Rheumatoid arthritis:

A heterogeneous disease with a heterogeneous response to treatment

Pr Pierre Miossec MD PhD

Clinical Immunology Unit

Hôpital Edouard Herriot

Lyon

miossec@univ-lyon1.fr

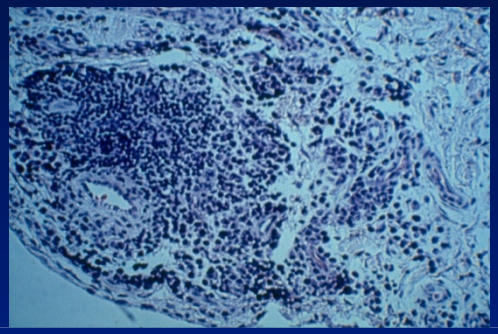
Pathogenesis of Rheumatoid Arthritis

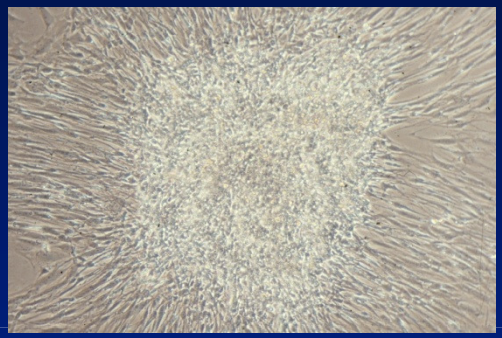
Exogenous factors

Bacteria ?
Virus ?
Smoking

Endogenous factors

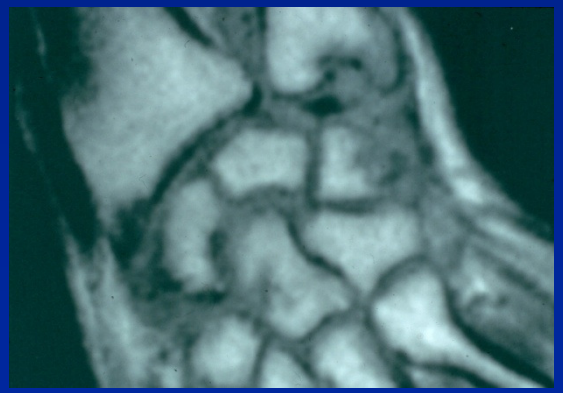
Sex
Hormones
Genetic factors





Blood-derived mononuclear cells in migration to joints

Mesenchymal cells in residence inside joints



Chronic inflammatory reaction

Production of proinflammatory factors

Bone and cartilage destruction

Rheumatoid arthritis: more than one picture

Early

Benign

Responder

Late

Severe

Non-responder

Rheumatoid arthritis: markers and heterogeneity in treatment response

- Environmental factors: smoking, food, parasites
- DNA markers: Shared epitope, other gene polymorphisms (SNP)
- RNA markers: from one gene to the whole genome
- Proteins: antibodies (rheumatoid factors, anti-CCP) cytokines, markers of formation/degradation of bone, cartilage, synovium; biopsies

Where to look for markers of heterogeneity and treatment response

- Environmental factors: population studies
- DNA markers: blood is fine
- RNA markers: blood or synovium?
- Proteins: reflection of local and systemic situation
- Structure of the synovium, cartilage, bone : synovial fluid, urine or blood markers

  synovium biopsy

Heterogeneity of rheumatoid arthritis

- Typical RA:

  HLA DR shared epitope, anti-CCP, smoking

- Atypical RA:

  lack of common markers

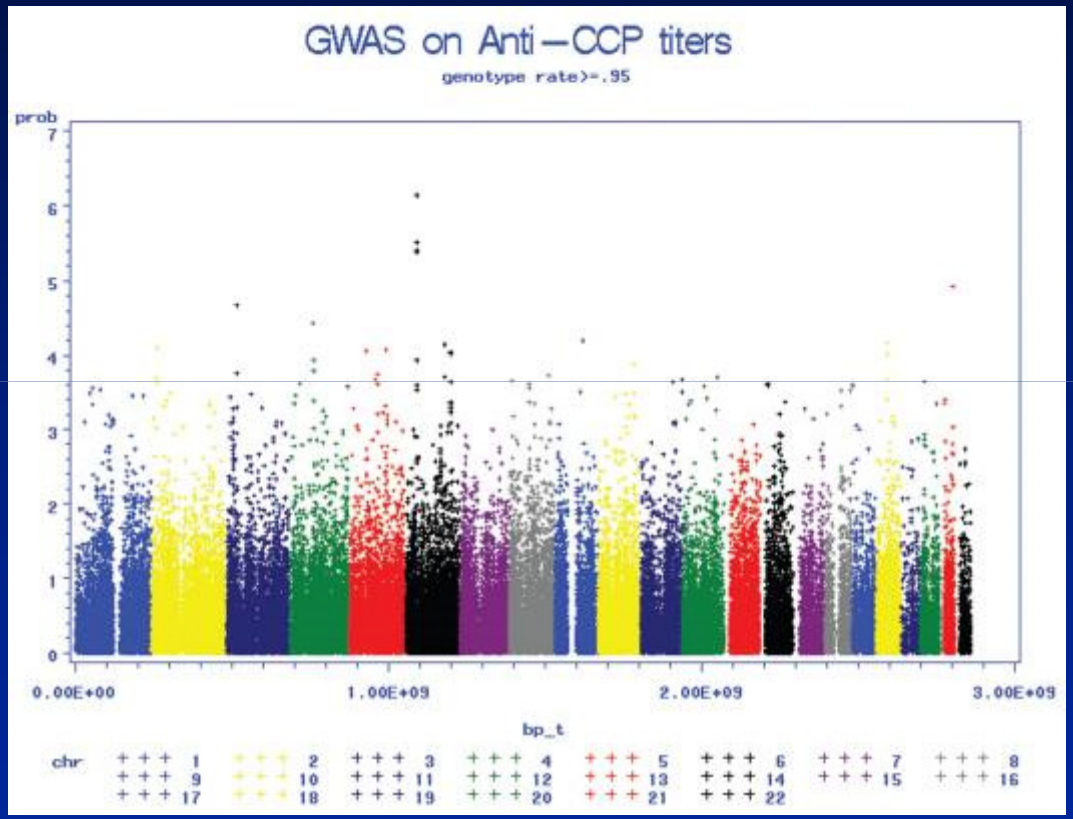
  less severe

  pathegenesis less understood

Markers of heterogeneity: 3 exemples

- DNA: Genetic markers and heterogeneity in disease expression and treatment response
- RNA: Synoviolin out of a large list of genes
- Protein: Levels of TNF and anti-TNF response: systemic vs. joint levels; bone, synovium, cartilage biomarkers; proteomic studies

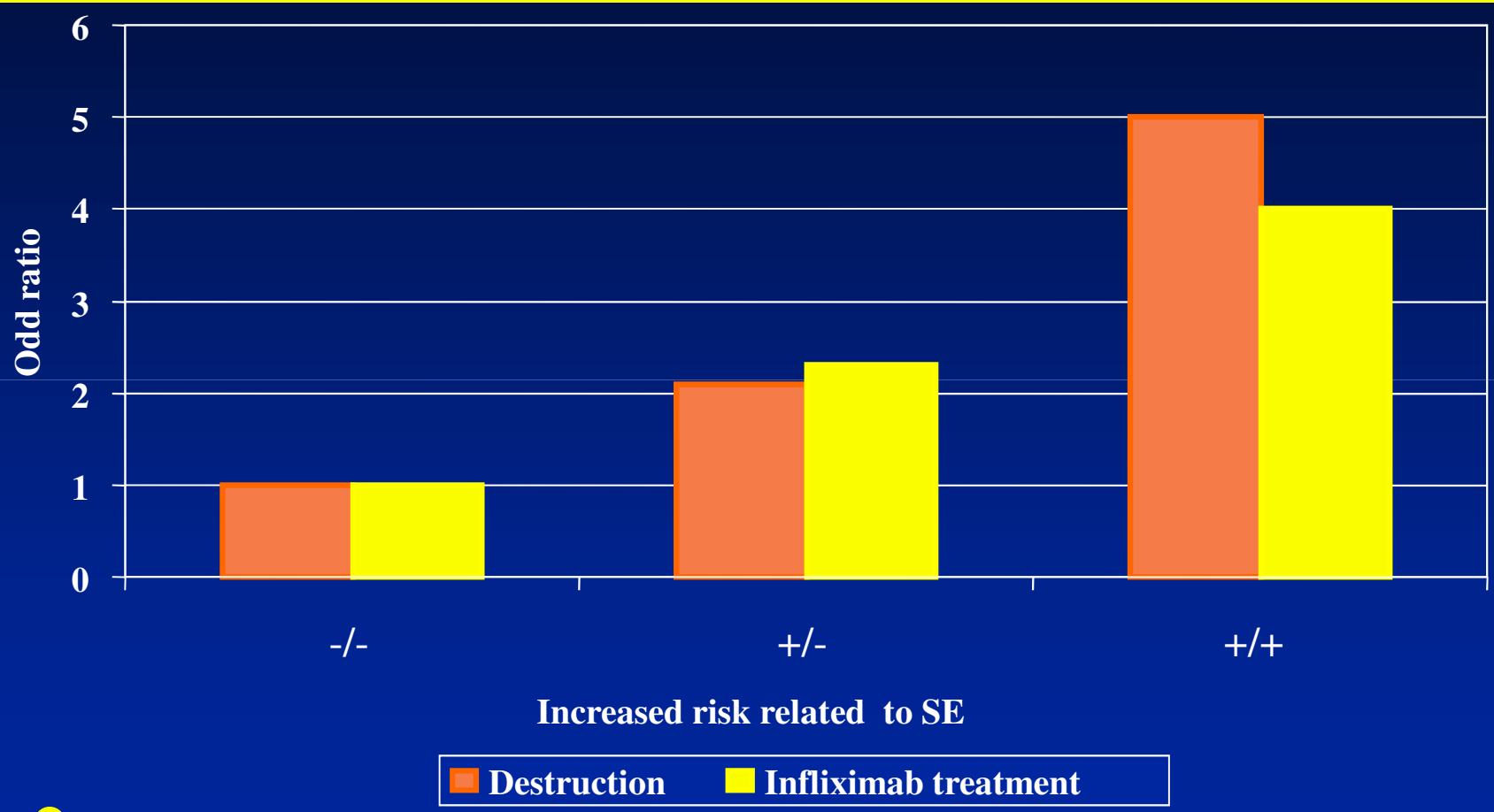
SNP analysis in RA: success or expensive failure?



