Ocular Surface Biomarkers and Inflammation

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Biomarkers in Inflammation

- Scope
- Definition of a biomarker
- Applications
- Risk factors v Screening
- Ocular Phenotypes
- Measuring Symptoms
- Signs v Symptoms
- Sampling variables

- Biomarker Technologies
- Monitoring - Candidates
- Diagnosis – Bioinformatics
- Duration of trials
- Conclusions
Scope

- Prenatal screening:
- Neonatal screening
  - endocrine and metabolic disorders, lysosomal storage dis.
- Adult diagnosis
- Alzheimer’s diagnosis:
  - CSF: Aβ and τ; FDG-PET scan
- HER2 – efficacy of HER2 blockade in treatment of metastatic breast cancer
- Huntingdon mutation in HD
- Serum anti-citrullinated peptide plus RhF in Rheumatoid arthritis diagnosis (PPV 100%)
- Prediction of morbidity/mortality in end stage renal failure.
Definition and Applications

- A disease-associated parameter
- Discriminates affected from unaffected
- Predicting Risk
- Screening
- Diagnosis
- Scaling severity
- Monitoring progress
- Predicting response to therapy
- Determining prognosis
- Understanding disease mechanism
Prediction of dry eye in at-risk groups?

- Contact lens wear
- Isotretinoin therapy - MGD
- LASIK - Refractive laser surgery – dry eye or LINE
- Chronic topical preservatives - in glaucoma therapy
- Bone marrow transplantation – GvH disease
- Connective tissue disease - 2° rheumatoid Sjögren
- Postmenopausal estrogen therapy
- Meds: antihistamines
- Androgen deficiency or receptor blockade
Is a strong risk factor of use in screening?

- The relative odds for the association of cholesterol (RO_{1-5}) with Ischaemic Heart Disease \(\cong 2.7\)

- This gives a \(\text{DR}_5 \cong 15\%\) which is poor for a screening test

\[
\text{DR}_5 = \text{Detection Rate at a False Positive rate of 5%}
\]

Wald et al. *BMJ* 1999; 319
Odds ratios and Detection Rates

- Emerging Risk Factors Collaboration: CRP and CHD
  Kaptoge et al. 2010
  - Odds ratio 3

- Rotterdam Coronary Calcification Study: CC and CHD
  Vliegenthart et al. 2005
  - Relative risk 8.3

- Atherosclerosis Risk in the Community: HbA1C- DM and CHD
  Selvin et al. 2010
  - Odds = 103.5 [for Diabetes]

See Wald and Morris 2011 Arch Intern Med 2011; 171: www.wolfson.gmul.ac.uk/rsc/
Odds ratios and Detection Rates

- **Emerging Risk Factors Collaboration: CRP and CHD**
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  - Odds ratio 3
  - $DR_5 = 9\%$

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  - $DR_5 = 22\%$

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- **Atherosclerosis Risk in the Community: HbA1C- DM and CHD**
  Selvin et al. 2010
  - Odds = 103.5 [for Diabetes]
  - $DR_5 = 32\%$

Wald and Morris 2011: www.wolfson.gmul.ac.uk/rsc/
Screening for Downs and Neural Tube Defect

- 2-step integrated test for Downs
- 1st Trimester – nuchal translucency and serum pregnancy-associated plasma protein A
- 2nd Trimester – AFP, hCG, unconjugated estriol, and Inhibin-A
- Risk result in 2nd tr.

\[ DR_5 = 90\% \]

Serum AFP raised in NTD
Nearly all NTD pregnancies can be identified by AFP screening.
\[ DR_5 = 91\% \text{ spina bifida} \]

Valuable diagnostic tests may take time to...
DRY EYE

Global features

Symptoms +

Hyperosmolarity +

Tear Instability +

Surface stain +

Tear allergy markers negative
DRY EYE

Global features

Symptoms +
> 20 OSDI

Hyperosmolarity +
≥ 316 mOsm/l

Tear Instability +
BUT ≤ 10 s

Surface stain +
≥ 3 or 4 VB

Tear allergy markers negative

Phenotypes
**DRY EYE - aqueous deficient**

- Symptoms $+$
  - $> 20$ OSDI
- Hyperosmolarity $+$
  - $\geq 316$ mOsm/l
- Schirmer $^+$
  - $\leq 5$ mm
- Tear Instability $+$
  - BUT $\leq 10$ s
- Surface stain $+$
  - $\geq 3$ or $4$ VB

