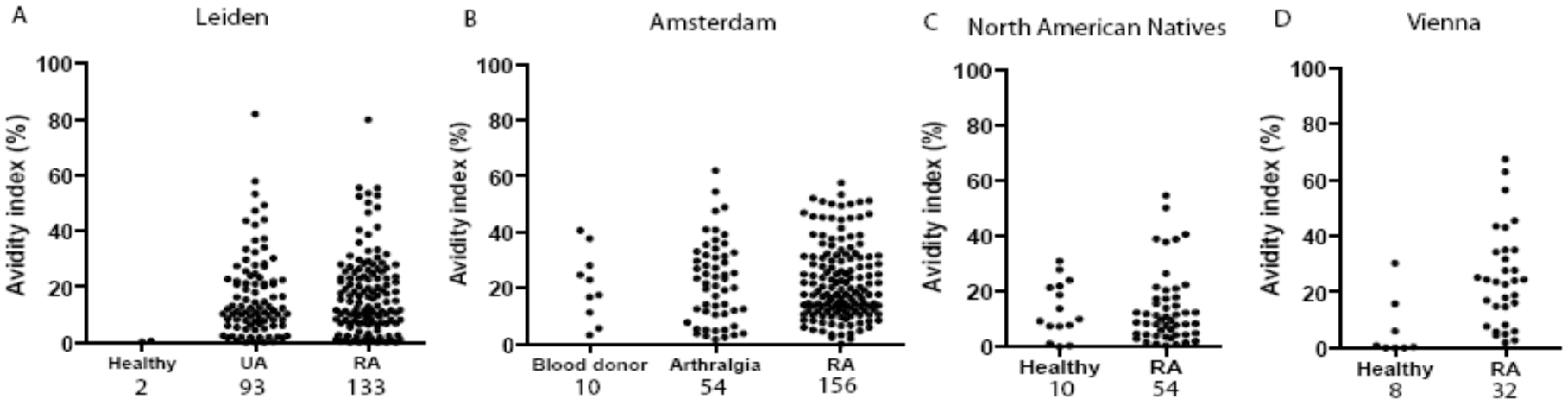


< 3 months RA

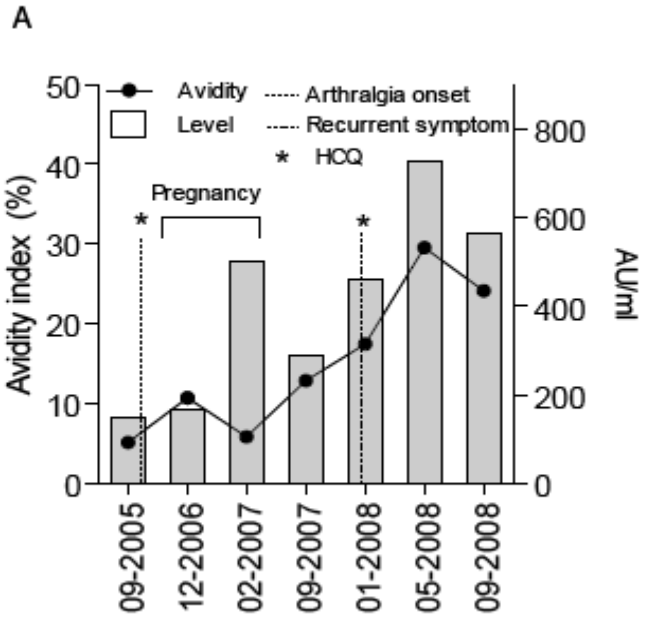
> 12 months still UA

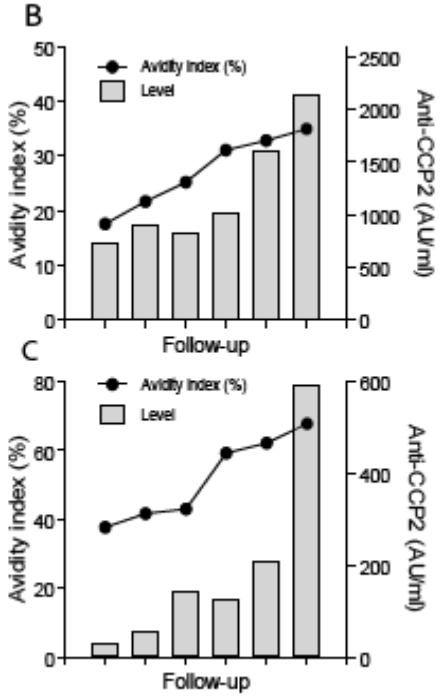


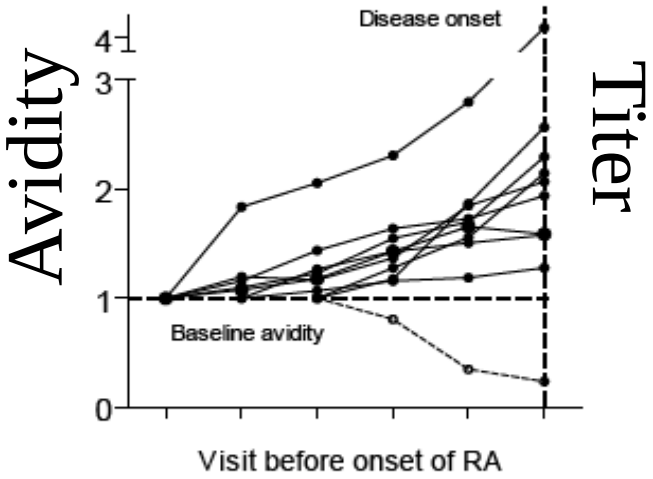
Avidity Maturation during disease development



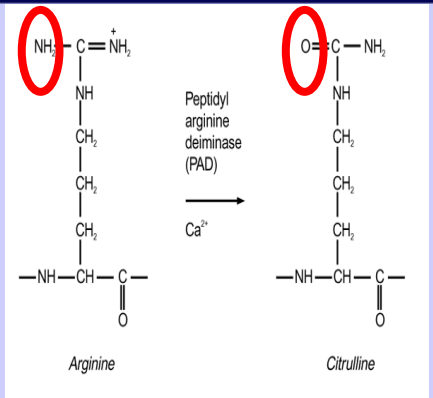
Avidity Maturation in longitudinal samples







ACPA characteristics :a biomarker of the window of opportunity



Few isotypes
limited epitope recognition
only low avidities

ACPA

Many isotypes
extensive epitope recognition
high and low avidities

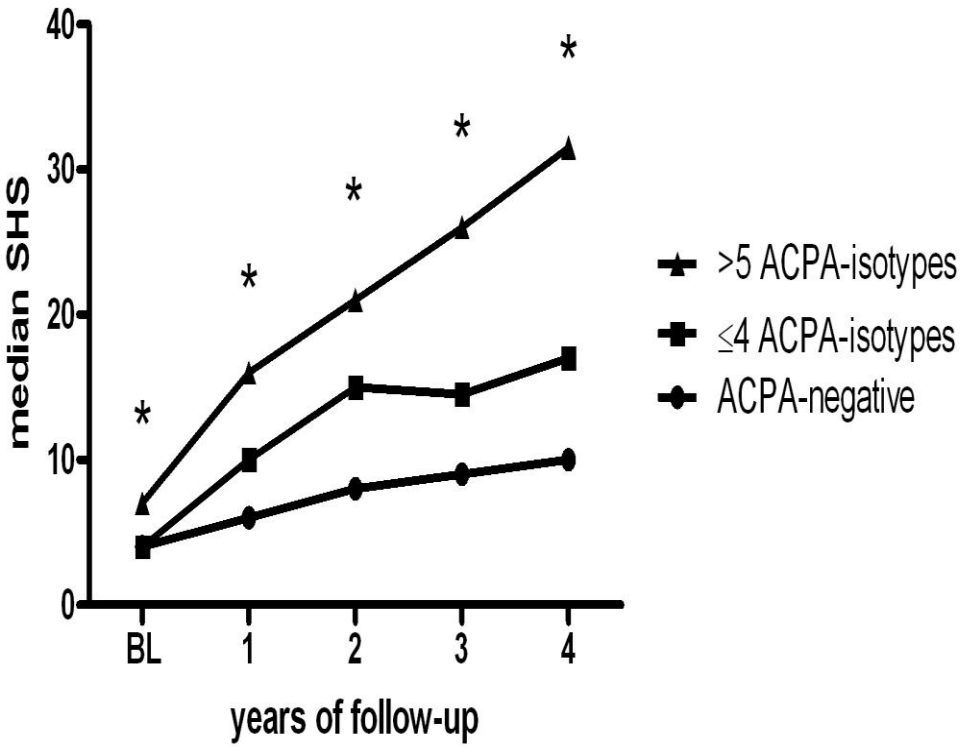
No changes in ACPA characteristics

Population ⇄ Undifferentiated Artritis ⇄ Reumatoide Artritis

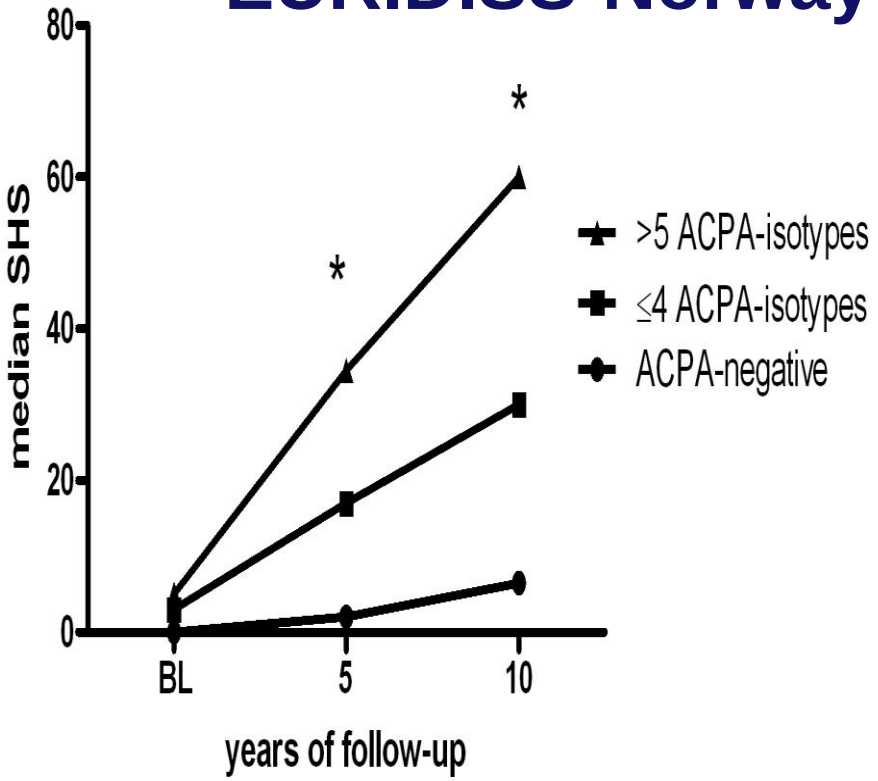
What is the relevance of this developing autoimmune response during early artritis?