- HLA-DR Shared Epitope 0401, 0404, 0101
- PTPN22
- TRAF1-C5 region
- STAT4
- PADI4 (Asia)

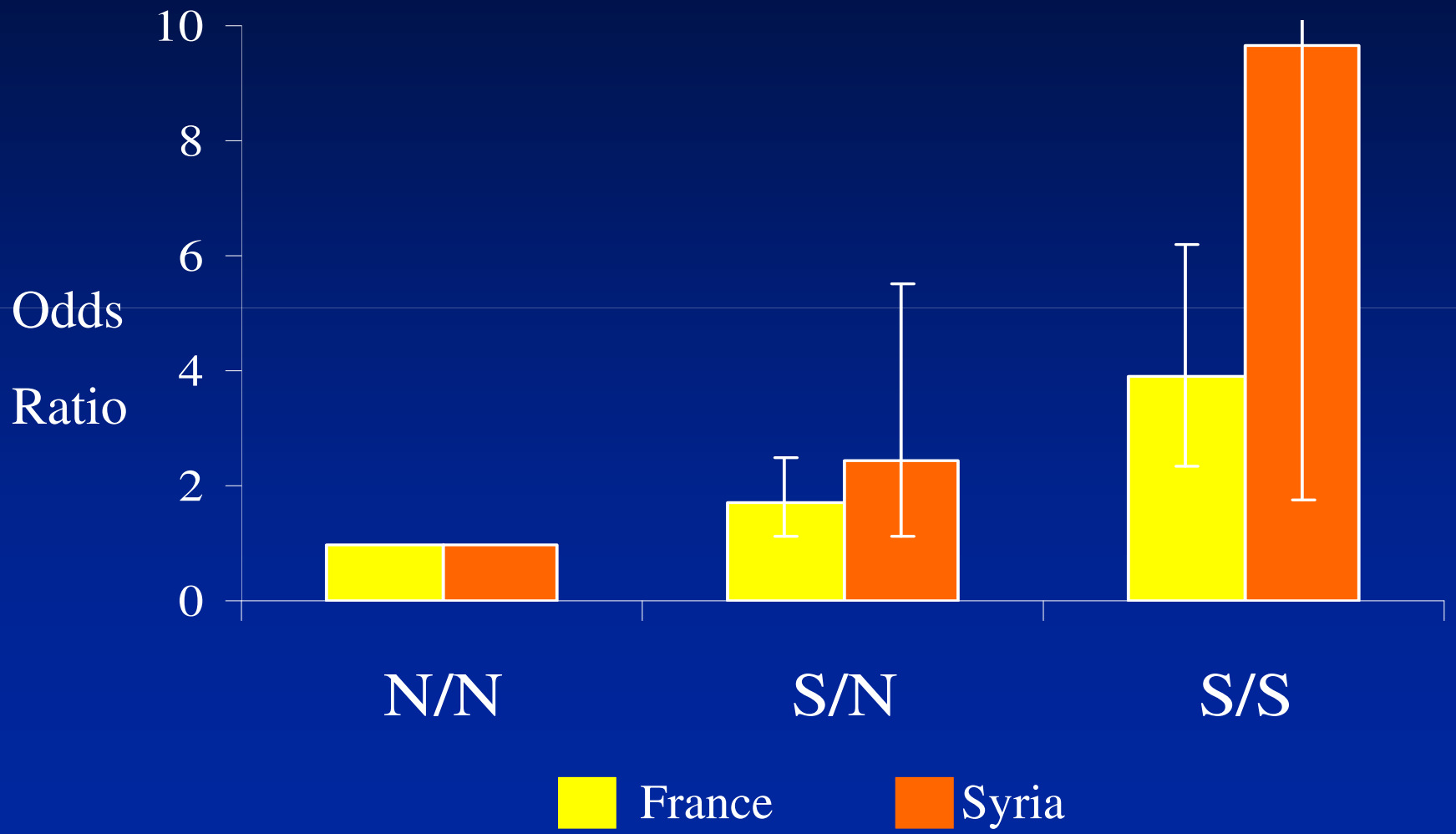
R Plenge 2009

Shared epitope association with increased risk of destruction and treatment with infliximab



- Risk for destruction was calculated in a population of 637 RA patients
- Risk to be treated with infliximab was calculated in a population of 165 treated patients

Association between the SE status and the risk for RA joint destruction in Syria and France



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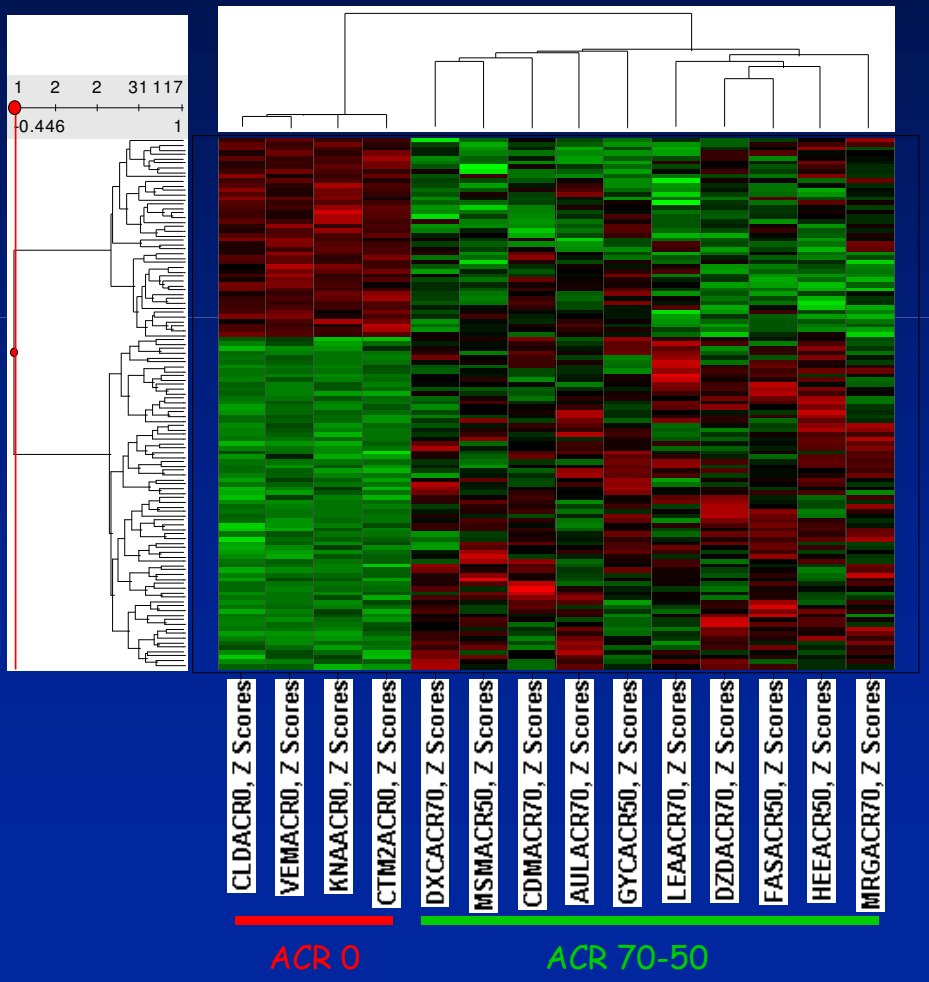
Non-hypothesis-driven single gene selection: responders vs. non-responders

Affymetrix U133A micro-array expression profile in whole blood
ACR 50-70 vs. ACR 0 response to infliximab



Hetergeneity of published signatures:
Paxgene, Whole blood, PBMC
Array technology
Kinetics t0, 6m

Synoviolin contribution to RA

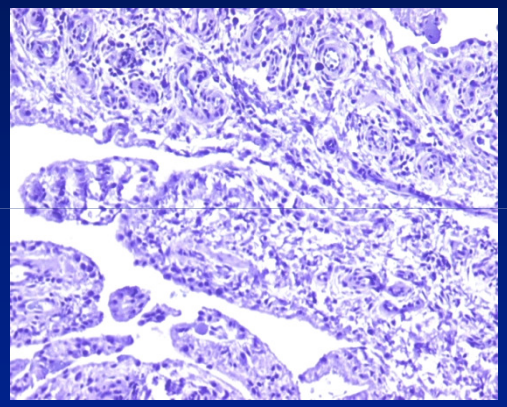
- Synovial hyperplasia
  - imbalance between synoviocyte proliferation and defective apoptosis
  - contributes to RA chronicity
- Synoviolin is a novel E3 ubiquitin ligase with anti-apoptotic effects and identified in RA synoviocytes and synovial lining tissue
- Mice transgenic for synoviolin develop spontaneous arthritis and KO mice are resistant
- Synoviolin mRNA levels are increased in RA blood using extensive micro-arrays

Expression of synoviolin in RA synovium

Control Ab

Anti-synoviolin Ab

A



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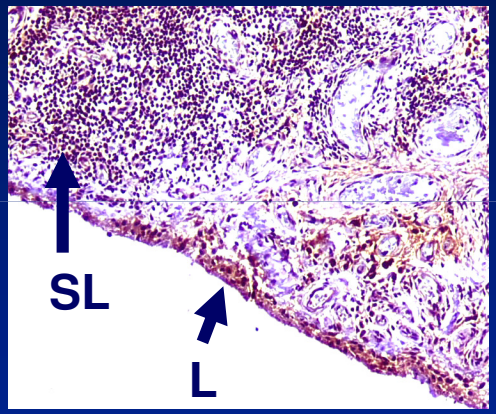

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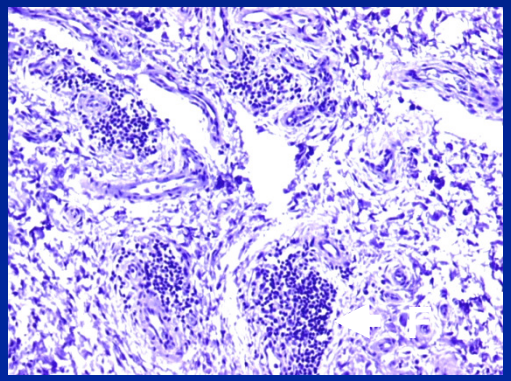

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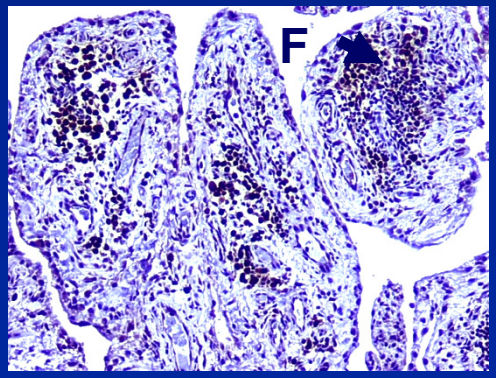
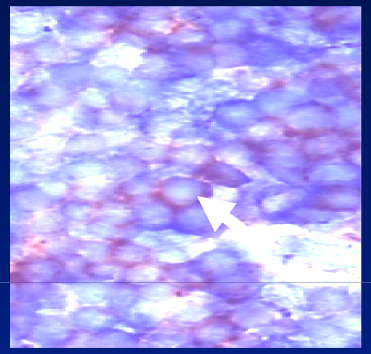


SL = sublining L = lining F = follicle

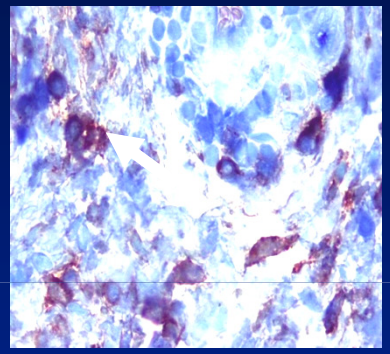
Synoviolin is expressed in RA synovium and blood immune cells