**Meniscus radius**
-Meniscus height
-Tear clearance
-Tear EGF
-Tear Lysozyme
-Tear Lactoferrin

**Tear allergy markers negative**

**Phenotypes**

- MGD $^-$
  - negative MGD surrogates
DRY EYE - aqueous deficient

Symptoms +
> 20 OSDI

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Tear Instability +
BUT ≤ 10 s

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≥ 3 or 4 \text{VB}

Schirmer +ve
≤ 5 mm

Meniscus radius
Meniscus height
Tear clearance
Tear EGF
Tear Lysozyme
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MGD -ve
negative MGD surrogates

Tear allergy markers negative
DRY EYE  Evaporative

Symptoms +
> 20 OSDI

Hyperosmolarity +
≥ 316 mOsm/l

Tear Instability +
BUT ≤ 10 s

Surface stain +
≥ 3 or 4 VB

Phenotypes

Schirmer
>5 mm
- negative LG surrogates

MGD +
MGD signs +
↑evaporation
TFLL changes
Meibum change
tear Calgranulin

Tear allergy markers negative
DYSFUNCTIONAL TEAR SYNDROME

- Symptoms +
  > 20 OSDI
- Hyperosmolarity +
  ≥ 316 mOsm/l
- Tear Instability +
  BUT ≤ 7 s
- Surface stain +
  ≥ 3 or 4 VB
- Schirmer < 10 mm
- MGD +
  Lack of expressable meibum ≥ 75% of glands
  2 or more of:
  Acinar atrophy
  Orifice metaplasia
  Vascular dilatation at posterior lid margin

Tear allergy markers negative
Endpoints – Signs versus Symptoms

Symptoms in dry eye
- Soreness, irritation
- Gritty, scratchy
- Burning, stinging
- Itching
- Dryness
- Tired eyes.

- Light Sensitivity,
- Visual Change

- Frequency
- Timing
- Intensity

- Provocations:
  - Low humidity-AC
  - Airflow – windy day
  - Fumes - smoke

eg D E Q – Begley et al 2002
Symptom Measurement

- In dry eye, whose major feature is symptoms, there is no surrogate for symptom measurement.
- Validated Questionnaires are available.
- Biomarkers whose levels correlate with symptom severity are of interest because they may be closer to symptom mechanisms.

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<thead>
<tr>
<th>Name</th>
<th># of questions</th>
<th>Author</th>
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<tr>
<td>Womens Health</td>
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<td>Schaumberg et al. 2003</td>
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<td>Sjögren Consensus</td>
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<td>Korb et al. 2005</td>
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<td>OCULAR COMFORT INDEX</td>
<td>31</td>
<td>Johnson Murphy 2007</td>
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<tr>
<td>IDEEL</td>
<td>57</td>
<td>Rajagopalan et al. 2005</td>
</tr>
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</table>


Symptom / Sign correlation is often poor

- Goren 1988
- Begley 2003
- Nichols 2004
- Saleh 2006
- Moore 2009
- Fuentes-Paez 2011

- Enriquez de Salamanca 2010
- No correlation with global scores:
- Some scattered correlations with individual CKs.
Symptom sources in dry eye

- **Hyperosmolarity**
  - **Diffuse:** meniscus sample
  - **Focal:** tear film break up
    
    [Ocular Protection Index - BUT/Blink interval]

- **Reduced lubrication**
  - frictional drag: loss of glycocalyx and goblet cell mucin
  - lid wiper epitheliopathy. ²
    
    [Shearing between lids and globe during blinks and eye movements]

- **Conjunctivochalasis**

- **Inflammatory mediators**
  
  [Prostanoids, cytokines, neurokinins, neuromediators]

- **Ocular surface damage**
  
  [Altered nerve excitability ³; neuropathic firing ⁴]

Symptom sources
- are dependent on-

Corneal sensory fibres
- Polymodal nociceptors
- Cold fibres\(^1\)

• Physiological
  - Surface stress - increased stimuli
  - increased excitability

• Neuropathic firing
  - cold fibres\(^1\)

Lid margin sensory fibres?