A broader isotype usage is associated with Radiographic progression

EAC-Netherlands



EURIDISS-Norway

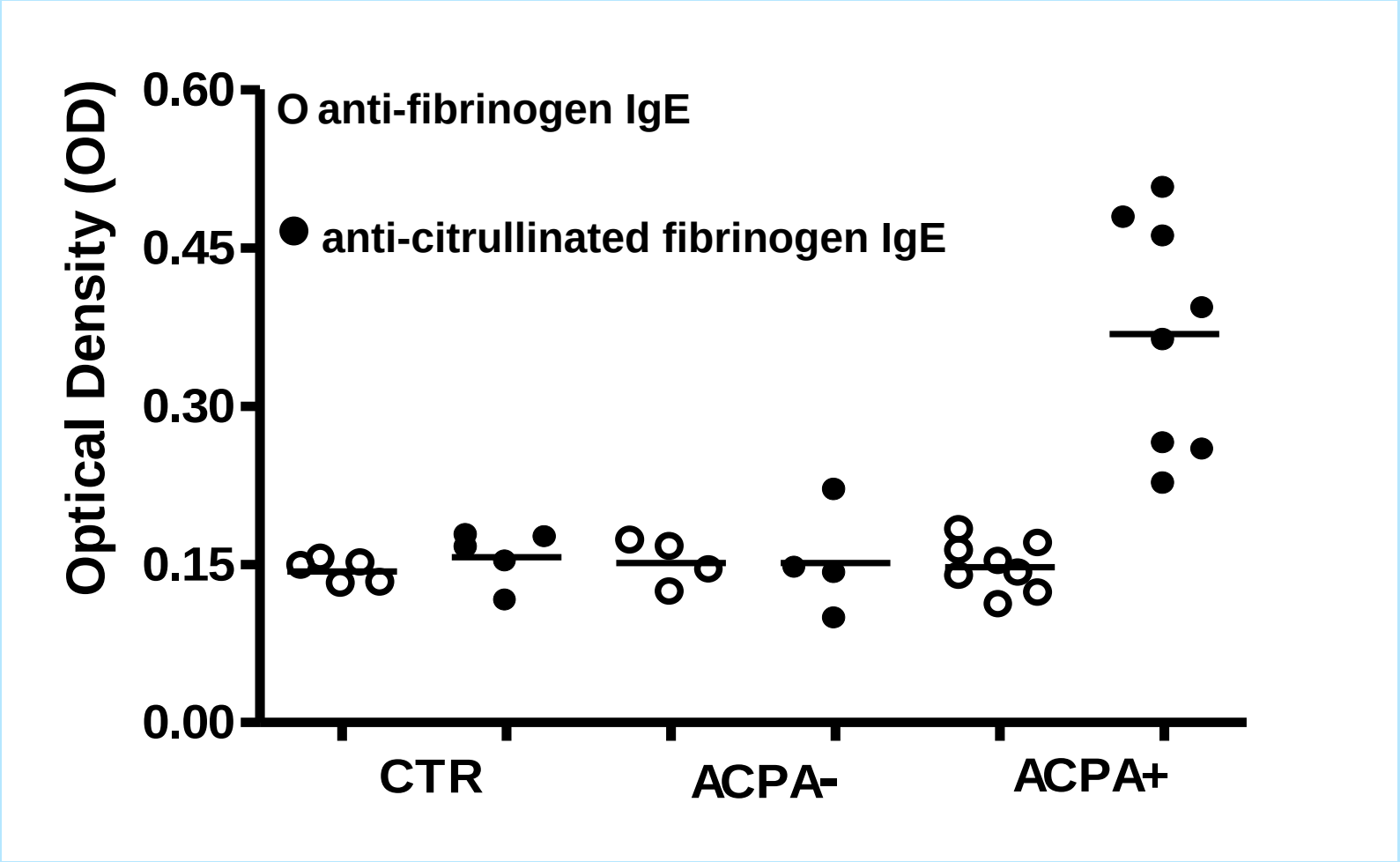


* comparison ≤4 isotypes versus ≥5 isotypes: $p<0.05$

What can be learned from RA as a prototype autoimmune disease?

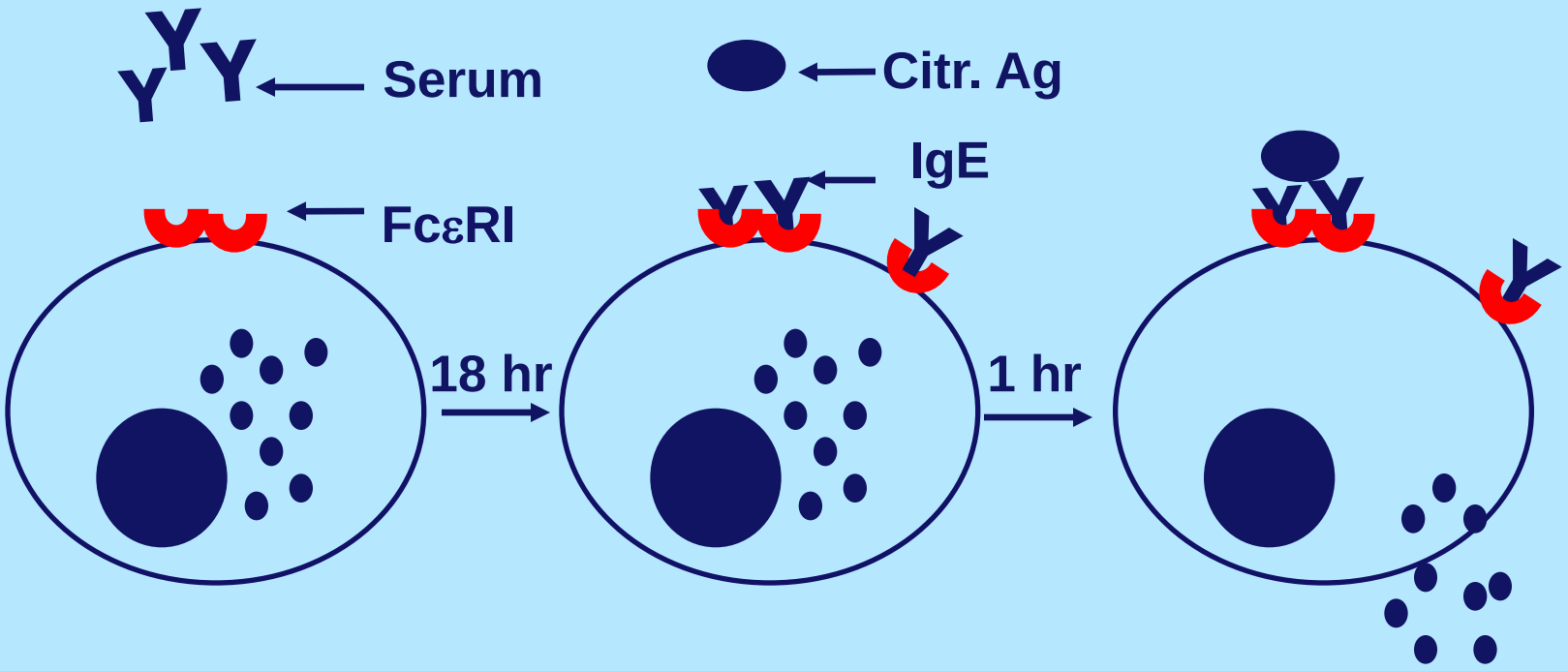
- Classification criteria classify a mix of patients
- Matters in intervention trials given different clinical course of different subsets
- Statistical methodology: CCP+ versus CCP- groups different subgroups
- Recognition of an arteficial peptide sofar the best way to group ACPA+ versus ACPA-negative RA
- Isotypes relevant.Are there specific pathways within the ACPA+groups?

IgE ACPA are present in ACPA-positive RA



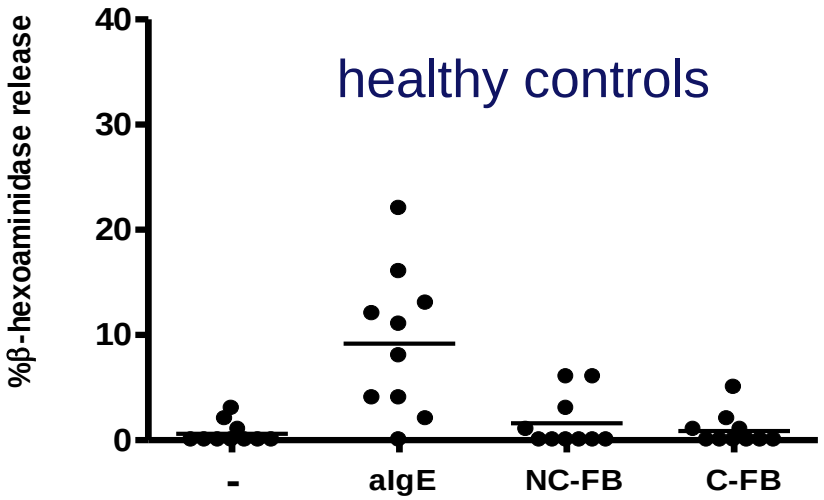
Measurement: cumbersome but possible: Depletion of IgG/A/M by protein G, ELISA, Coating with CCP2/ cit Fibrinogen / fibrinogen, detection with anti-IgE

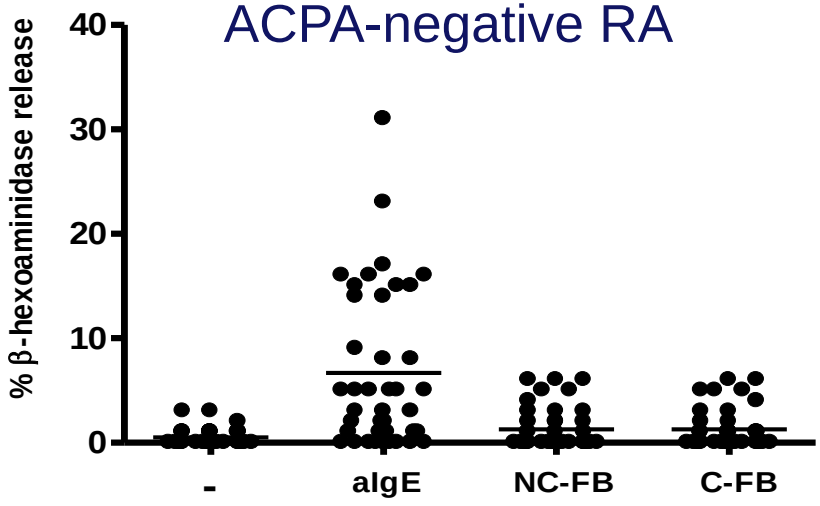
Sensitisation and activation of a FcεRI-transfected Rat Basophil Line: IgE ACPA is functional