Synovium

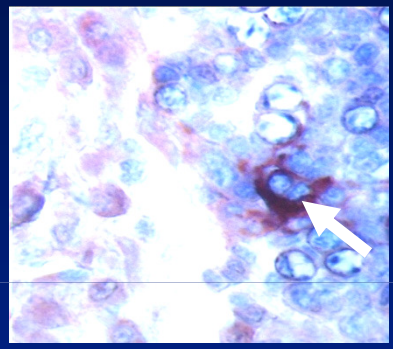
CD3 + synoviolin +



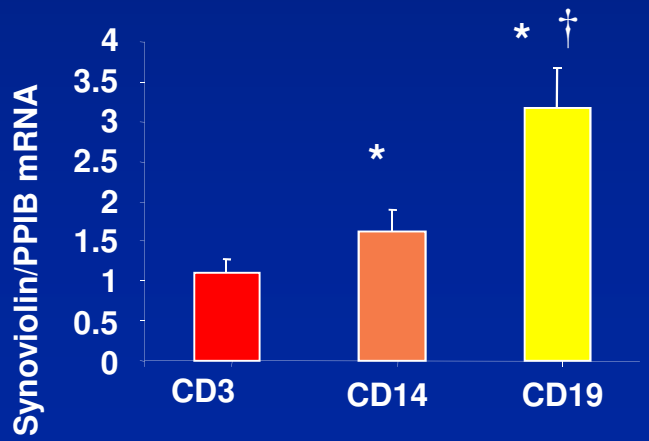
CD14 + synoviolin +



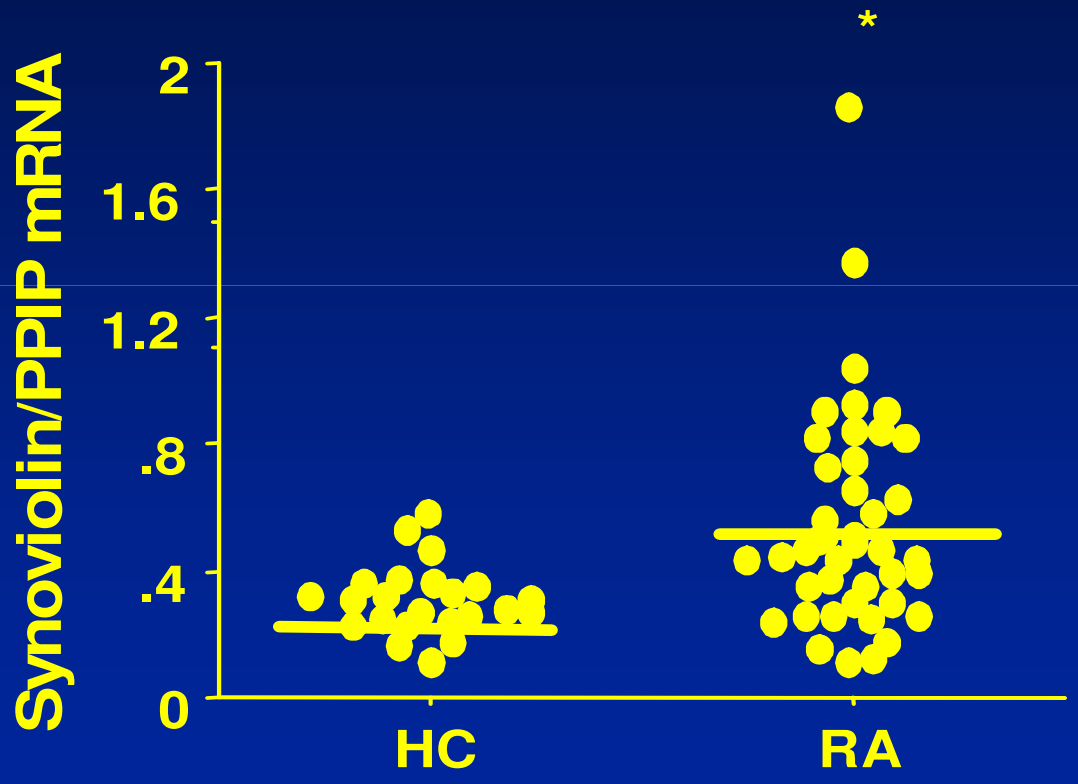
CD20 + synoviolin +



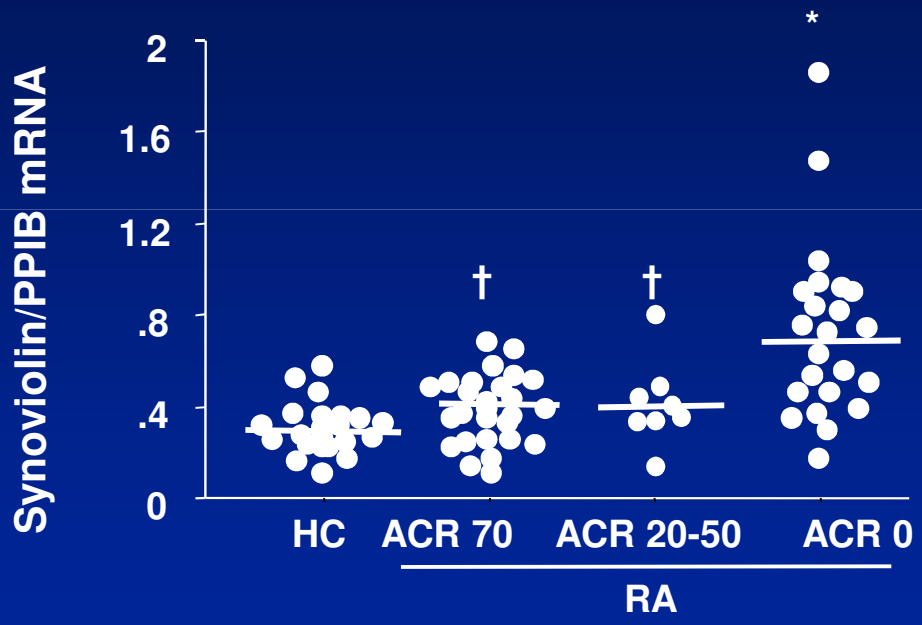
Blood

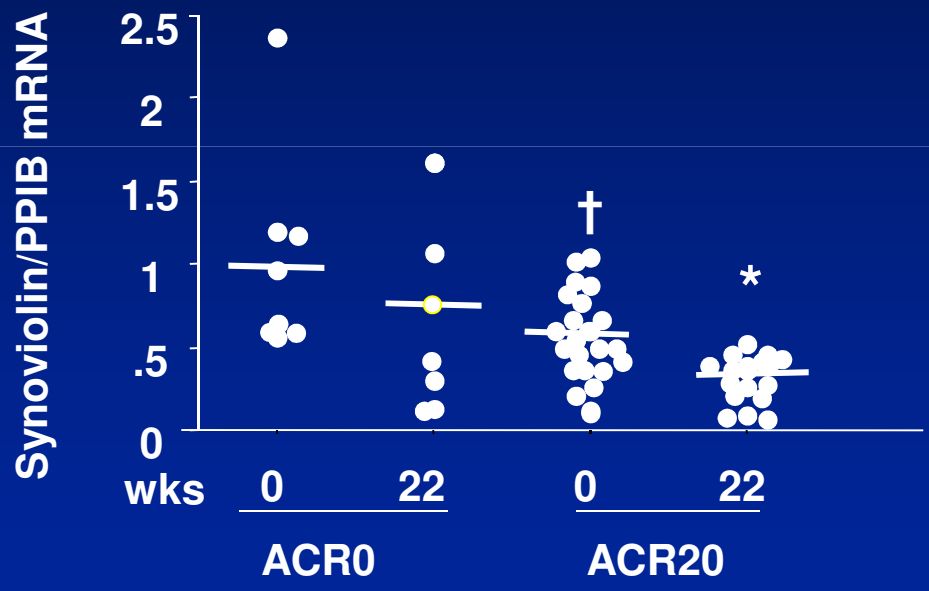


Synoviolin is over-expressed in RA blood

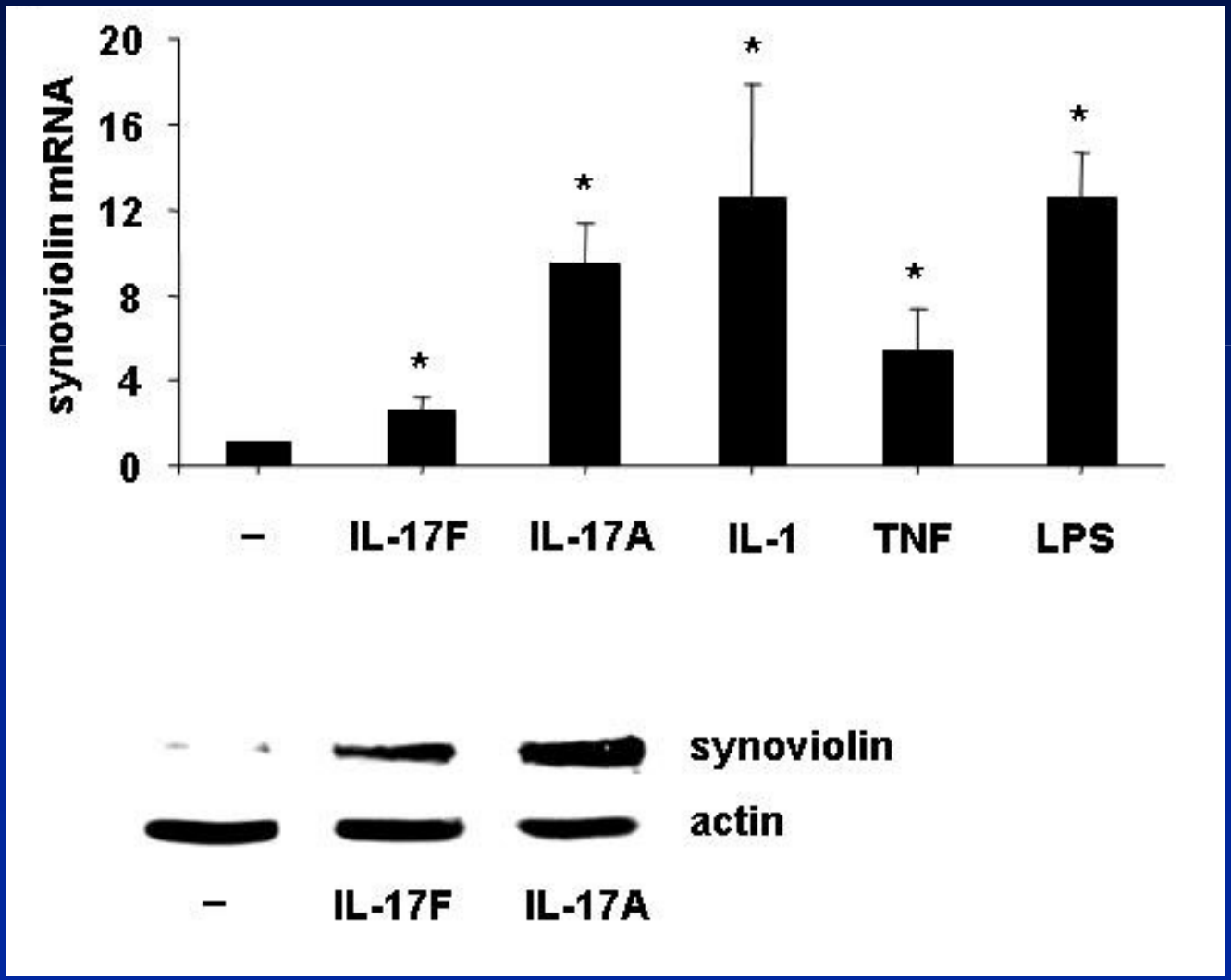


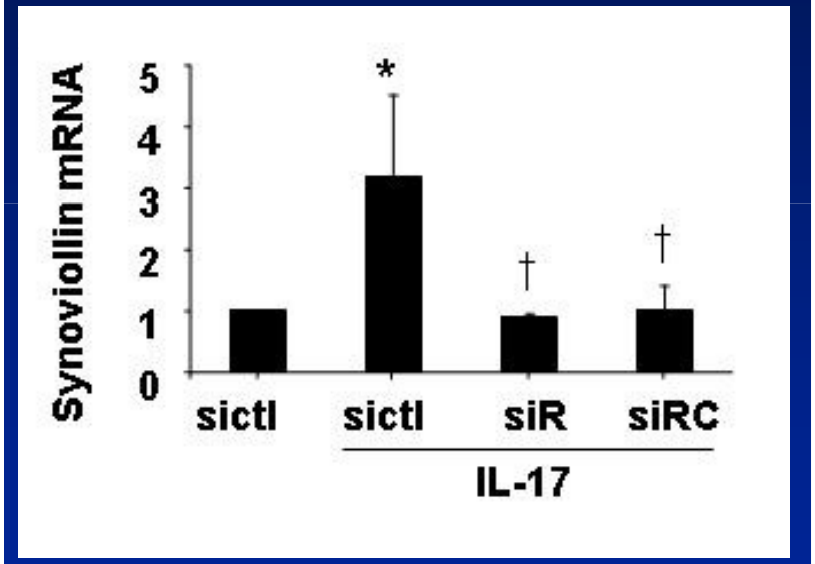
Synoviolin is over-expressed in infliximab non-responders



