Ocular Surface Biomarkers and Inflammation

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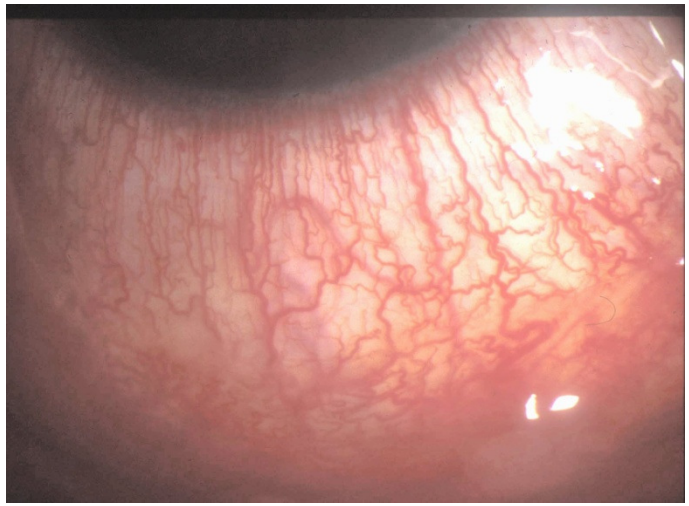
Image -freedoncurrent.com

EMA 27/28th October

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

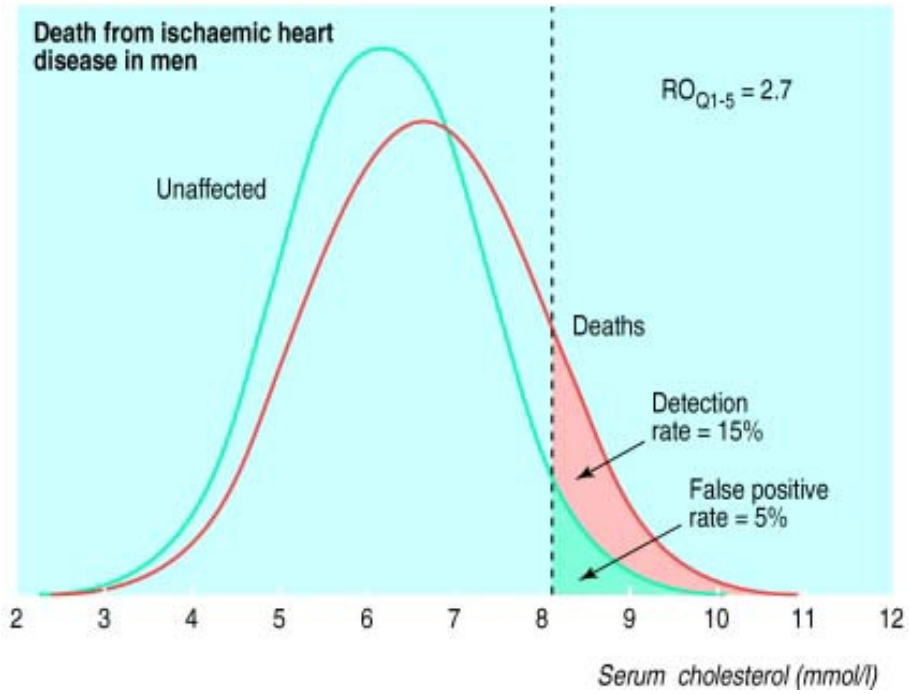
- Predicting Risk
- Screening
- Diagnosis
- Scaling severity
- Monitoring progress
- Predicting response to therapy
- Determining prognosis
- Understanding disease mechanism

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 – G v H 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 ≅ 2.7
- This gives a DR_5 ≅ 15% which is poor for a screening test



DR_5 = 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**:

Odds ratios and Detection Rates

- Emerging Risk Factors Collaboration: CRP and CHD
  Kaptoge et al 2010
  - Odds ratio 3
  - DR_5 = 9%
- Rotterdam Coronary Calcification Study: CC and CHD
  Vliegenthart et al. 2005
  - Relative risk 8.3

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Wald and Morris 2011: 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%
- Rotterdam Coronary Calcification Study: CC and CHD
  Vliegenthart et al. 2005
  - Relative risk 8.3
  - DR_5 = 22%
- Atherosclerosis Risk in the Community: HbA1C- DM and CHD

  Selvin et al. 2010
  - Odds = 103.5 [for Diabetes]

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Odds ratios and Detection Rates