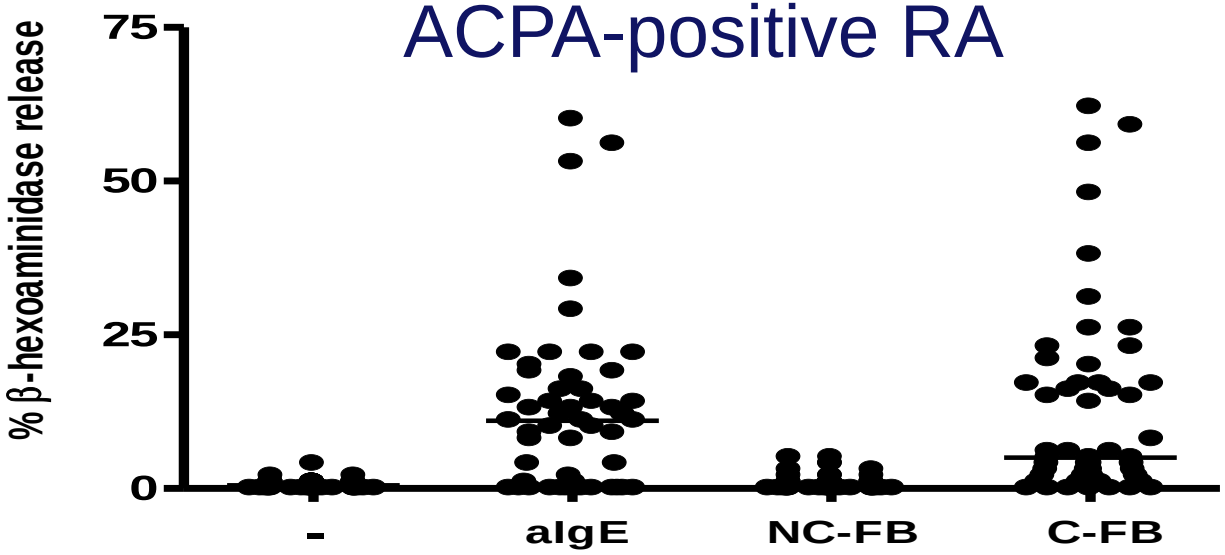


β hexoaminidase release

Sensitisation and activation of a FcεRI-transfected Rat Basophil Line

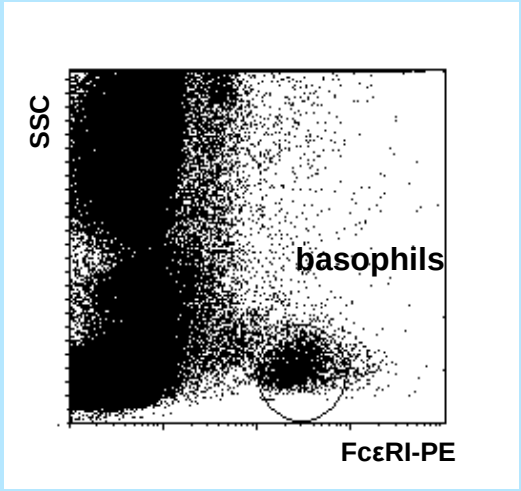


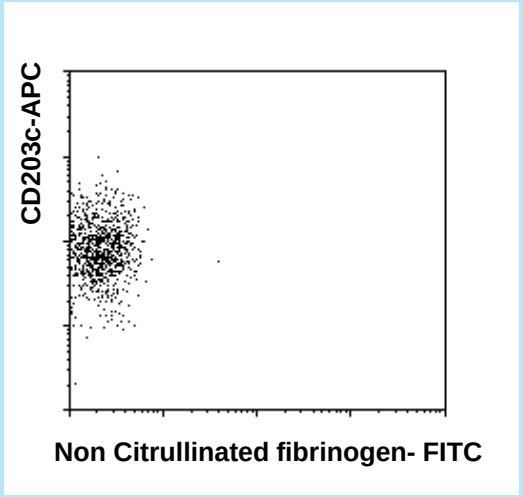


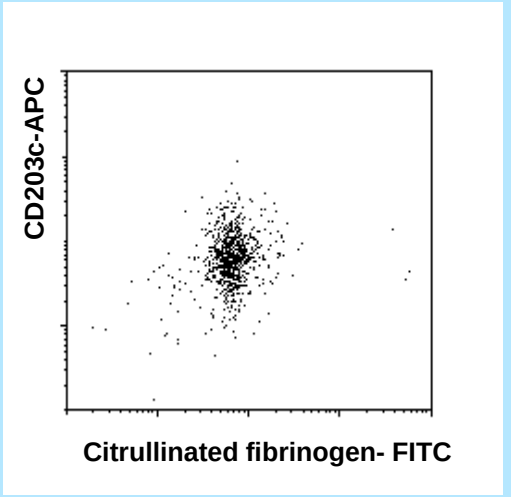


FcεR-positive cells bind Citrullinated antigen

ACPA+ donor

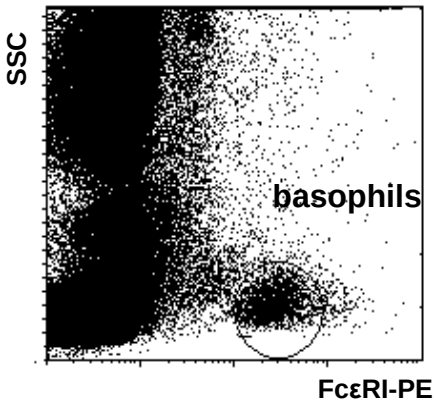


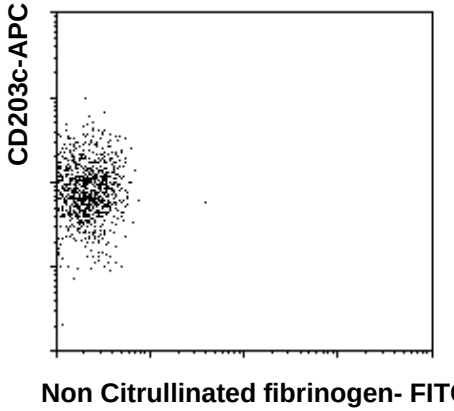


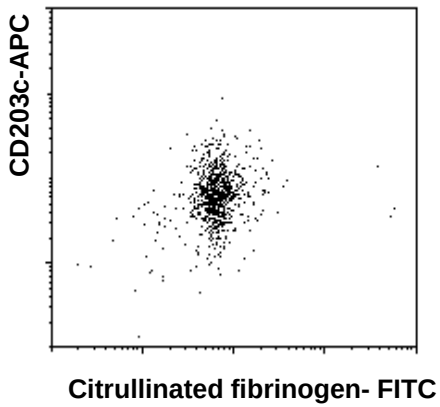


FcεR-positive cells bind Citrullinated antigen

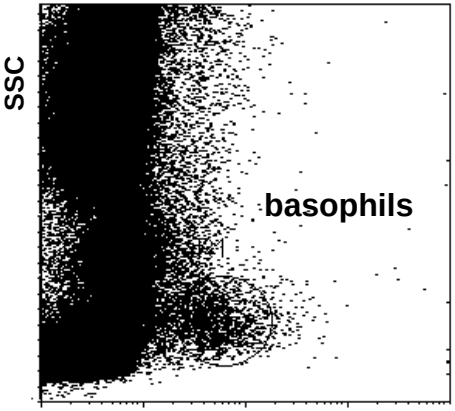
ACPA+ donor

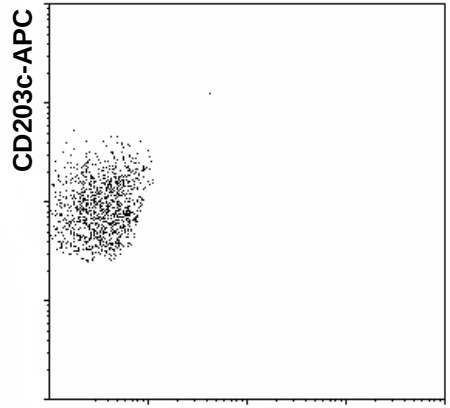


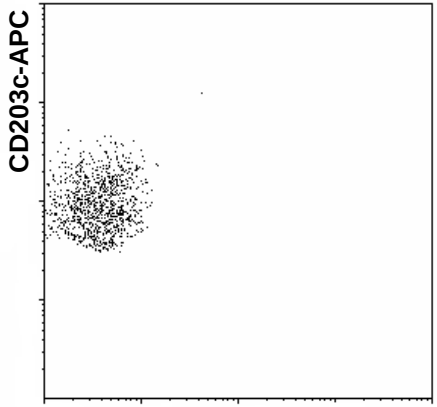




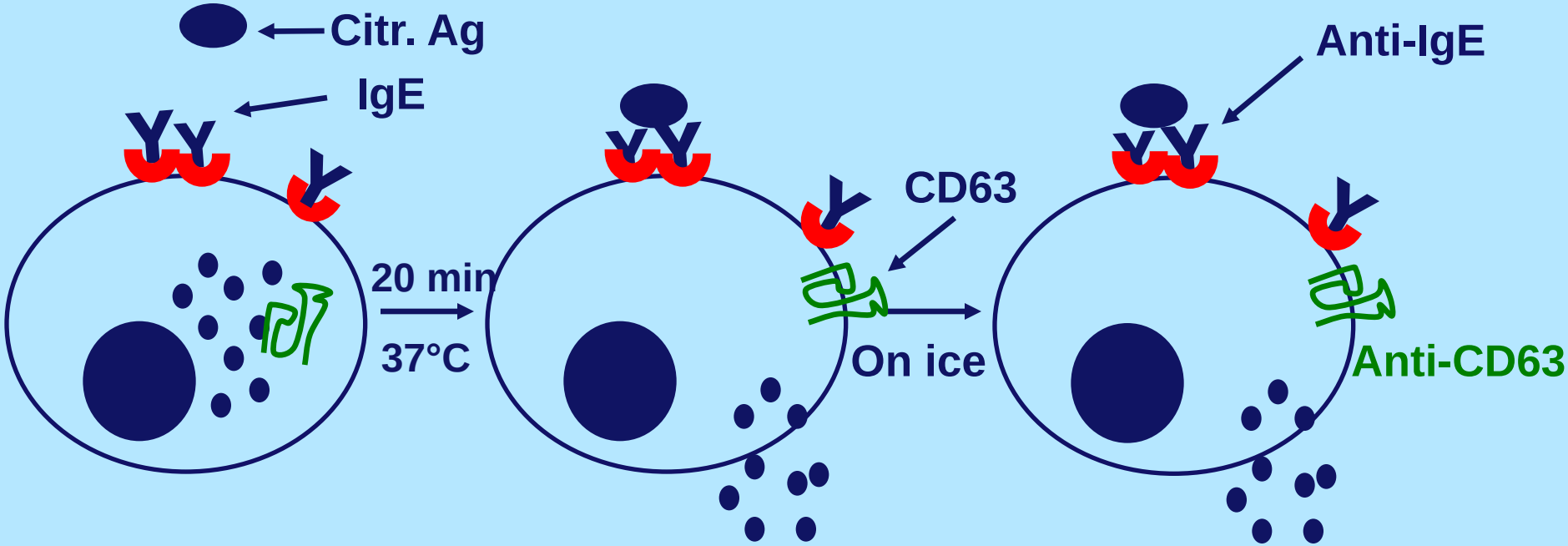
ACPA- donor

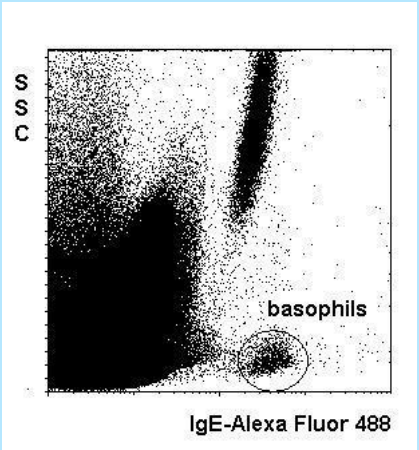


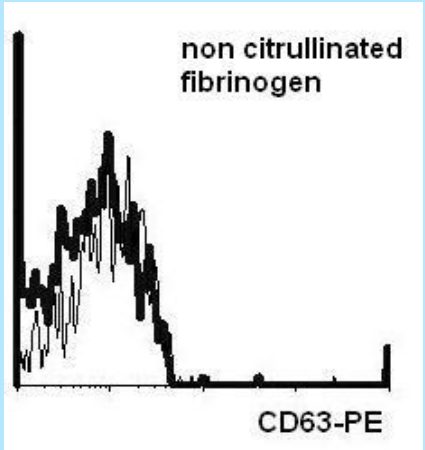


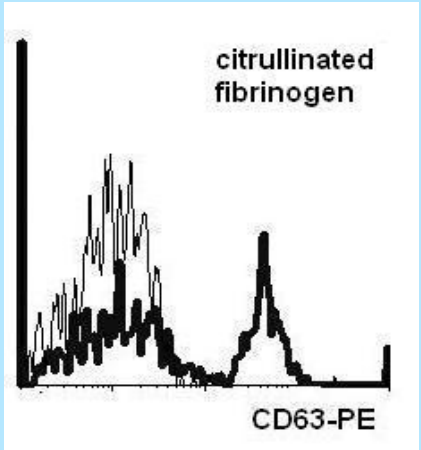


Direct activation of IgE-positive basophils by citrullinated Antigens measured by a “Basophil Activation Test”