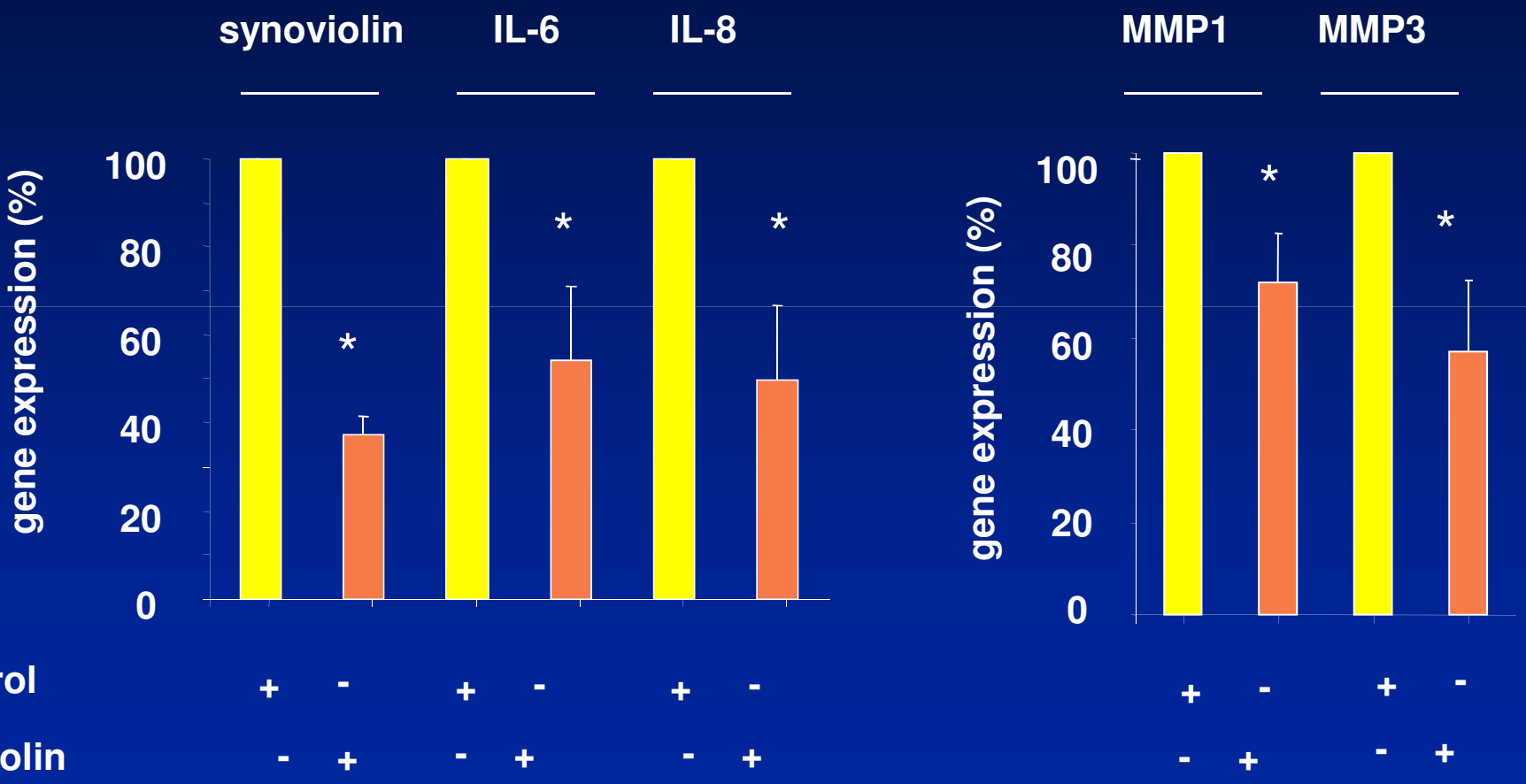


Induction of synoviolin in RA synoviocytes by proinflammatory cytokines





Endogenous synoviolin regulates cytokine, chemokine and MMP expression in RA synoviocytes



Markers of heterogeneity: 3 exemples

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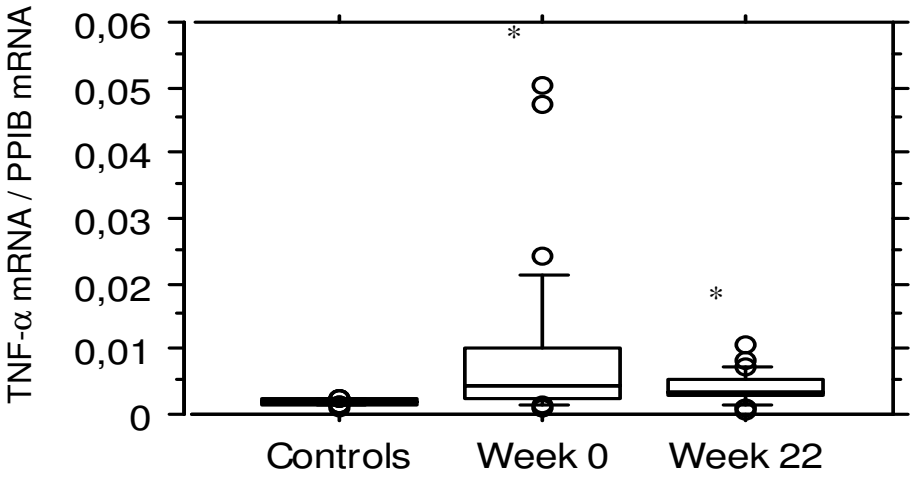
TNF as a marker of response

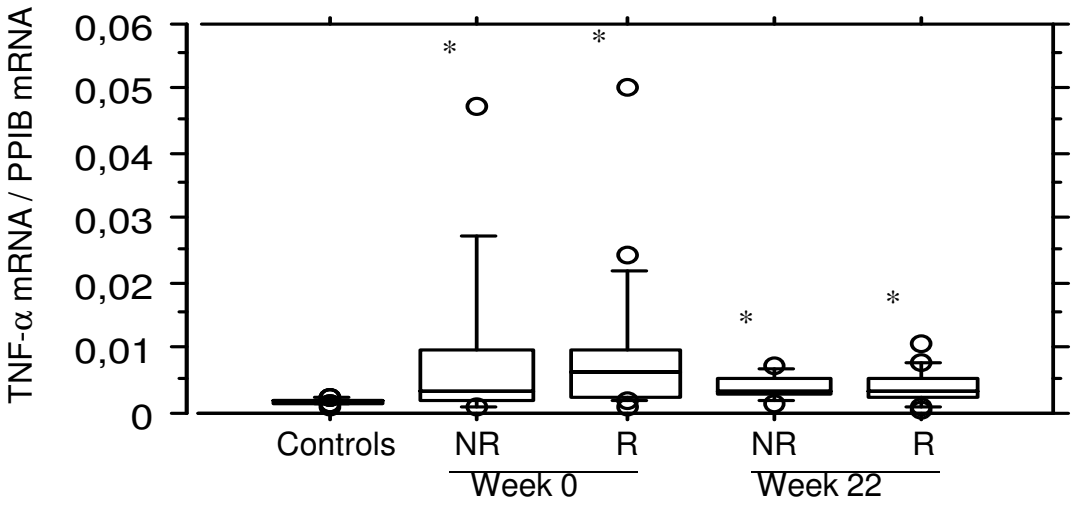
- Contribution of TNF has been shown at the site of inflammation
- Blood TNF could reflect joint-derived TNF
- Detection is complex because of endogenous inhibitors
- ELISA: protein levels (pg/ml), free or complexed with sR
- Bioassay: functional activity reflecting free levels (U/ml); addition of exogenous TNF to measure circulating inhibitors (sR, Ab)
- Differences reflect ligand receptor interactions

