Biomarkers and personalized medicine: Towards a new European law

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Biomarkers in medicine

Patient heterogeneity and contribution to clinical presentation and disease prognosis
More recently, role in response to and safety of treatments
Application first to cancer, then to chronic diseases
Progress in technology, high throughput microarrays for DNA, RNA, proteins
Rheumatoid arthritis: more than one picture

Early
- Benign
- Responder

Late
- Severe
- Non-responder
Heterogeneity of rheumatoid arthritis

- **Typical RA:**
  
  HLA DR shared epitope, anti-CCP, smoking

- **Atypical RA:**
  lack of common markers
  less severe
  pathogenesis less understood
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: markers and heterogeneity in treatment response

- Environmental factors: smoking, food, infections
- DNA markers: Shared epitope, other gene polymorphisms (SNP), whole genome (GWAS)
- 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
What marker do we need?

- Pre-clinical: anti-CCP
- Early diagnosis (from early signs to at/before birth)
- Prognosis / severity (matrix destruction, CV)
- Treatment
  - Safety
  - Lack of response
  - Response
Collections

- Critical issue: no samples, no results
- Long-term collections needed to evaluate:
  - matrix destruction
  - cardio-vascular events
  - cancer
- Legal aspects, IP issues, site of storage, source of funding
Chronic myeloid leukaemia and tuberculosis in a patient with rheumatoid arthritis treated with infliximab

F Brousais, M Kawashima, H Marotte, P Miossec

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
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
GWAS analysis in RA: success or expensive failure?

- HLA-DR Shared Epitope 0401, 0404, 0101
- PTPN22
- TRAF1-C5 region
- STAT4
- PADI4 (Asia)
Markers of heterogeneity: 3 exemples

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  Protein: Levels of TNF and anti-TNF response: systemic vs. joint levels
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

Heterogeneity of published signatures:
Paxgene, Whole blood, PBMC
Array technology
Kinetics t0, 6 months
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
Synoviolin is over-expressed in infliximab non-responders.

Synoviolin/PPIB mRNA
Endogenous synoviolin regulates cytokine, chemokine and MMP expression in RA synoviocytes

**siRNA control**

- Synoviolin
- IL-6
- IL-8

**siRNA synoviolin**

- + - + - + -
- - + - + - +

**gene expression (%)**
Markers of heterogeneity: 3 exemples

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- Protein: Levels of TNF and anti-TNF response: systemic vs. joint levels
TNF as a marker of response

- Contribution of TNF has been shown at the site of inflammation (PP Tak)
- 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
Response to infliximab and IL-6 and OPG production by TNF-stimulated synoviocytes

A

p = 0.001

Delta IL-6 production (ng/ml)

Good clinical responders

Poor clinical responders

IL-6
Levels of biactive TNF are higher in patients with the A/A or A/G -308 TNF SNP genotypes

A

B

Bioactive TNF

TNF protein by ELISA
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
- Relative importance of markers: safety; non response; response
- Such markers have to be tested during clinical trials
An ideal picture of interactions between partners: A proposal from the GREES working group