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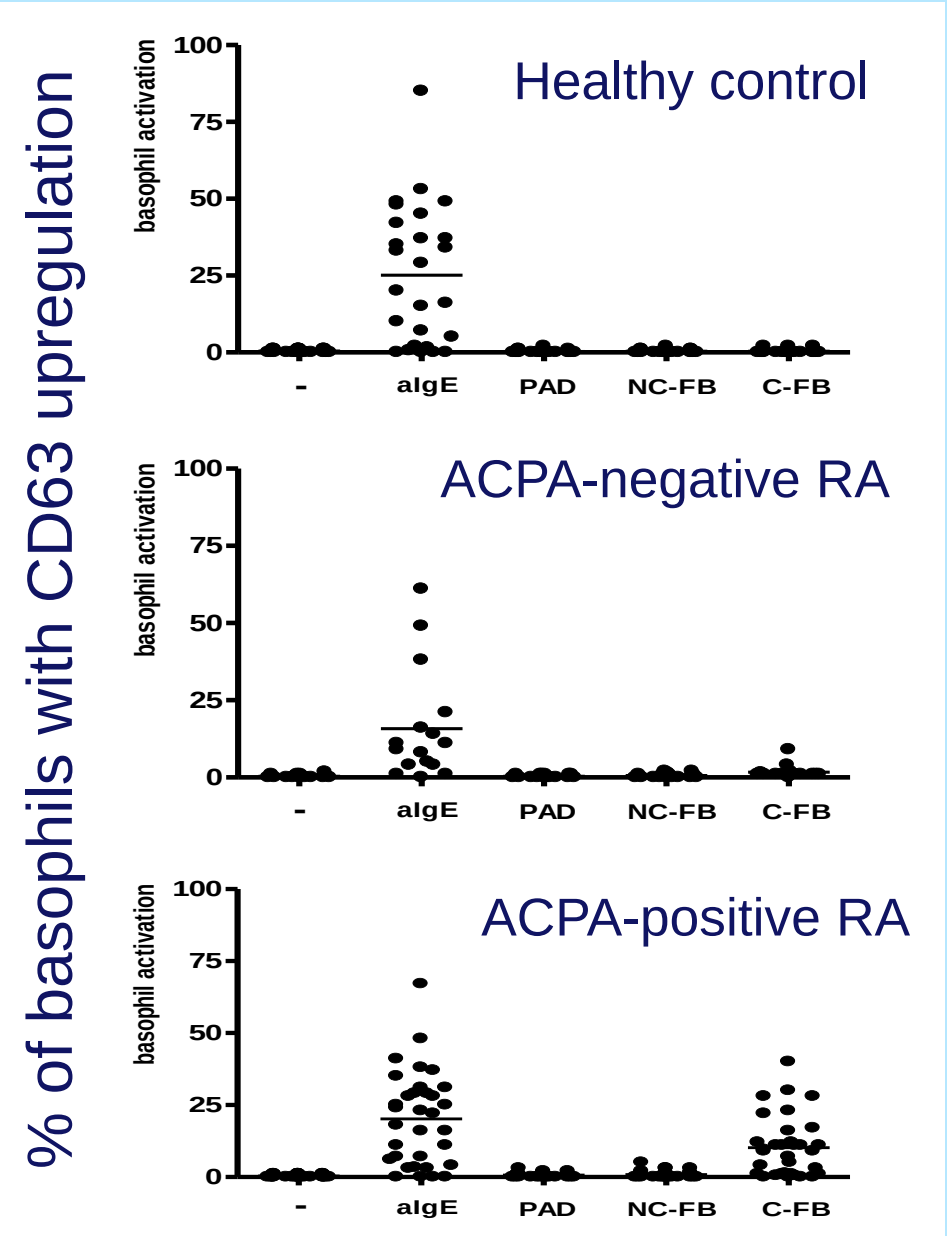
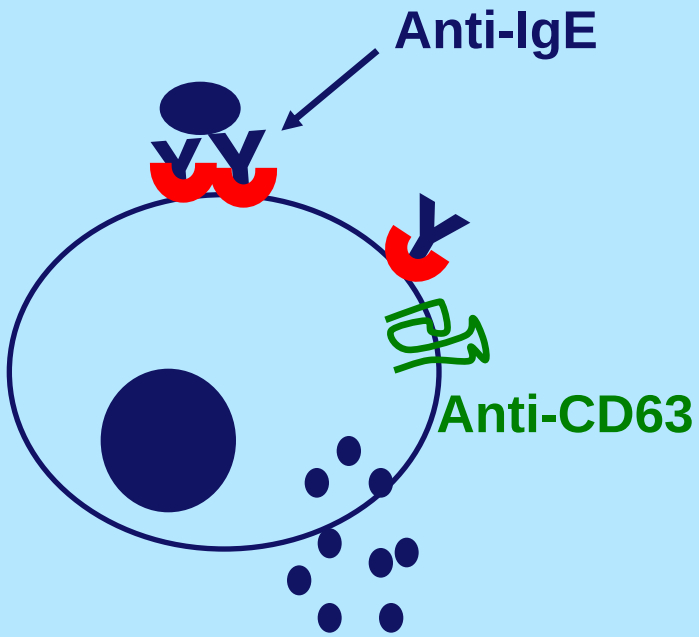
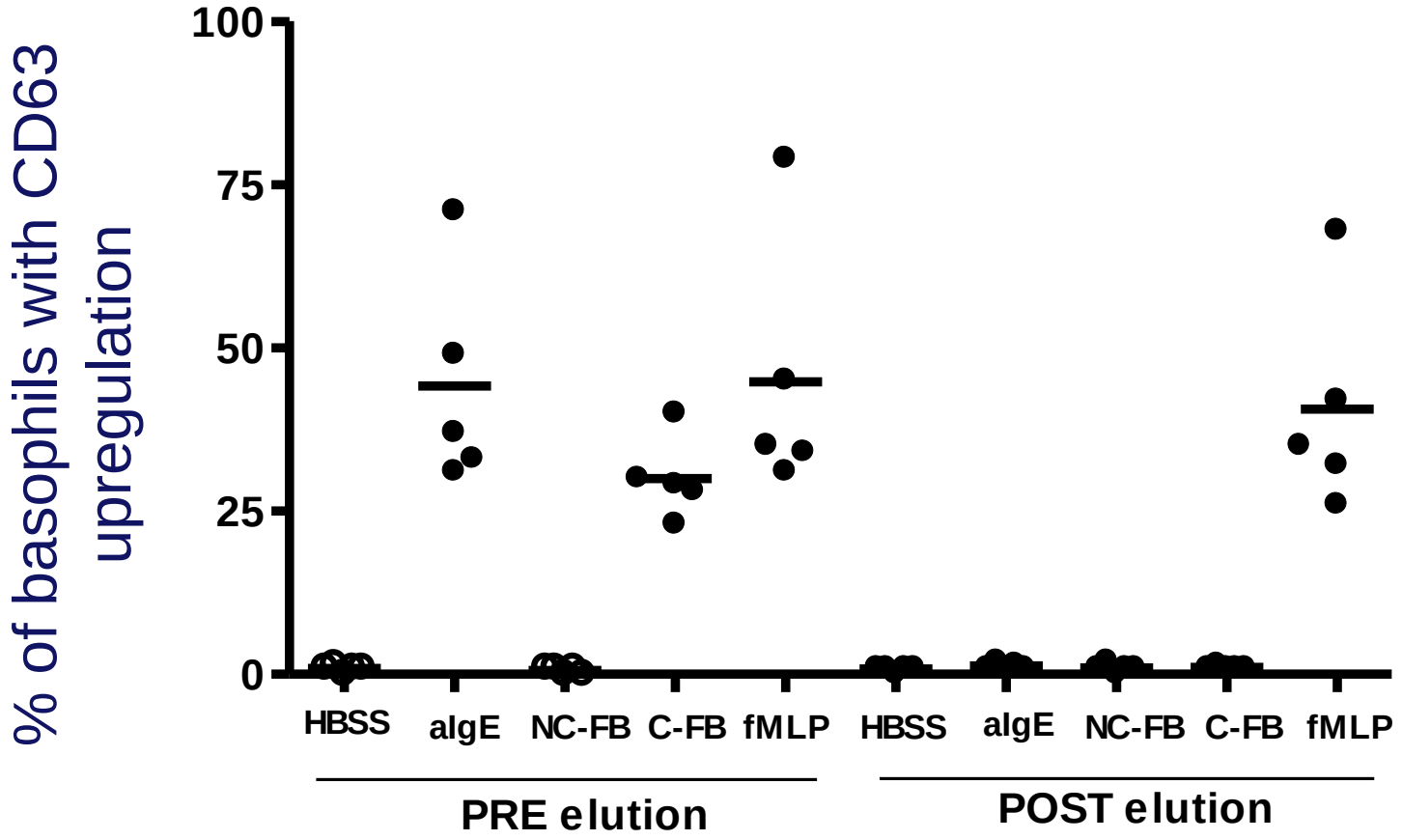
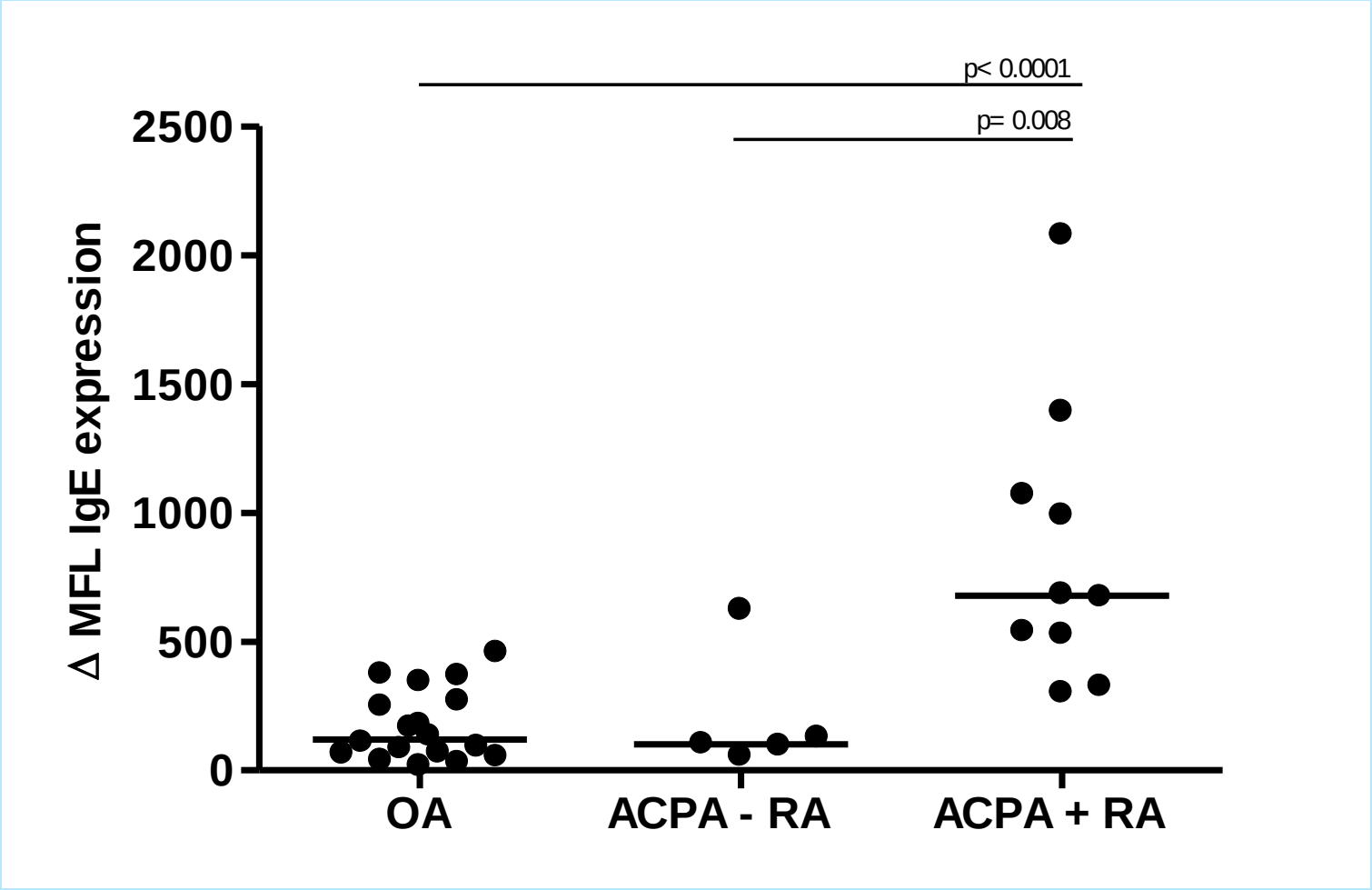