TNF mRNA levels in RA blood and response to TNF inhibition

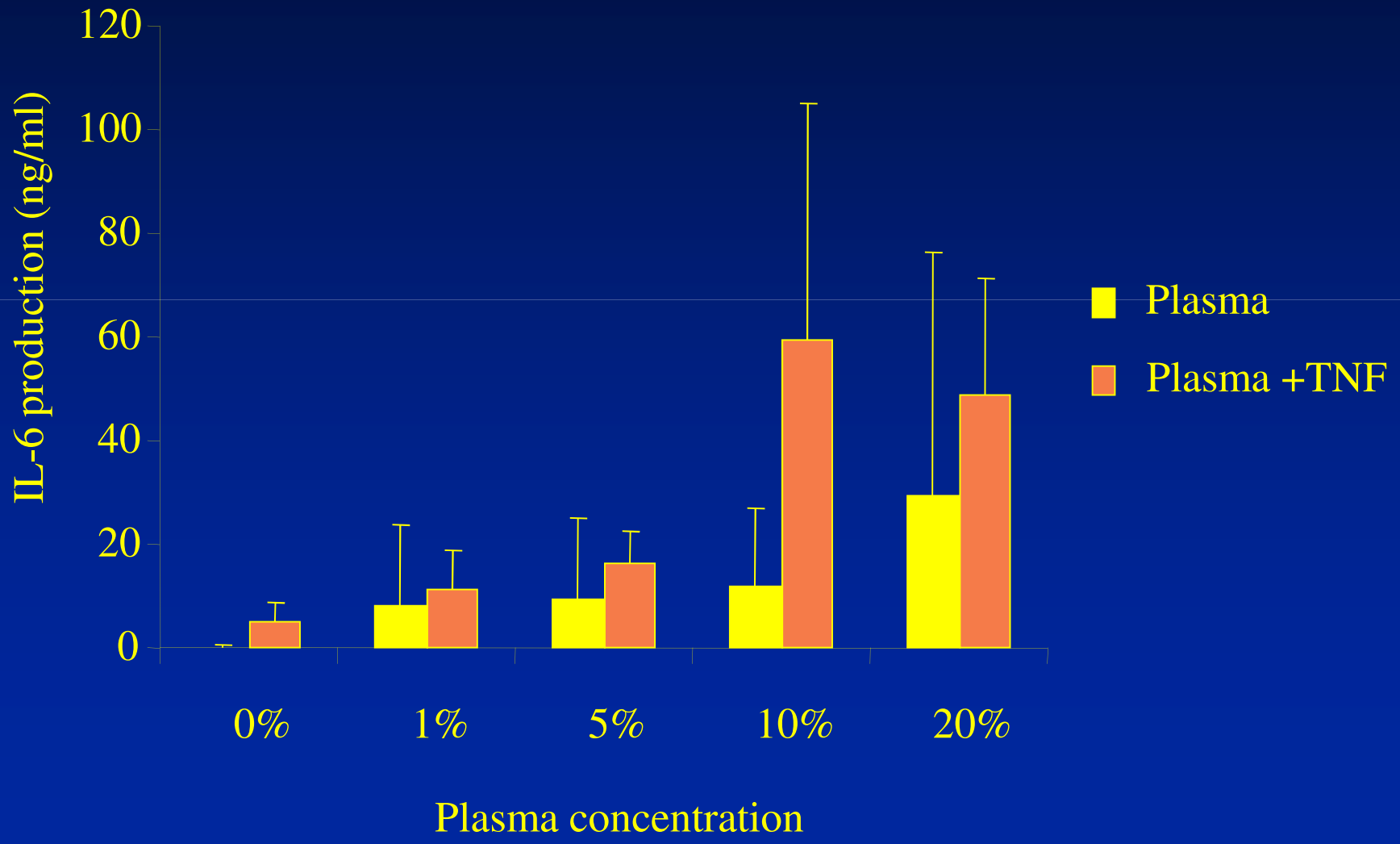
Changes at 22 weeks



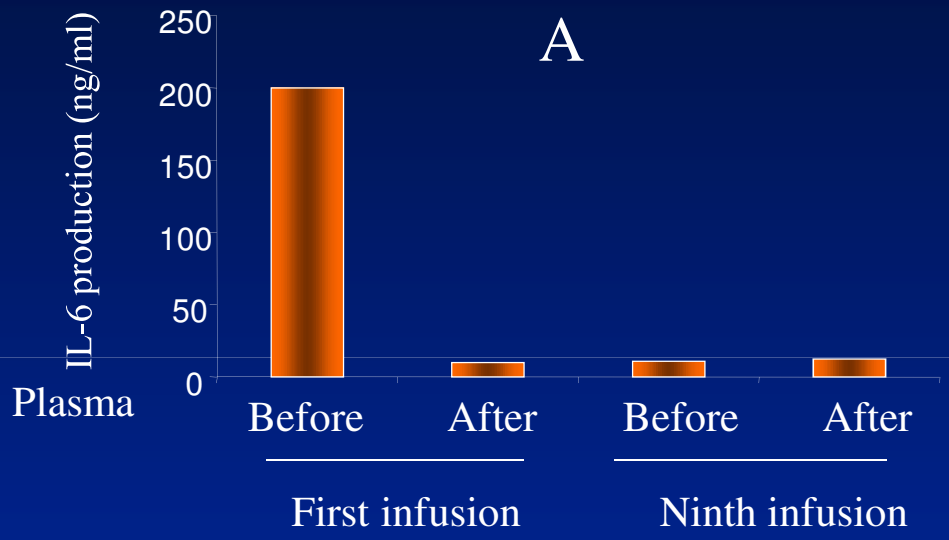
Link to treatment response

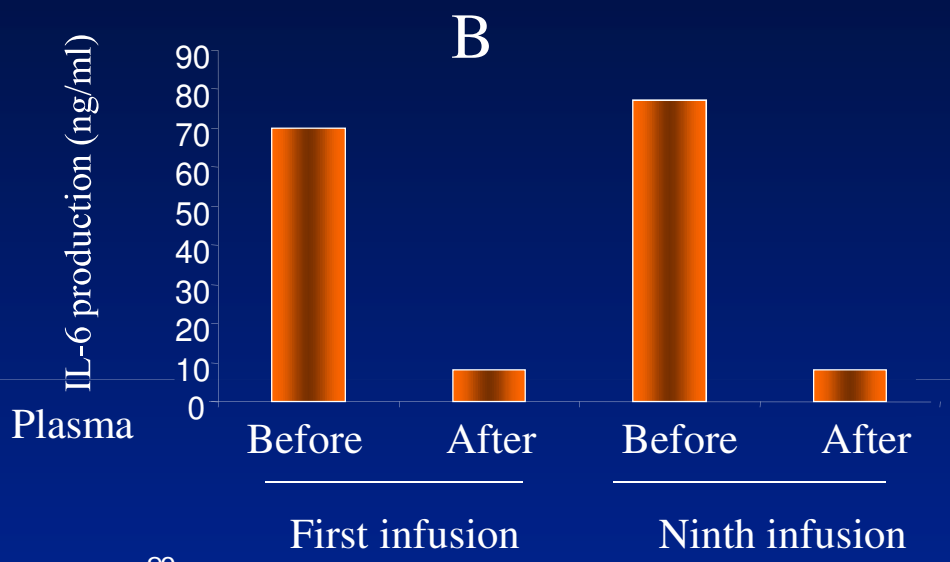


Effect of addition of RA plasma on IL-6 production by TNF-stimulated synoviocytes



TNF bioactivity in plasma and response to infliximab





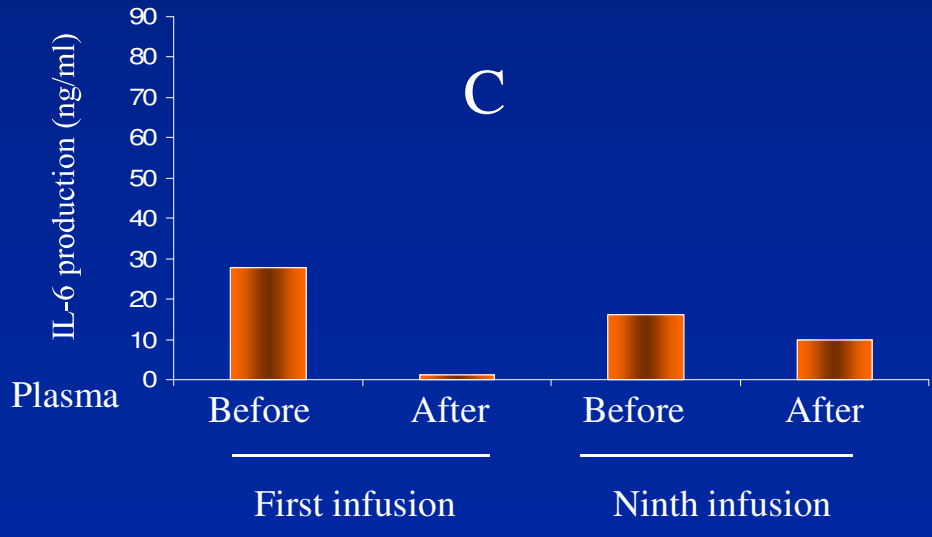
Clinical response ACR 50

A: 6/6

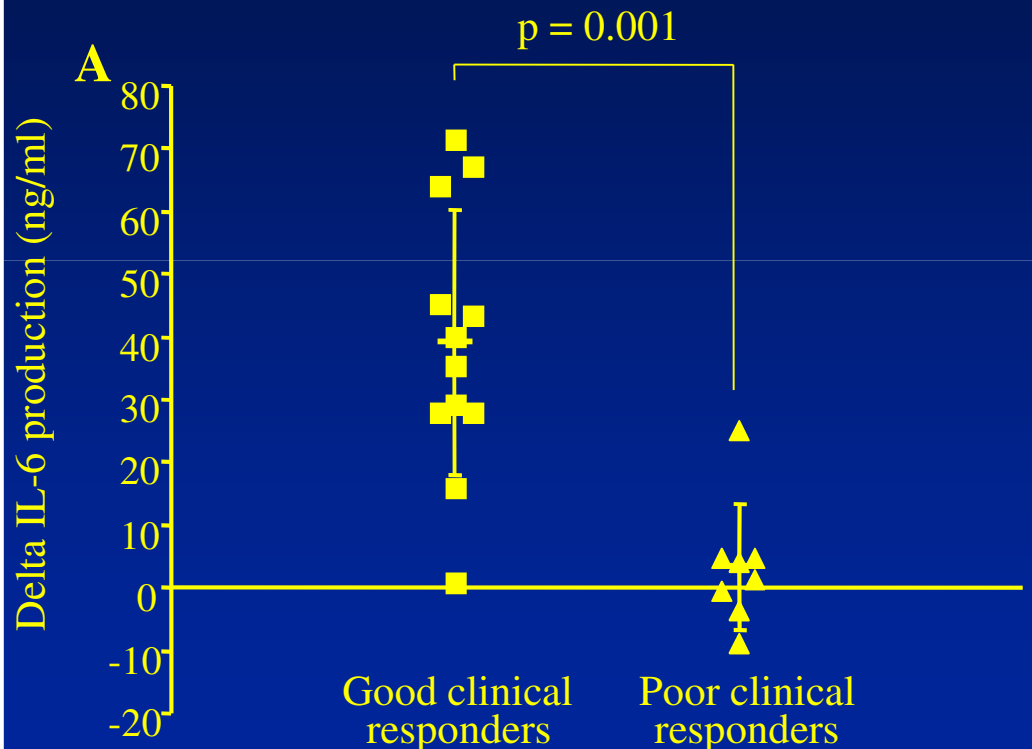
B: 5/6

C: 1/6

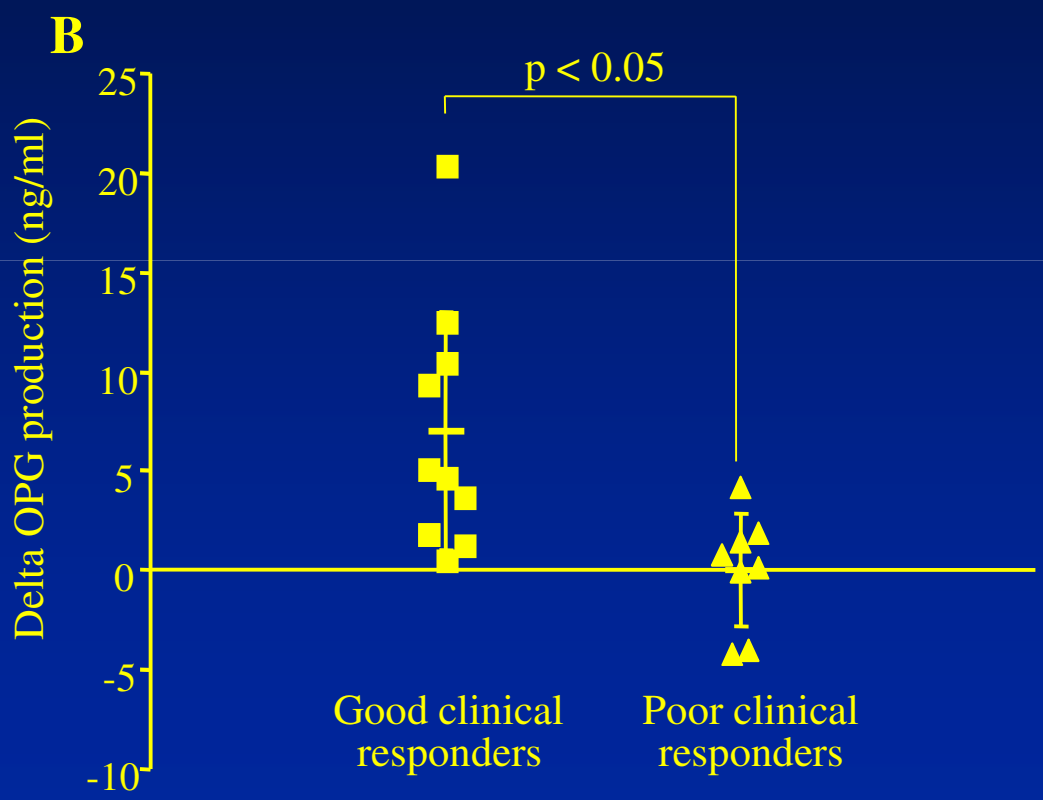
90 % correct prediction



Response to infliximab and IL-6 and OPG production by TNF-stimulated synoviocytes