1. Belmonte Gallar IOVS 2011, Vol. 52, 3888
the source of symptoms in dry eye

- Lack of a powerful association between a biomarker and dry eye symptoms at diagnosis should not discourage its use to track the efficacy of a drug,
- particularly where it reflects a causal hypothesis or could provide proof of principle of drug action
Tissue sampling - variables affecting measurement

Epithelial Cell Samples

- Impression cytology
  - Instant, regional sample of surface cells
- Brush cytology
  - Global sample
- Analysis
  - Immunocytochemistry
  - Flow cytometry
  - HLA-DR; mRNA; cytokines; transmembrane mucins
- Standardisation is the key – optimize techniques to enhance repeatability.

Biomarker ratios in single samples
Molecular Biomarker Technologies

- Electrophoresis: 1D; 2D gels
- ELISA sandwich
- Protein arrays (beads, blots)
- Western blot
- LC-MS
- SELDI/TOF
- MALDI/TOF
- LC MALDI
- LC-MS/MS
- iTRAQ proteomics
Candidate Tear Proteins

Multiplex Bead Assay / Microwell and membrane antibody Arrays

- R Sack

- **CYTOKINES**
  - IL-1α; IL-1β; 2; 4; 5; 6; 8; 10; 12P70; 13; 15; 17; 23
  - INFγ; TNFα; TNFβ

- **CHEMOKINES**
  - Eotaxin; GROα; I-309; IL-8; IP10; MCP-1,2; RANTES; TARC

- **ADHESION** molecules
  - ICAM-1, 3; VCAM-1;

- **OTHER** molecules
  - Soluble receptors: IL-1RI, II; IL-2R, γ; IL-4R; IL-6R; IL-6R; IL-13Ra1; TNF-R1; TNF-RII;
  - Sgp 130; gp340

- **α2-M**
Candidates: Chemokines in Dry Eye:
Th-1-dependent inflammation

Yoon IOVS 51 643 2010

<table>
<thead>
<tr>
<th>Chemokine type</th>
<th>CXC [α]</th>
<th>CC [β]</th>
<th>C [γ]</th>
<th>CXXXC [δ]</th>
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<tr>
<td>ELR +</td>
<td>EMR -</td>
<td>IFNγ</td>
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<tr>
<td>CXCL9 [MIG]</td>
<td>CXCL10 [IP-10]</td>
<td>CXCL11 [I-TAC]</td>
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<td>CXCR 1 and 2</td>
<td>CXCR 3 &amp; 5</td>
<td>CCR 3 &amp; 4</td>
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<td>PMNS</td>
<td>T-cells *</td>
<td>PMNS</td>
<td>NK cells</td>
<td>Th-1 related inflammation</td>
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Candidates: Chemokines in Dry Eye
Th-1-dependent inflammation

- Capillary tears: ELISA; CIC flow cytometry.
- Increase in:
  - IFNγ-inducible ELR^- CXC chemokines in DE tears. CXCL 9, 10 esp 11, and
  - CXCR3^+ Th 1 type cells in conj. epithelium.
- CXCR3^+ CD4^+ conj. cells — main effectors of lac. and conj. epithelial damage?
- CXCL 11 levels correlated with
  - low basal Schirmer,
  - low tear clearance,
  - kerato-epitheliopathies,
  - reduced goblet cell density.
Subjects: 30 DTS; 14 control
2-eye, pooled 0.5 µl tear capillary samples
Luminex Bead array

These cytokines & MIP-1α correlated with DEWS severity grade:
- IL-6 correlated with severity of symptoms and signs
- EGF levels correlated with the Schirmer value and inversely with corneal staining.

Candidates: Cytokine profiles in Dysfunctional TS
Lam et al. 2008
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Lam et al. 2008

- Subjects: 30 DTS; 14 control
- 2-eye, pooled 0.5 µl tear capillary samples
- Luminex Bead array

- IFN$\gamma$ / IL-13 ratio ↑ in DTS
- IFN$\gamma$ a marker for Th-1 inflammation and IL-13 for Th-2 inflammation
- The ratio correlates with goblet cell loss and metaplasia in DE model
Candidates: **MMP9 in Dysfunctional TS**  
Chotikavanich et al. 2009

- **Subjects:** 19 DTS; 16 control (+subset)
- **2-eye, pooled 0.5 µl tear capillary samples**
- **Tear immunoassay, CIC RNA real-time PCR**
Candidates: MMP9 in Dysfunctional TS
Chotikavanich et al. 2009

• Tear MMP9 activity ↑ in DTS patients; correlated with:
  – Increases in -IL-1β; IL 6 ; TNFα AND TGFβ1 CIC epithelial transcripts.
  – Clinical severity controls = 8.4 pg/ml DTS grade 4 = 381.2 pg/ml P<0.001

Also correlates with:
• Surface stain; confocal epithel. score; surface irregularity; low contrast sensitivity.