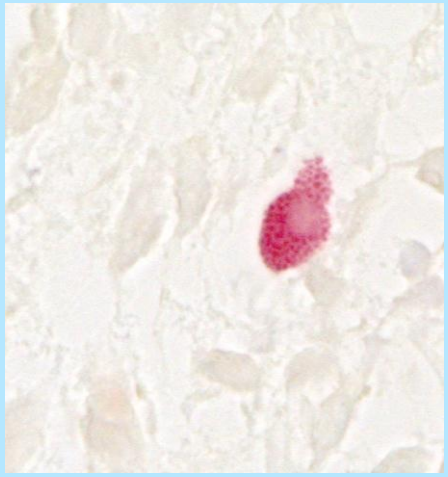
Basophils are no longer activated by citrullinated proteins after elution of Immunoglobulins

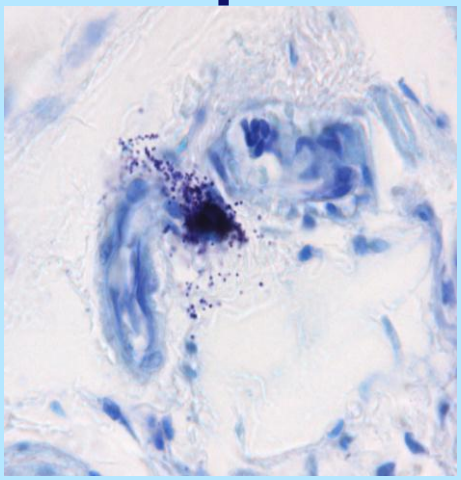


Elevated IgE expression on ACPA+ Synovial Mast Cells



Increased number of degranulated mast cells in synovial tissue of ACPA+ RA-patients





	All patients N=50	ACPA positive N=27	ACPA negative N=23	p-value
CD117 + Mast Cells (MC)	*27 (0-178)*	*33 (0-178)*	*11 (0-100)*	*0.003*
degranulated MC CD117+ CAE-	*21 (0-173)*	*28 (0-173)*	*6 (0-67)*	*0.005*
% degranulated MC	*82 (0-100)*	*89 (0-100)*	*58 (0-100)*	*0.03*

Conclusion:

- IgE ACPA are present in ACPA+ RA
- FcεR+ cells from ACPA+ RA-patients are activated by citrullinated Antigens
- enhanced degranulated mast cells in ACPA + RA

- *Moreover*
- Elevated histamine-levels in ACPA+
- Synovial fluid
  Histamine-levels correlate with IgE-levels in ACPA+ Synovial fluid
- All point to function for IgE-ACPA in RA

Proc Natl Acad Sci U S A. 2010 Feb 9;107(6):2586-91. Epub 2010 Jan 25.

Evidence for a functional role of IgE anticitrullinated protein antibodies in rheumatoid arthritis.

Schuerwegh AJ, Ioan-Facsinay A, Dorjée AL, Roos J, Bajema IM, van der Voort EI, Huizinga TW, Toes RE.

TIGER-study

- Omalizumab inhibits the binding of IgE to the high-affinity IgE receptor by binding to an epitope on IgE that overlaps with the site to which FcεRI binds.
- Thus surface FcεRI-bound IgE cannot be crosslinked and mediator release from basophils and mast cells is prevented.
- The epitope to which omalizumab binds is sterically hindered by the receptor when IgE is bound to the receptor and is therefore not accessible to omalizumab binding- no anaphylaxis.
- By binding to IgE in solution, omalizumab prevents IgE binding to cell surface receptor (both FcεRI and FcεRII). This limits the degree of release of mediators.

Tiger-study

- A prospective open **single-center, phase IIa study** investigating anti-IgE
- therapy (Omalizumab□) in refractory IgE-ACPA+ RA patients.
- 2 x 40 patients placebo versus verum

- Outcome measurement reduction in inflammation & basophil activation via IgE-ACPA by citrullinated proteins

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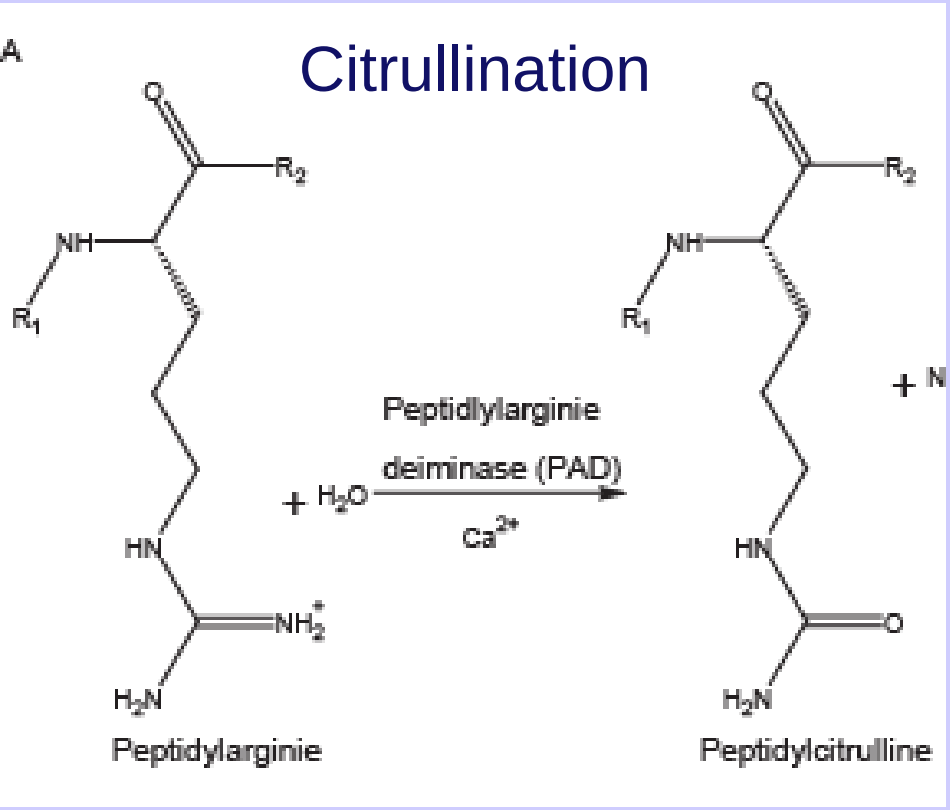
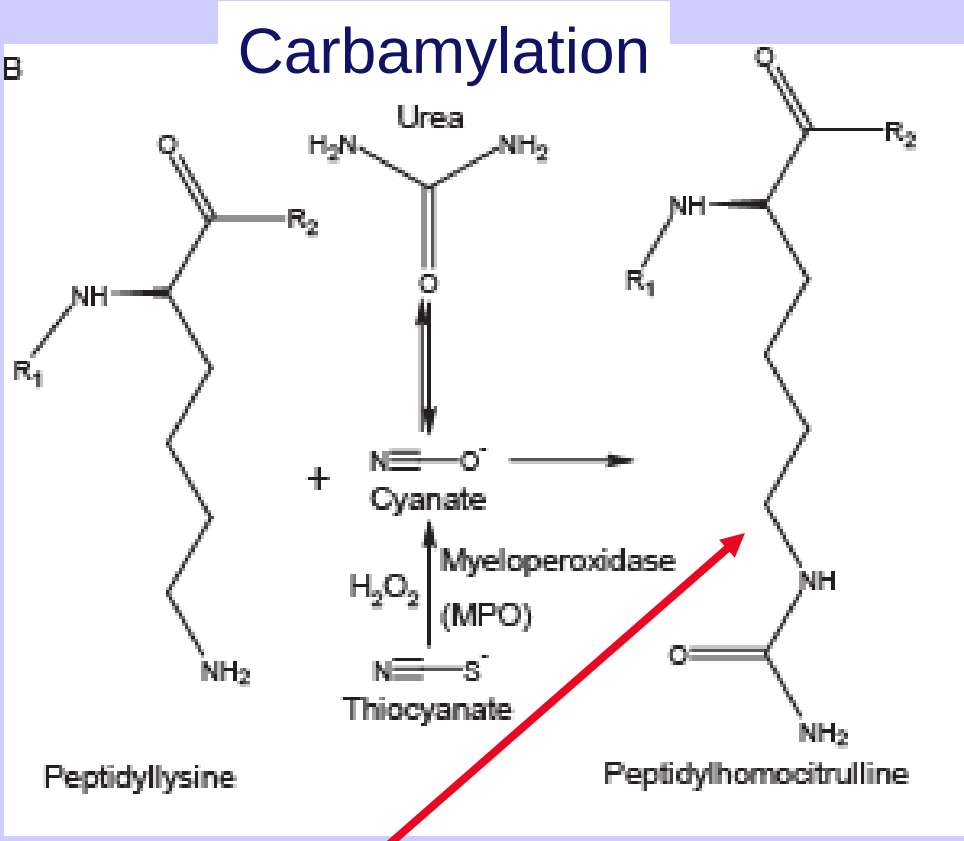
- Classification criteria classify a mix of patients
- Matters in intervention trials given different clinical course of different subsets
- Statistical methodology: CCP+ versus CCP- groups different subgroups
- Recognition of an arteficial peptide sofar the best way to group ACPA+ versus ACPA-negative RA
- IgE-CCP exploits FcεR+ cells in RA pathogenesis in this subgroup allowing personalized medicine
- Are there other antibody systems that define subgroups?

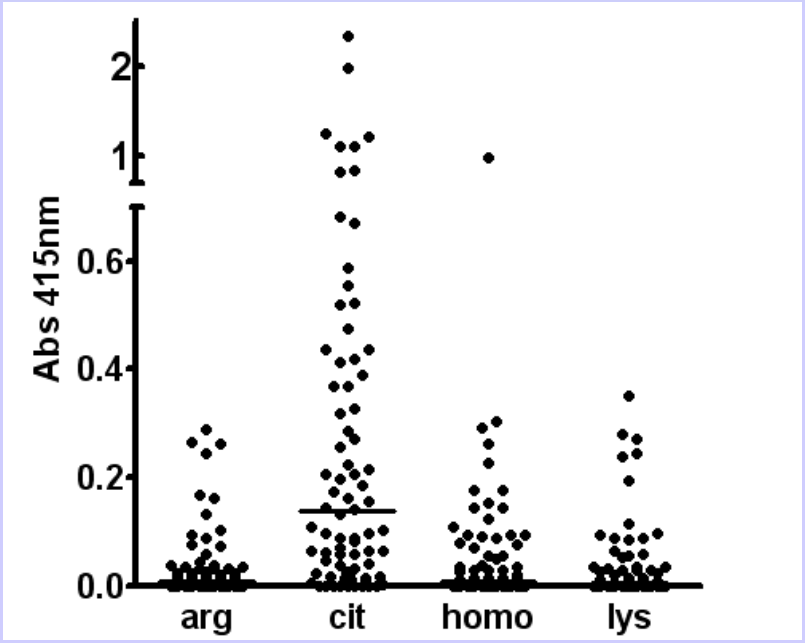
Posttranslational modifications: Citrulline and Homocitrulline





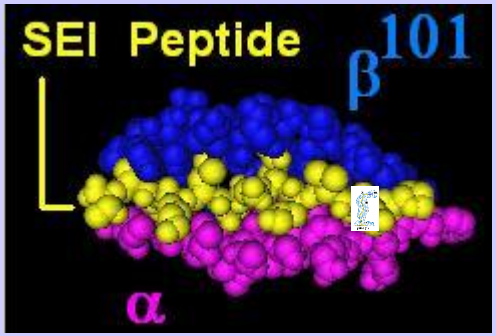
Difference only 1 C-atom

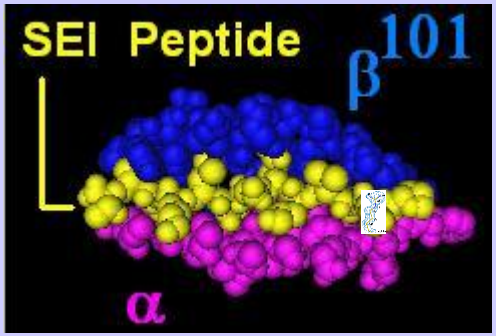
ACPA can discriminate between citrulline and homocitrulline in the same peptide