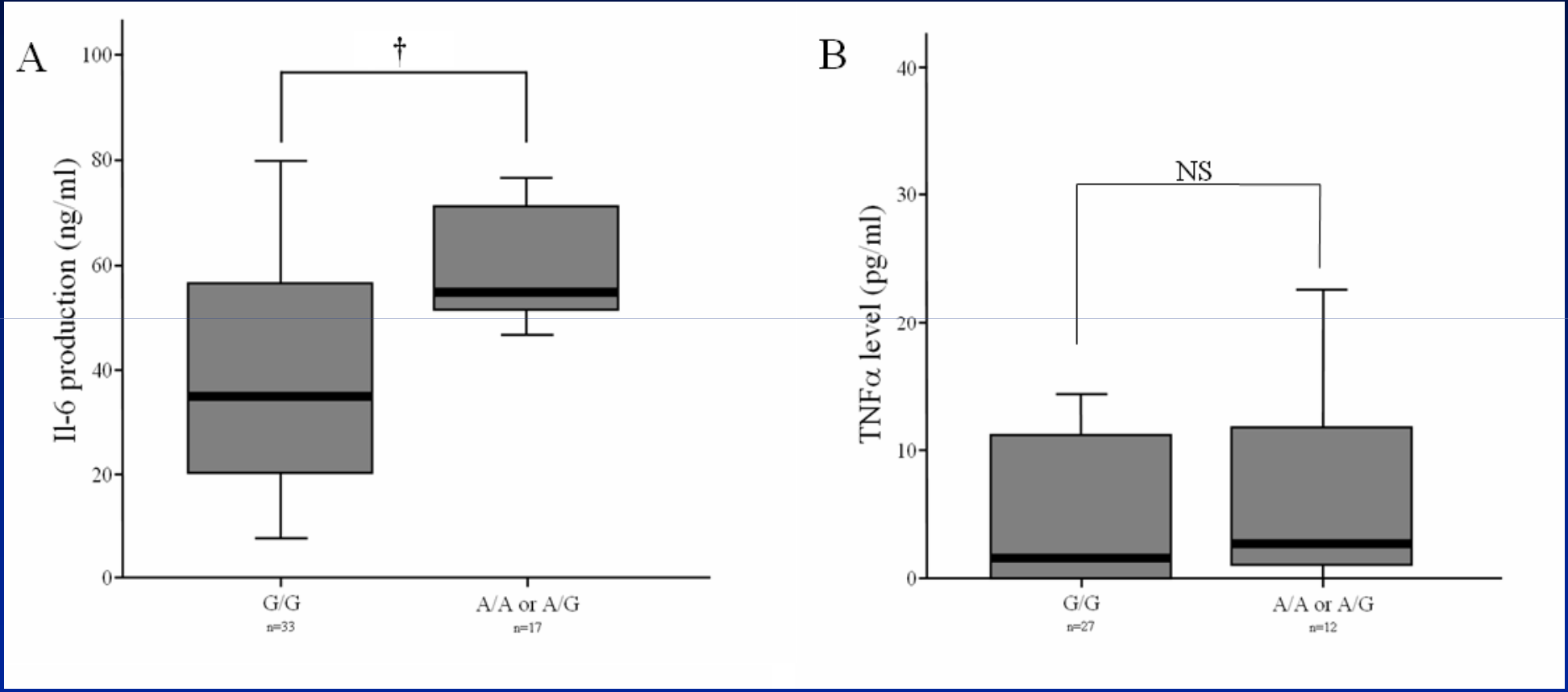


IL-6



OPG

Levels of biactive TNF are higher in patients with the A/A or A/G -308 TNF SNP genotypes



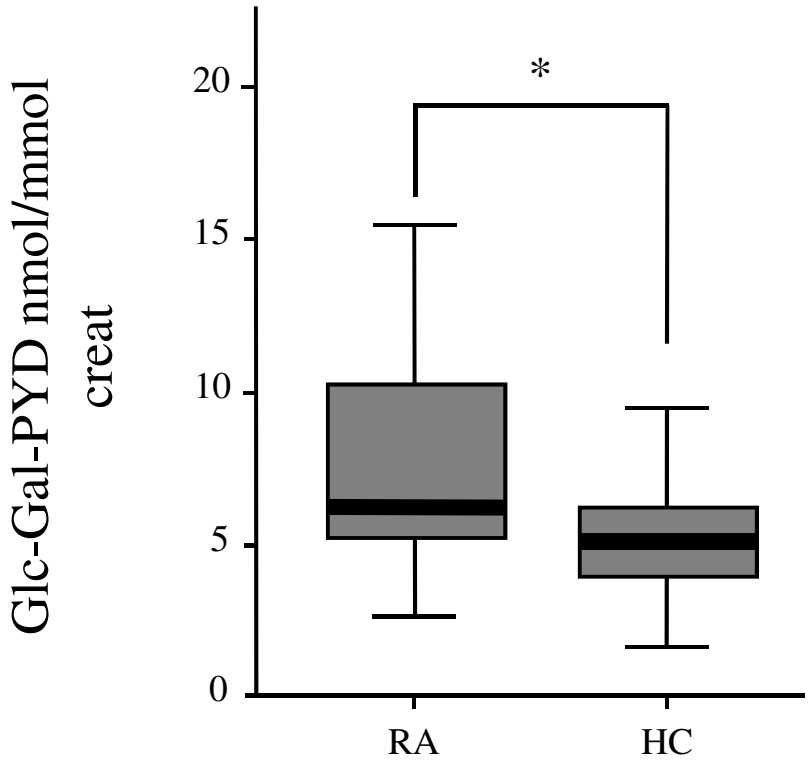
Bioactive TNF

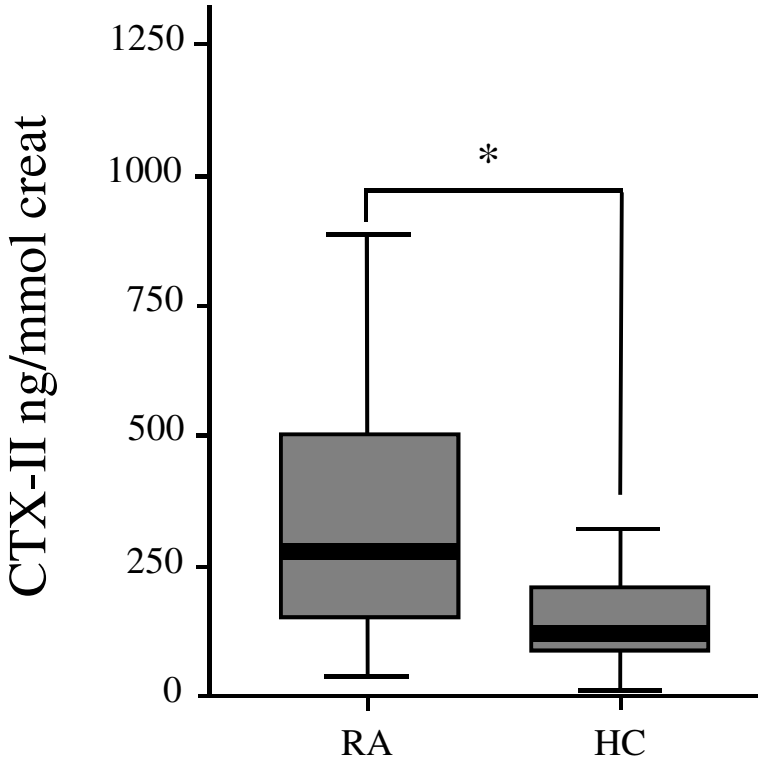
TNF protein by ELISA

TNF levels in rheumatoid arthritis and response to treatment

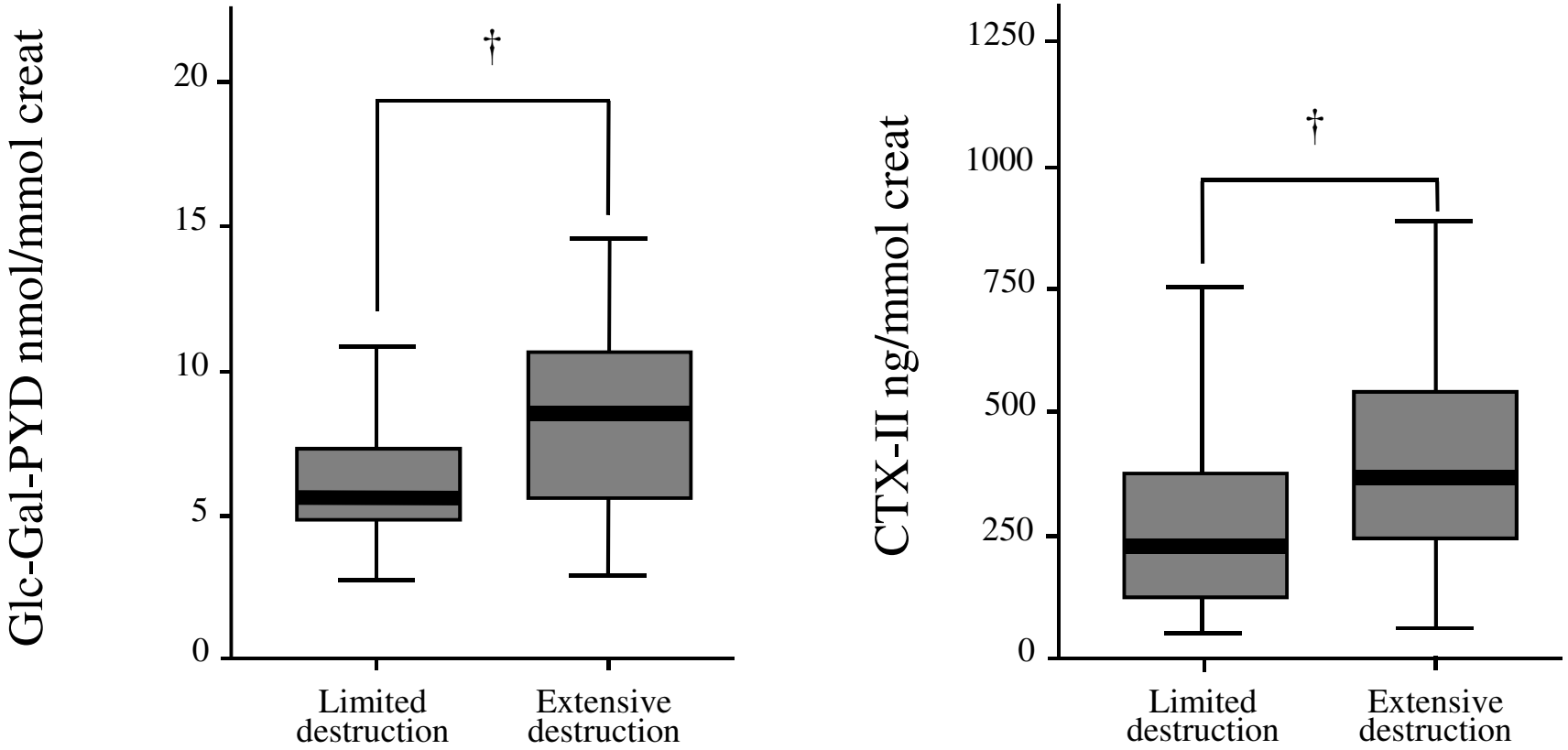
- Bioactive circulating TNF may reflect production in the joint
- Local TNF expression in synovium biopsies is associated with response (PP Tak)
- Other sites such as lymph nodes are probably involved
- Thus to respond to a TNF inhibitor, disease has to be higly TNF-driven
- Increase of TNF inhibitor dosage has only a modest effect