• No corrln with BUT.

but -MMP9 also increased in patients with MGD and with SS [Solomon 2001 IOVS 42 2283]. and proMMP9 is increased in rosacea [Afonso 999 40 2506; Sobrin IOVS 2000, 41 1703]
Candidates: tear and membrane bound MUC1

- The trans membrane mucin MUC 1 is a key component of the ocular surface glycocalyx.
- Cleavage of the exodomain releases soluble MUC1 into the tears.

Ocular surface mucins are: MUC1, MUC2, MUC4, MUC5AC, MUC7, MUC13, MUC15, MUC16, and MUC17.
Candidates: tear and membrane bound MUC1

- Subjects: 25 primary SSDE; 25 NSDE; 26 controls
- Eye wash and pooled CIC samples

- Tear MUC1 and MUC1 expression highest in SSDE. Tear MUC1 also higher in NSDE
Candidates: tear and membrane bound MUC1

- Subjects: 38 NSDE; 43 controls.
- Individual CIC samples

- Expression of MUC 1, 2, 4 and 5AC lower in NSDE
- Using MUC1 expression in dry eye diagnosis:
  \[ \text{DR}_{12.5} = 83.3 \% \]
- Validated in additional control and DE groups.

Corrales 2011
SELDI-TOF-MS Protein Chip Array in dry eye diagnosis

- Focus on Mass < 14 KDa.
- Multivariate discriminant analysis used to identify 50 peaks differing between ADDE and normals

Grus 2005
SELDI-TOF-MS Protein Chip Array in dry eye diagnosis

- Focus on Mass < 14 KDa.
- Multivariate discriminant analysis used to identify 50 peaks differing between ADDE and normals
- Further analysis revealed a cluster of 7 polypeptides
- Dry eye detection rate
iTRAQ technology with 2D-nanoLC-nano-ESI-MS/MS

Zhou
Proteome Res 2009

- Subjects: 56 DE: Symptoms+; Sch $\leq$10 mm; FBUT $\leq$ 10s; Cr Stain $>2$ Oxf
- 40 control
- 10 mm Schirmer strip sample
- 93 tear proteins identified, 10 differentially expressed
- 6 up-regulated proteins,
- α-enolase,
- S100 A4 and
- α-1-acid glycoprotein 1,
- S100 A8 (calgranulin A),
- S100 A9 (calgranulin B),
- S100 A11 (calgizzarin)
- 4 down-regulated
  - lactoferrin and lysozyme.
  - prolactin-inducible protein (PIP),
  - lipocalin-1

Diagnosis with a 4 protein biomarker panel:

\[ DR_{10} : 91\% \]

- 3 proteins:
  - α-1-acid glycoprotein 1,
  - S100 A8 (calgranulin A),
  - S100 A9 (calgranulin B),
- Correlated with severity
• Subjects: 24 DE: Symptoms+; Sch ≤10 mm; FBUT ≤ 10s; Cr Stain > 2 Oxf;
• MGD severity scale 0-3
• 18 control
• Schirmer strip sample

• Calgranulin A and B ratios correlated with:
• MGD severity and
• Symptoms: Redness; transient blurring
• Lipocalin-1 was associated with heaviness of the eyelids and tearing
• “MGD may independently contribute to the symptoms of dry eye patients”.

Tong et al. 2011
Subjects: 
- 35 DRYaq;
- 36 DRYLip;
- 34 mixed 
- 38 Controls.

Eluted Schirmer strips 

Antibody microarray

↑ IL-1,-6,-8 TNFα, IFNγ,
LCN-1, Cystatin SN, α1-AT
In aqueous deficient not lipid deficient dry eyes

Boehm IOVS 2011
Recommendations

- **Establish**:  
  - Rigorous criteria for each phenotype  
  - Validated Questionnaires  
  - Measures of severity  
- **Optimize tissue sampling**:  
  - nano volumes; cell snapshots  
- **Select biomarker technology with low variance in field**  
- **Apply** to broad population samples with dry eye and other ocular surface disease.  
- **Establish** cut offs.  
- **Validate** key biomarkers or panels  
- **Refine** diagnostic and severity criteria
Thank You for your Attention