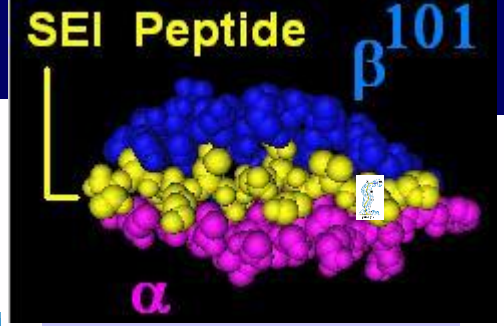
Sequence of different forms

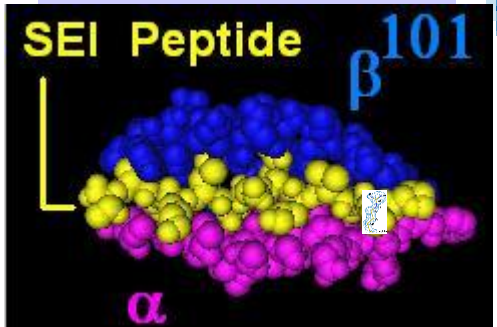
Arg	***NEE GFF SAR GHR PLD KK***
Cit	***NEE GFF SACit GHR PLD KK***
Homo	***NEE GFF SAHomoGHR PLD KK***
Lys	***NEE GFF SAK GHR PLD KK***

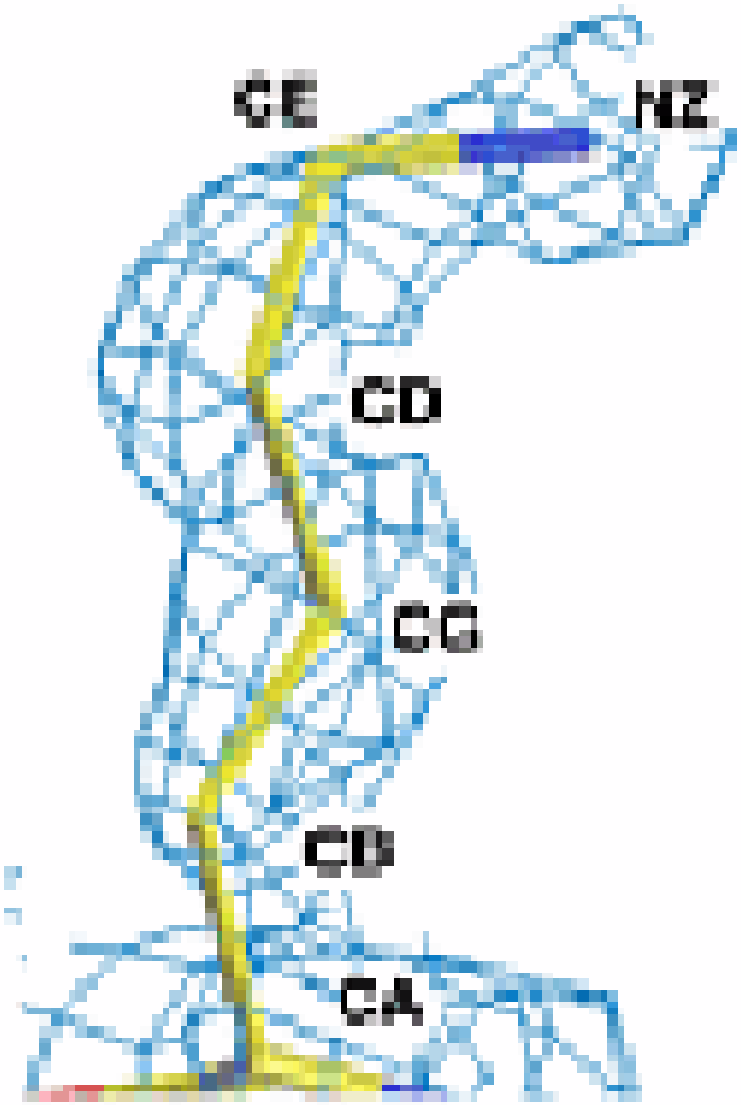




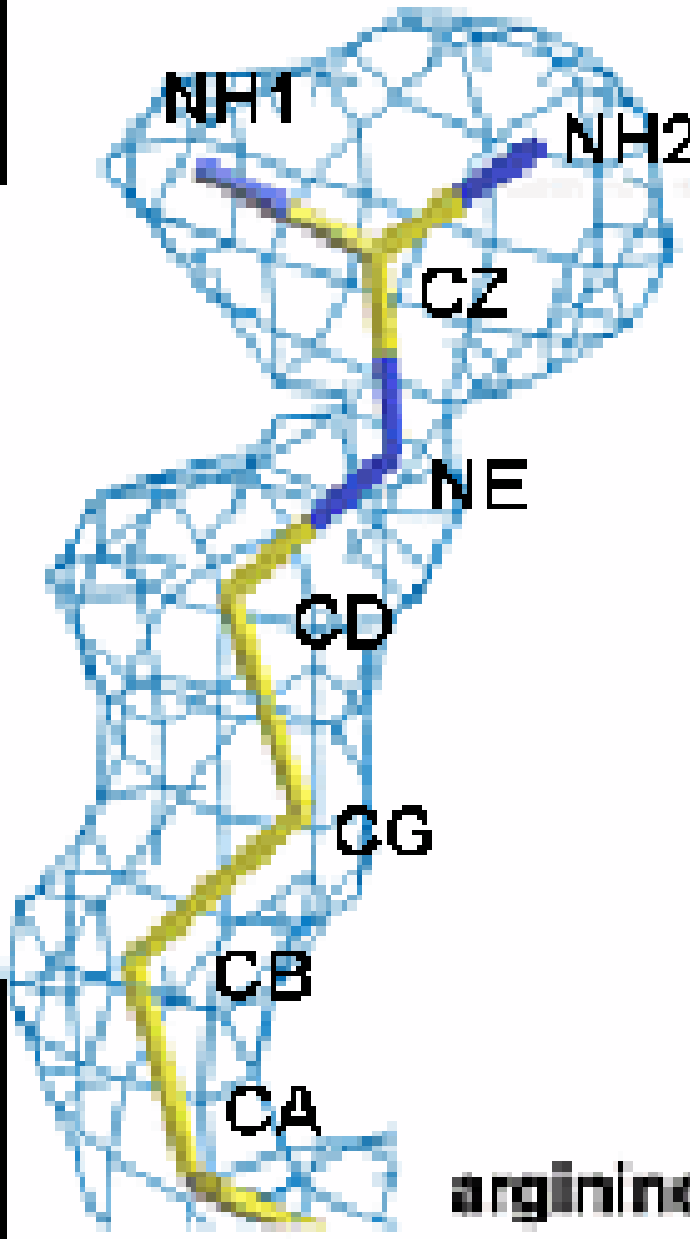
Amazing that one C-atom difference can be seen by the immune sysem





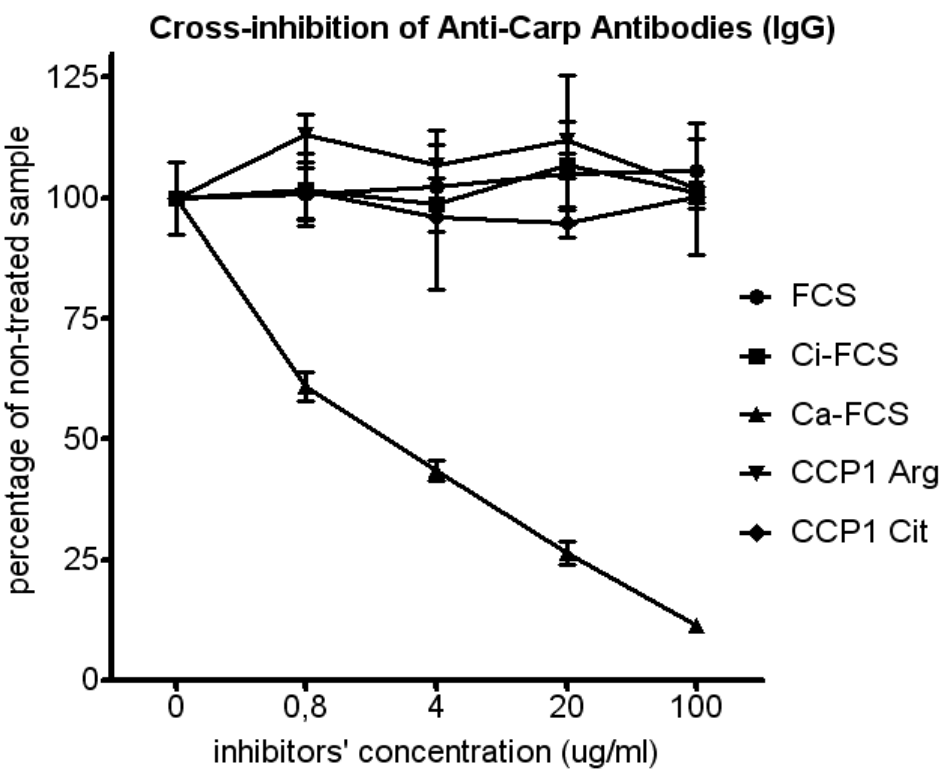


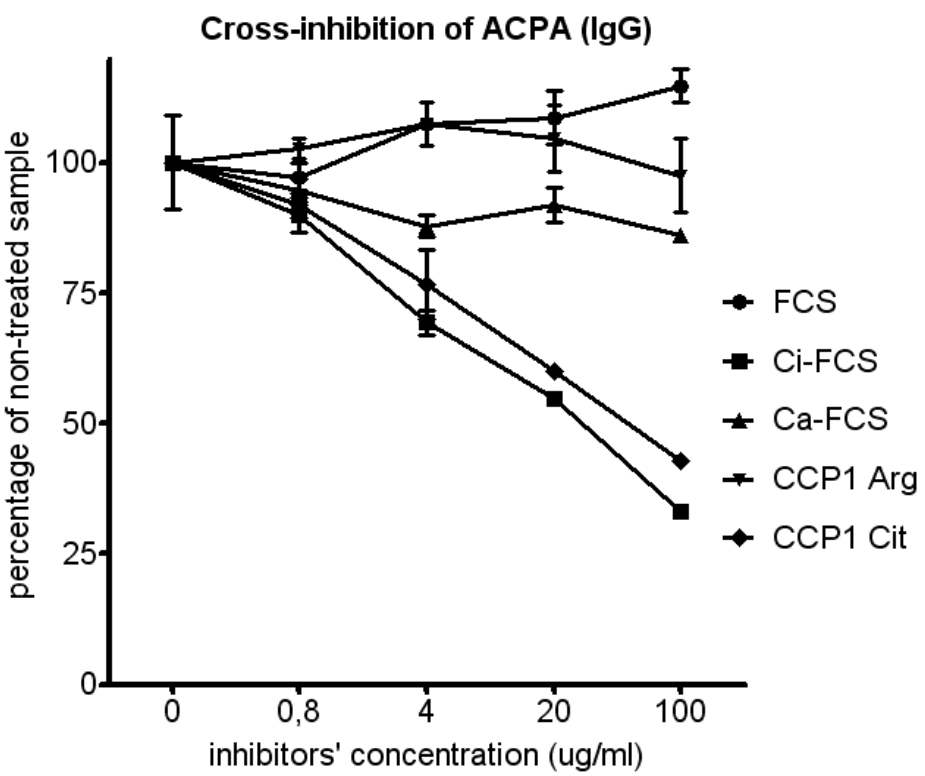
lysine (K)