Increased levels of urinary Glucosyl-Galactosyl-Pyrridinoline Glc-Gal-PYR (synovium) and CTX-II (cartilage) in RA patients

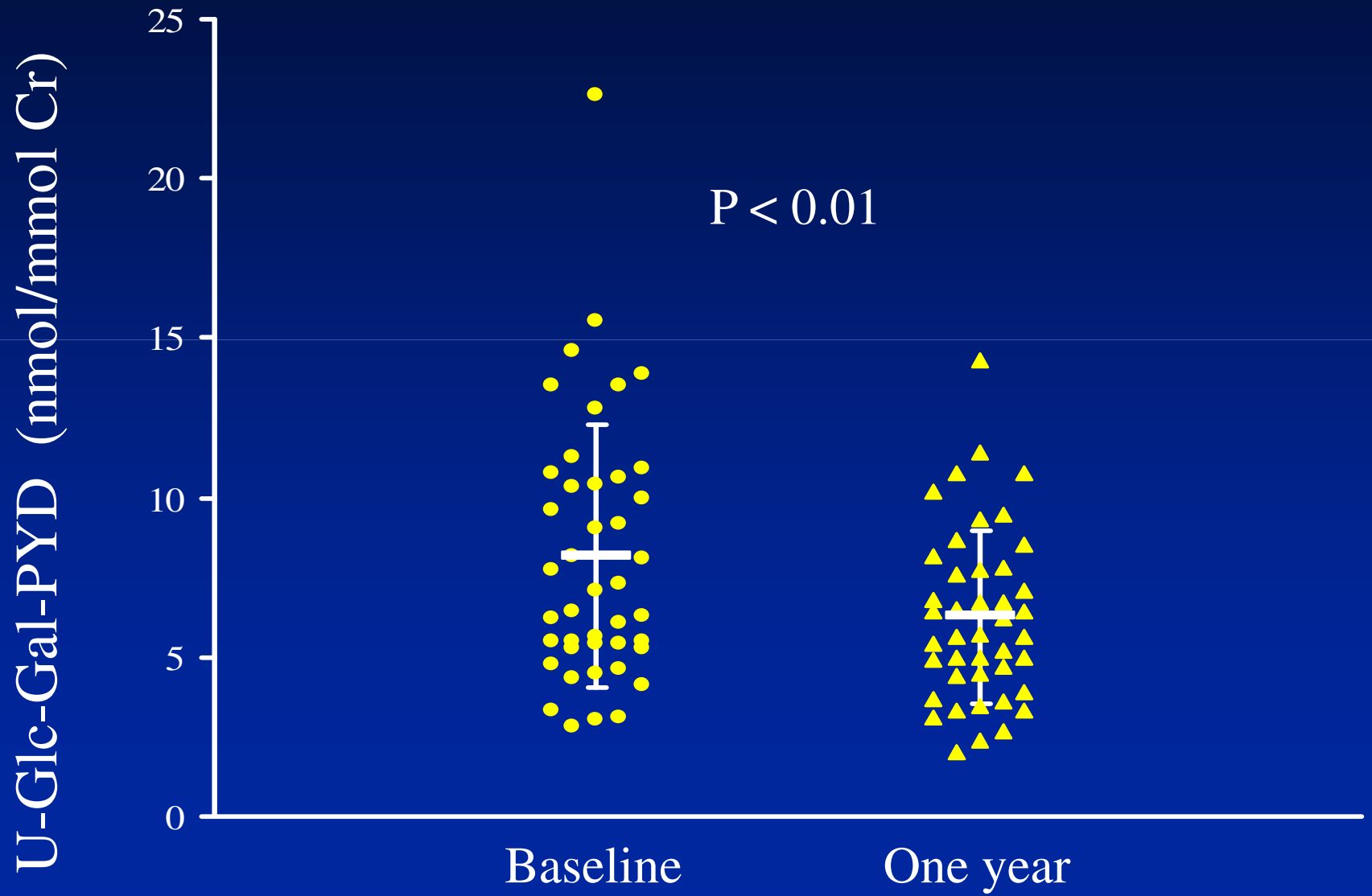




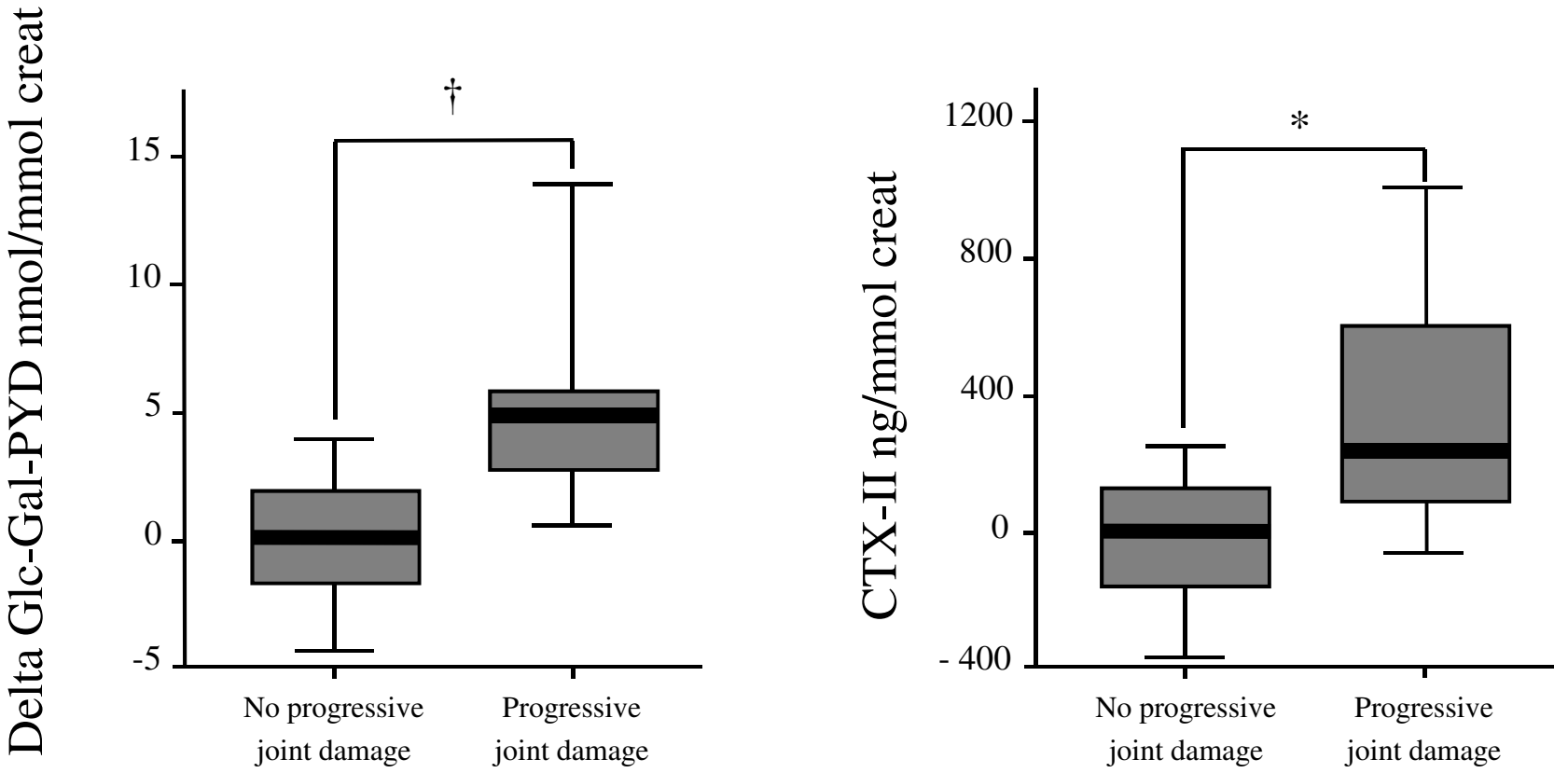
Increased levels of Glc-Gal-PYR and CTX-II in destructive RA



Reduction of urinary Glucosyl-Galactosyl-Pyrridinoline levels during infliximab treatment

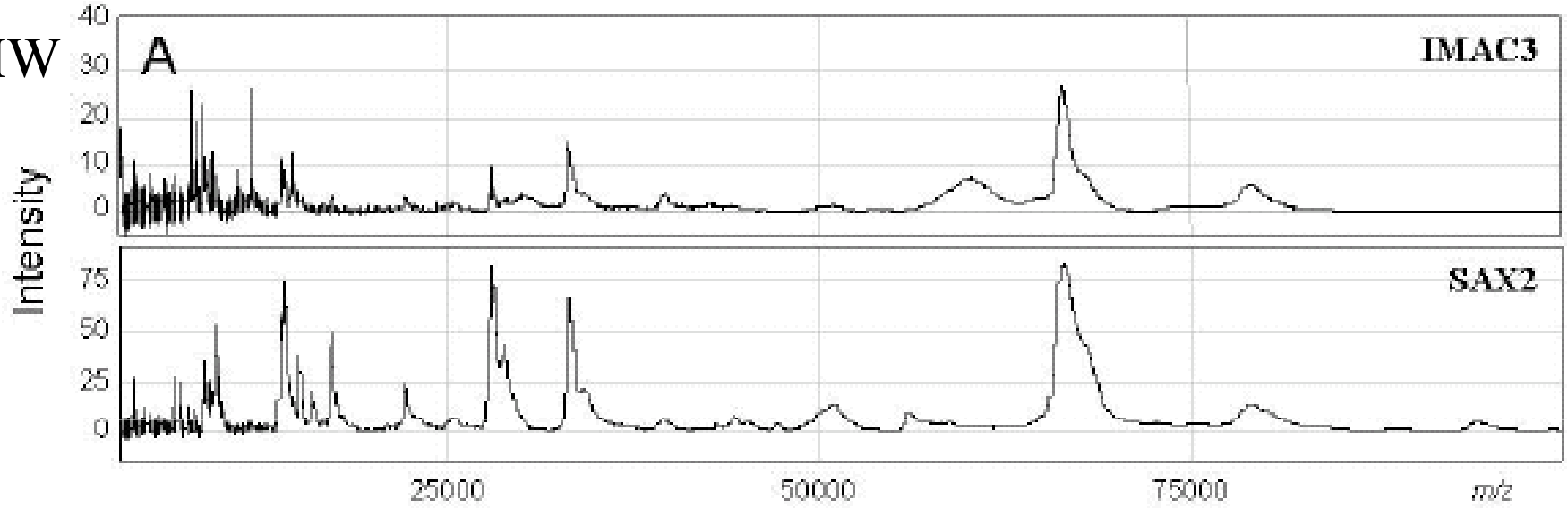


Only RA with progressive joint damage show changes in levels of Glc-Gal-PYR and CTX-II after infliximab