arginine

Anti-CarP antibodies and ACPA are not cross reactive





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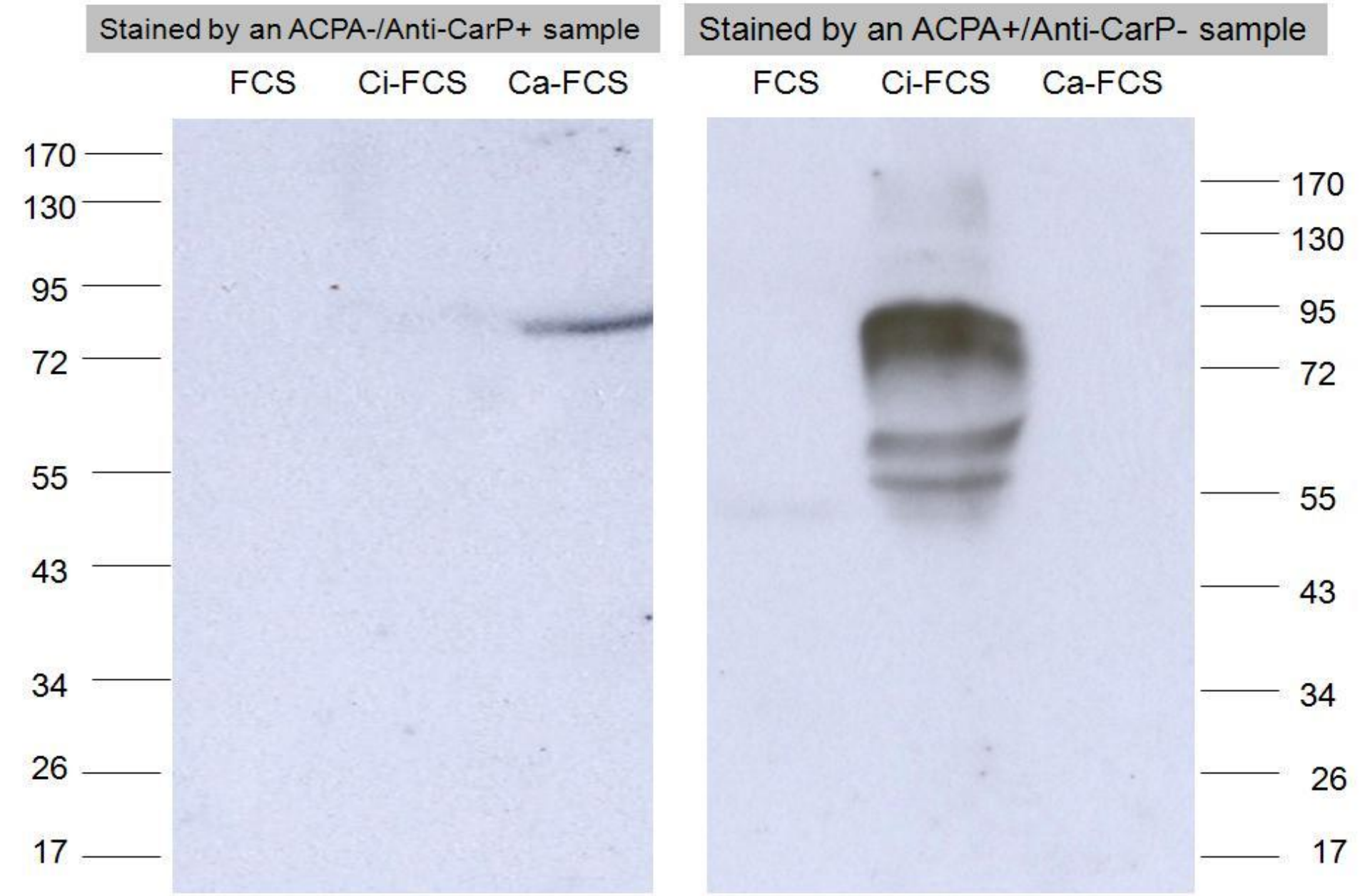
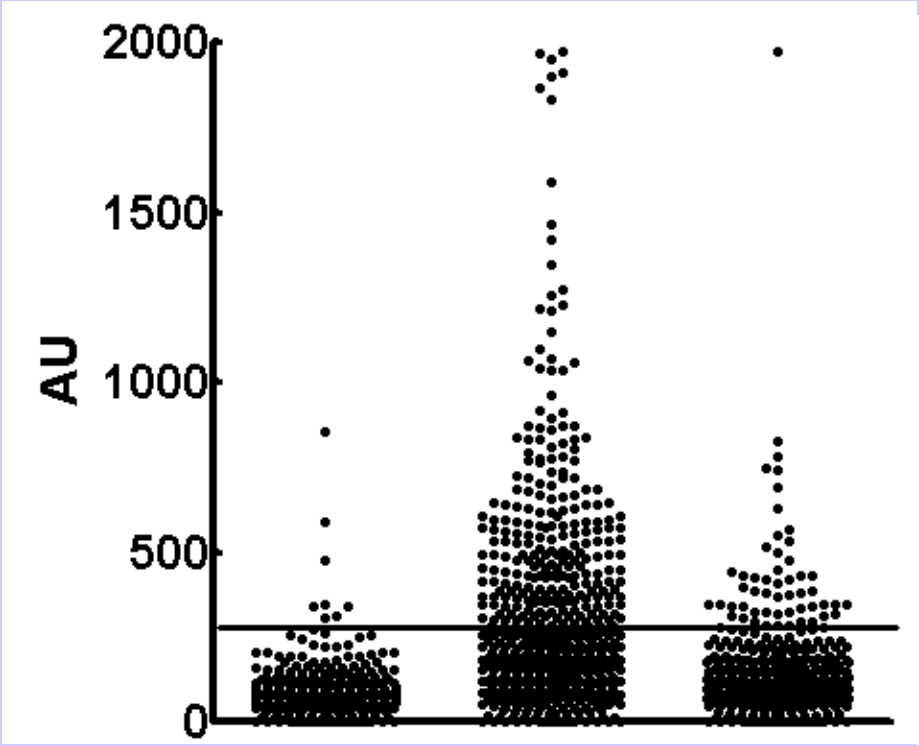
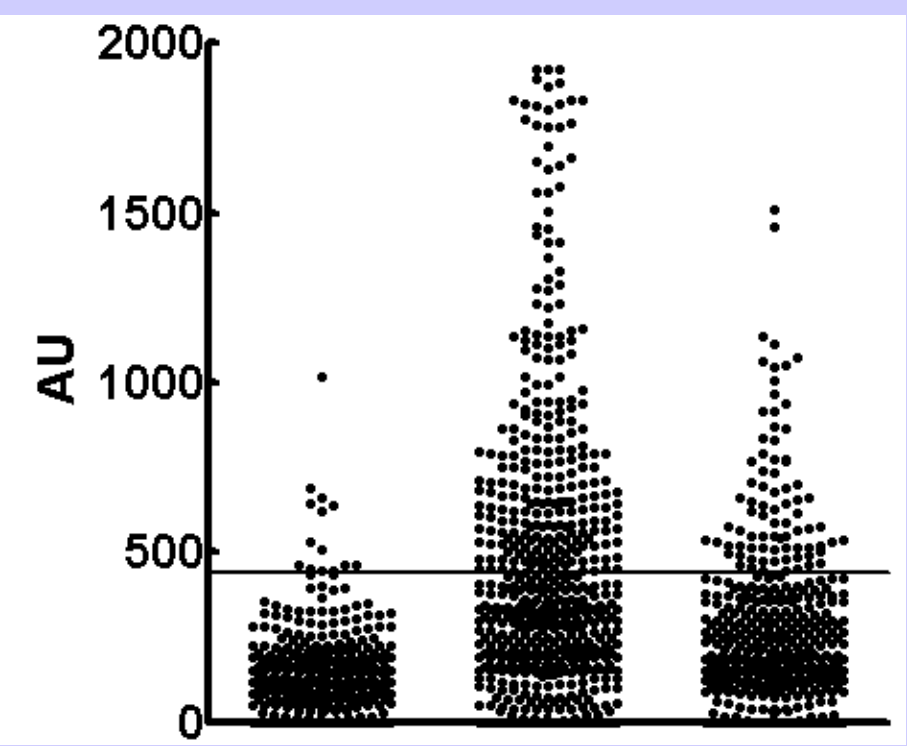


Anti-CarP IgG and IgA are present in RA

IgG



IgA

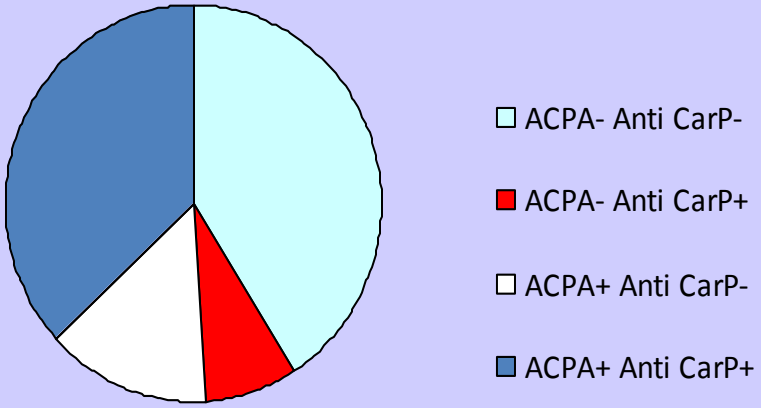


disease	*NHS*	*RA*	*UA*
number	*305*	*557*	*403*
% positive	*2.95%*	*44.9%*	*14.1%*

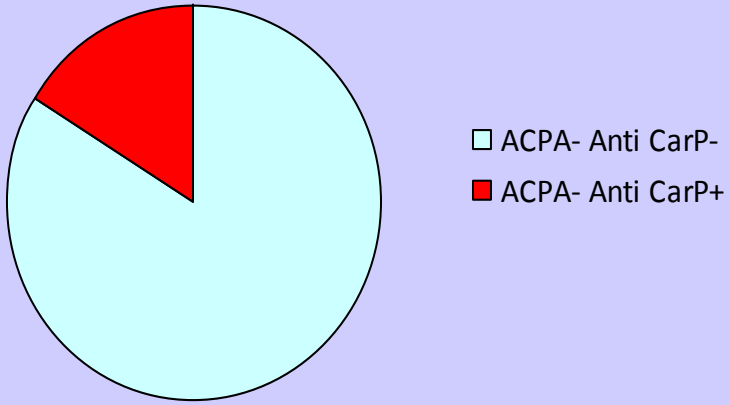
	NHS	*RA*	*UA*
number	*305*	*557*	*408*
% positive	*5.24%*	*43.0%*	*19.9%*

Anti-CarP versus ACPA in established RA (87)

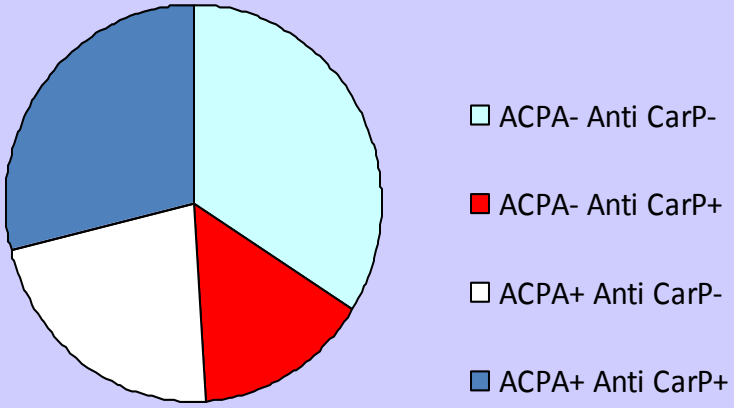
% pos in all RA pt (IgG)



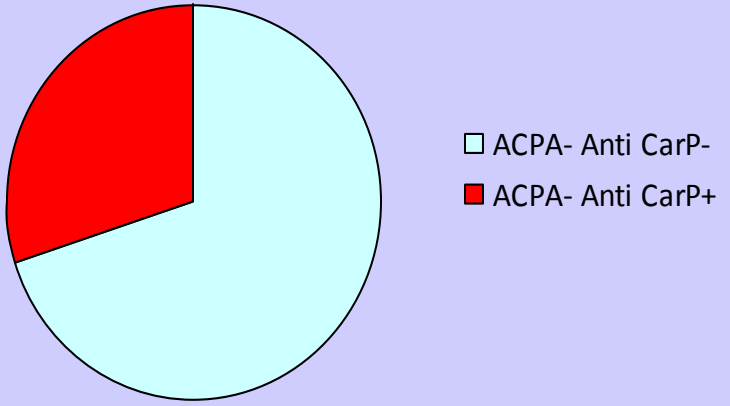
% in ACPA neg RA patients (IgG)



% in all RA pt (IgA)

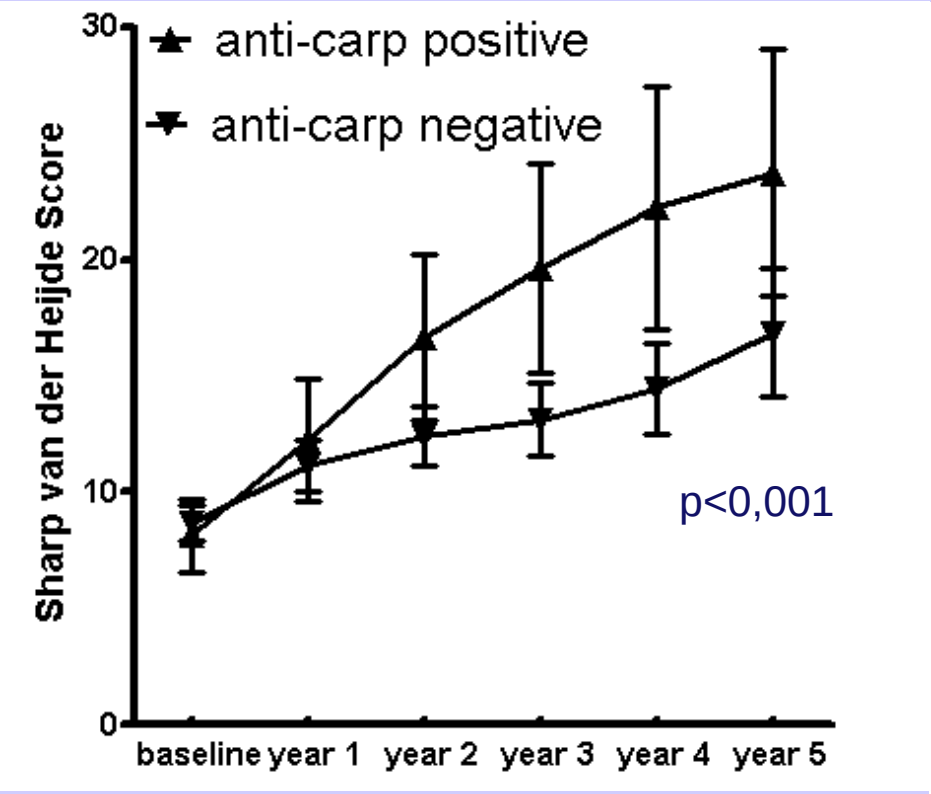


% in ACPA neg RA patients (IgA)

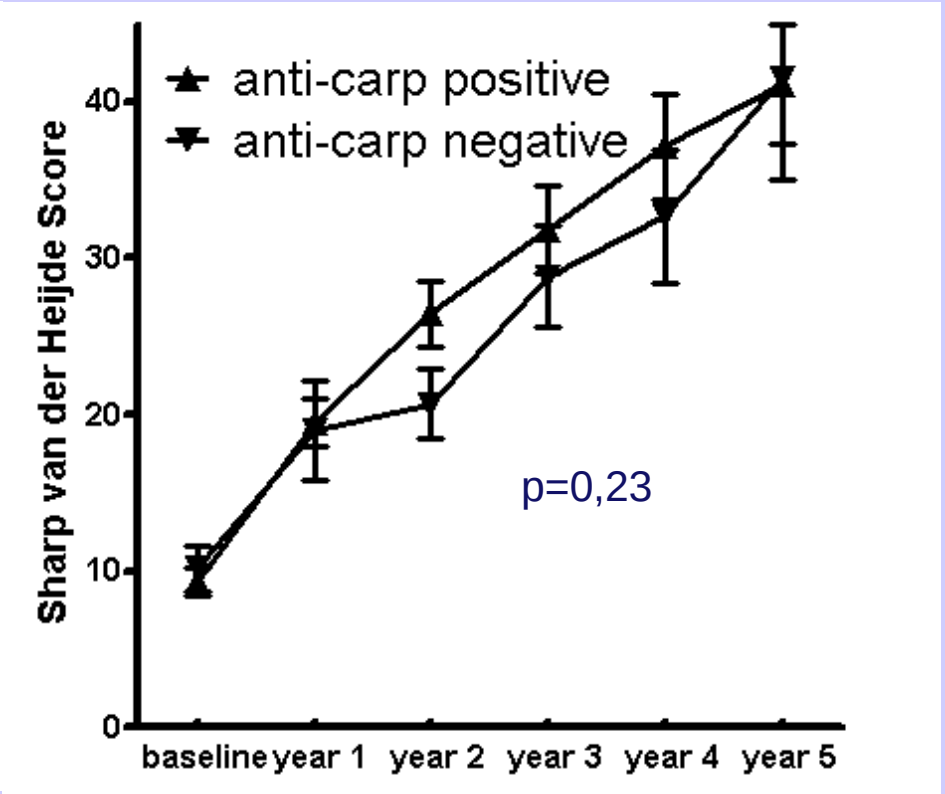


Association between Anti-CarP and radiographic damage

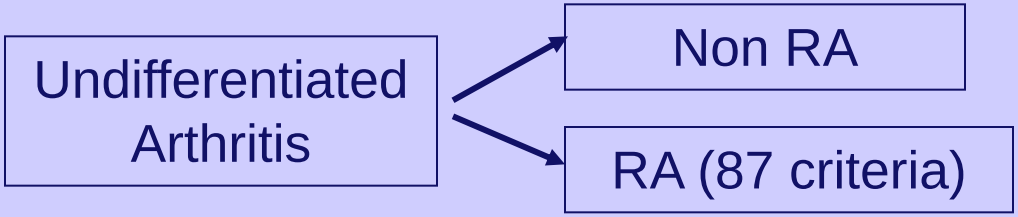
ACPA-negative

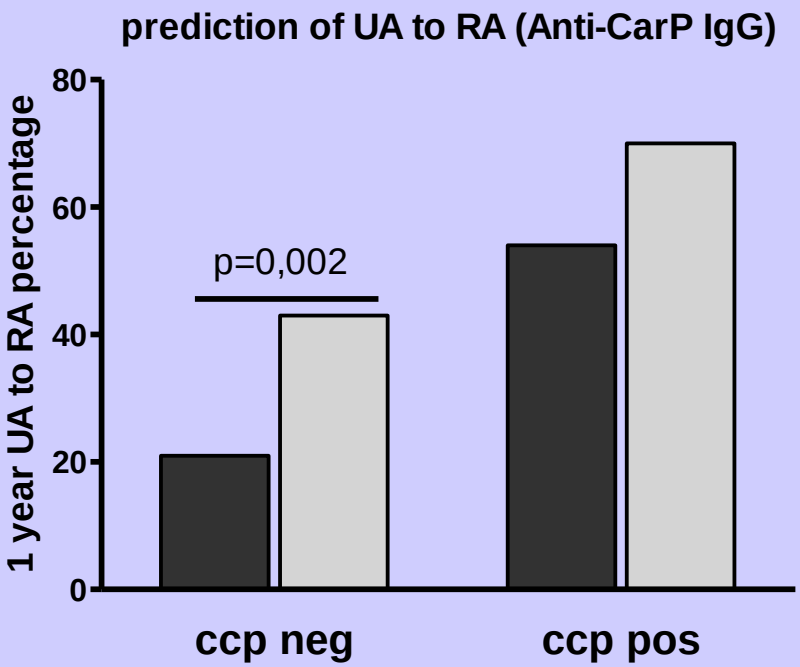


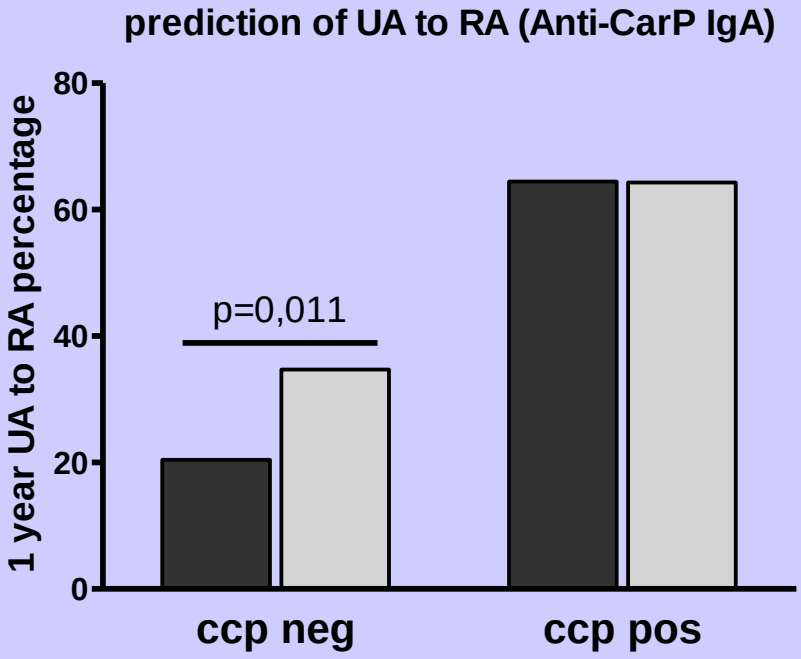
ACPA-positive



Anti-CarP antibodies predictive for conversion UA to RA







What can be learned from RA as a prototype autoimmune disease?

- Classification criteria classify a mix of patients
- CCP+ versus CCP- groups different subgroups relevant for clinical trials and relevant for studying pathogenesis
- Usage of isotype ACPA matters: IgE-CCP exploits FcεR+ cells in RA pathogenesis in this subgroup allowing personalized medicine
- Autoantibodies can discriminate between a citrulline and a homocitruline containing antigen
- Antigenic specificity matters: Anti-CarP antibodies have an impact on the clinical presentation of RA independent of ACPA
- Anti-CarP identify a distinct group within ACPA neg RA

Acknowledgements

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Diane van der Woude
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Kirsten de Boer
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Lotte van der Stadt

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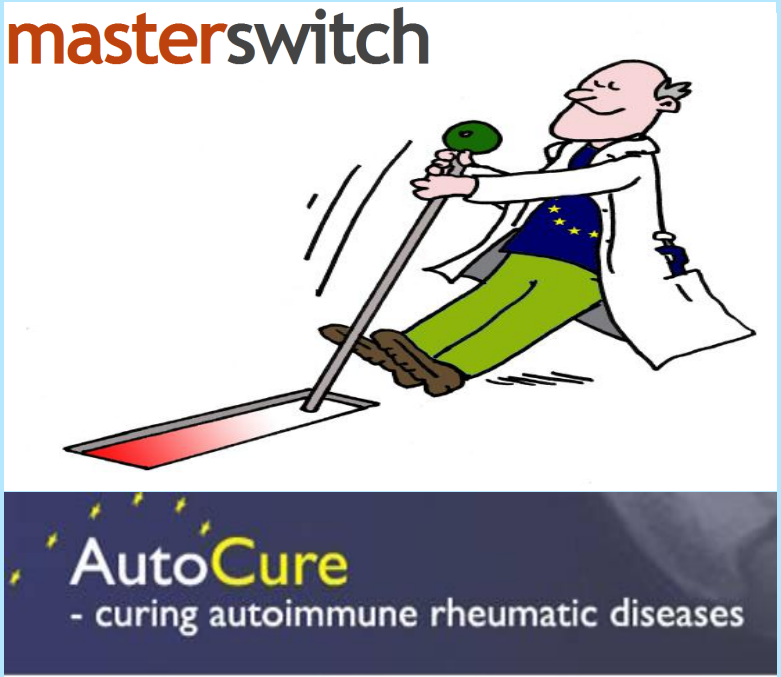

The Dutch Arthritis Association

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