Proteomic analysis of plasma from RA patients

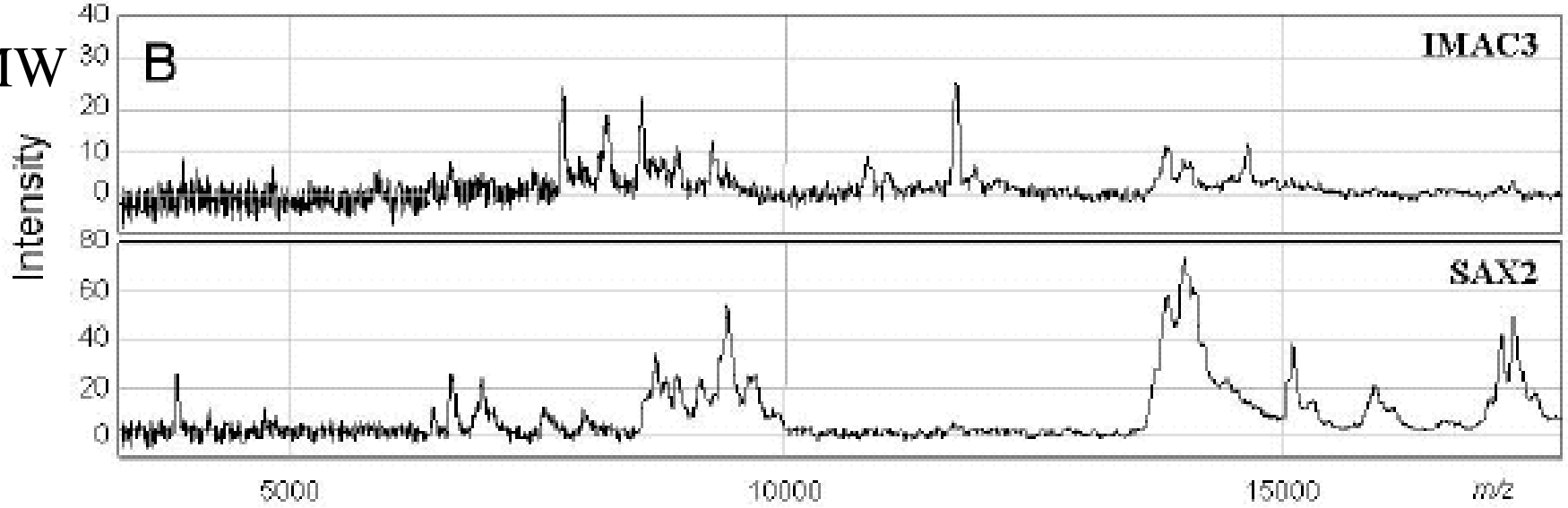
High MW



NR

R

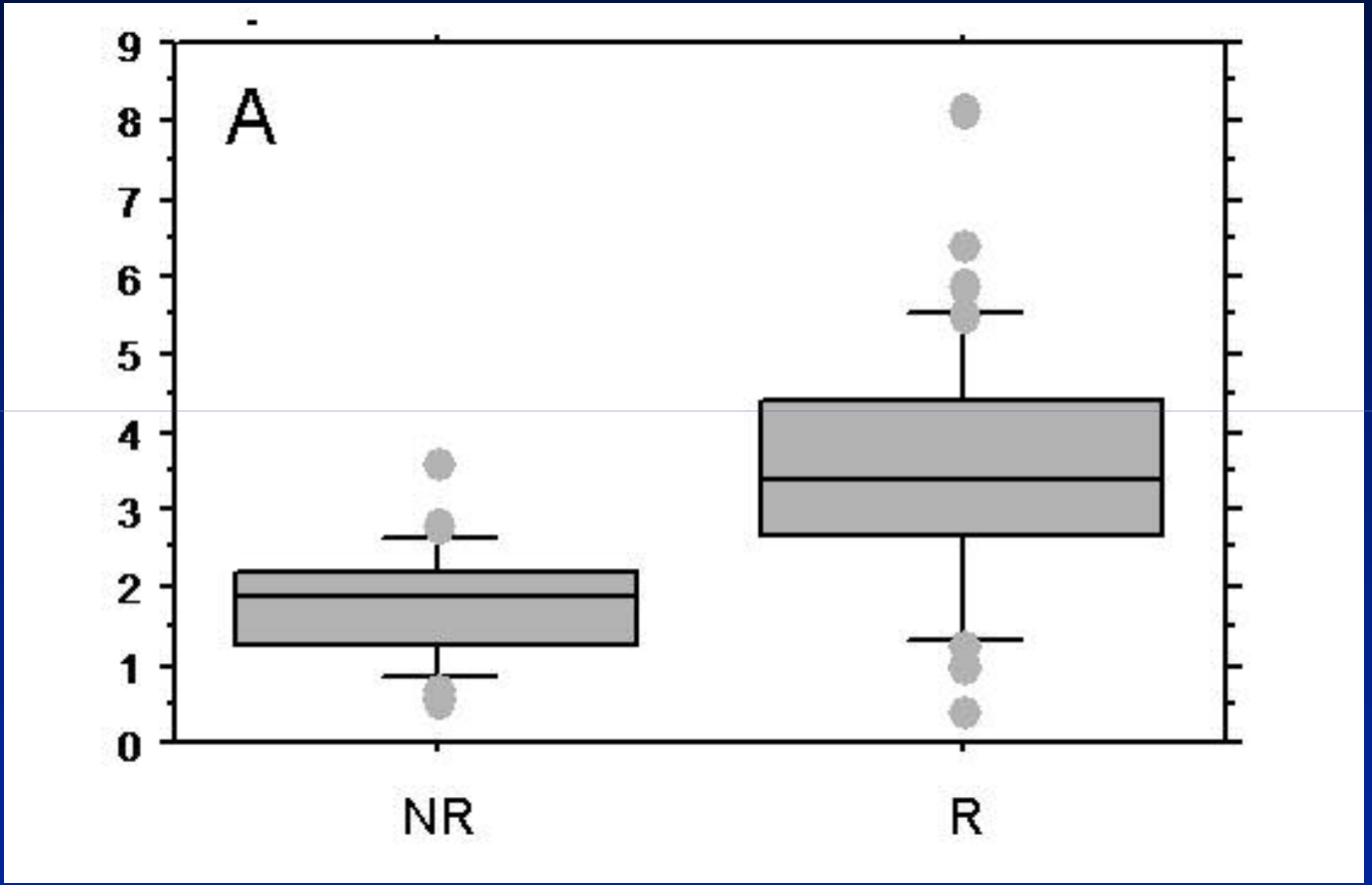
Low MW



NR

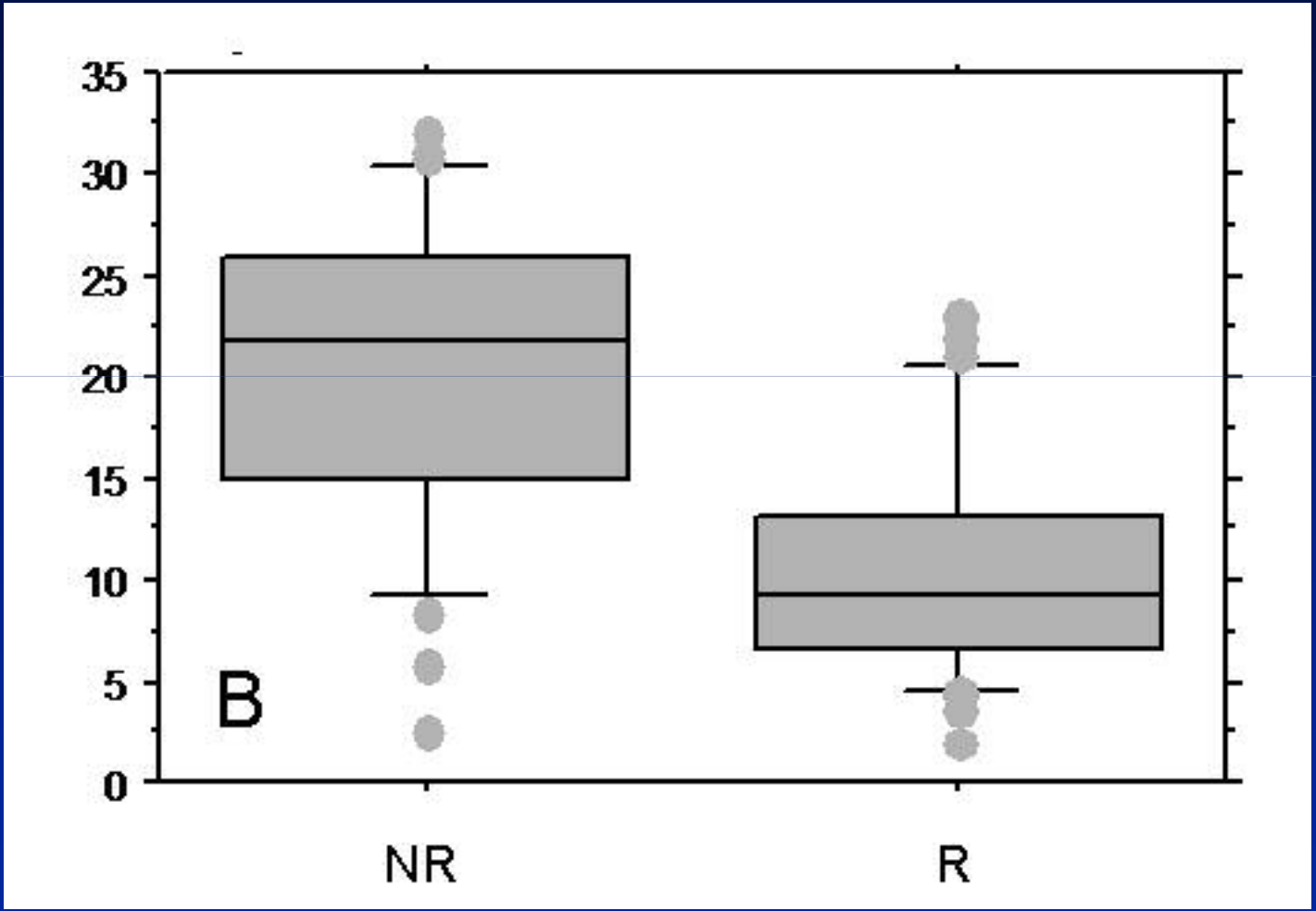
R

Apolipoprotein A1 as a marker of response to infliximab



The 28 kDa peak of apolipoprotein A1 at baseline was increased in the responders

Platelet factor 4 as a marker of response to infliximab



The 7.77 peak of platelet factor 4 at baseline was decreased in the responders

Rheumatoid arthritis: a heterogeneous disease

- Different levels of RA heterogeneity have been defined and better markers are needed
- Because destruction is irreversible, sooner remains better
- Early definition of prognosis will improve management
- Prediction of treatment response and tolerance will improve early choice and schedule of administration
- A marker of disease severity cannot be at the same time a marker of treatment response
- Relative importance of markers: safety; non response; response
- Such markers have to be tested during clinical trials