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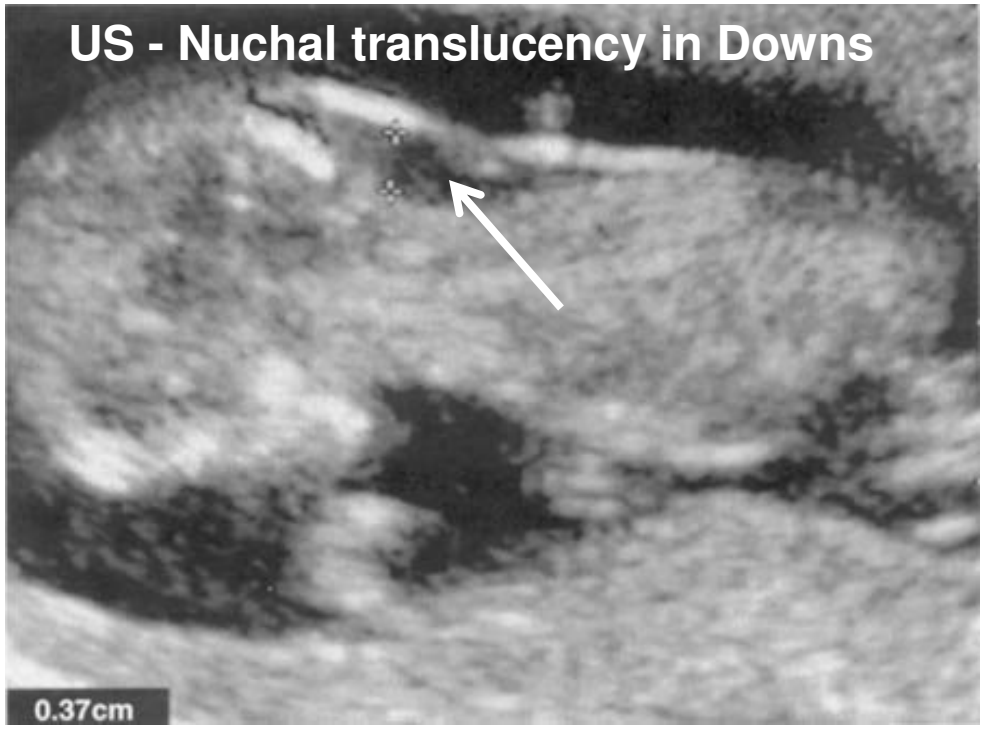
- Rotterdam Coronary Calcification Study: CC and CHD
  Vliegenthart et al. 2005
  - Relative risk 8.3
  - DR_5 = 22%
- Atherosclerosis Risk in the Community: HbA1C- DM and CHD

  Selvin et al. 2010
  - Odds = 103.5 [for Diabetes]
  - DR_5 = 32%

Wald and Morris 2011:

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% spina bifida

Wald 2010

Valuable diagnostic tests may take time to

DRY EYE

Global features

Phenotypes

Symptoms +

Hyperosmolarity +

Tear Instability +

Surface stain +

Tear allergy markers negative

DRY EYE

Global features

Phenotypes

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 +
≥ 316 mOsm/l

Schirmer $^{+ve}$
≤ 5 mm

Tear Instability +
BUT ≤ 10 s

MGD $^{-ve}$

negative MGD surrogates

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

Surface stain +
≥ 3 or 4 ^{VB}

Tear allergy markers negative

Phenotypes

DRY EYE - aqueous deficient

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

Surface stain +
≥ 3 or 4 ^{VB}

Tear allergy markers negative

Phenotypes

DRY EYE Evaporative

Symptoms +
> 20 OSDI

Hyperosmolarity +
≥ 316 mOsm/l

Schirmer
>5 mm

- negative LG surrogates

Tear Instability +
BUT ≤ 10 s

Surface stain +
≥ 3 or 4 VB

MGD +

MGD signs +
↑evaporation
TFLL changes
Meibum change
tear Calgranulin

Tear allergy markers negative

Phenotypes

DYSFUNCTIONAL TEAR SYNDROME

Symptoms +

> 20 OSDI

Hyperosmolarity +

≥ 316 mOsm/l

Schirmer

< 10 mm

Tear Instability +

BUT ≤ 7 s

MGD +

Lack of expressable meibum ≥ 75% of glands

Surface stain +

≥ 3 or 4 ^{VB}

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

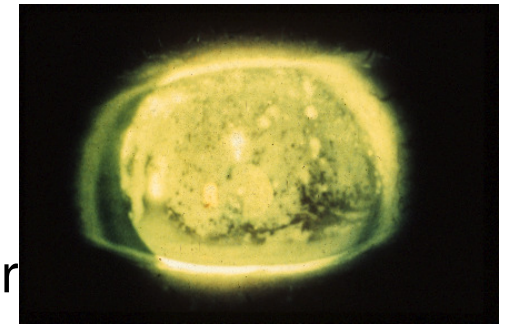
Name	# of questio	Author
Womens Health	3	Schaumberg et al. 2003
Sjögren Consensus	3	Vitali et al. 2002
Schein	6	Schein et al. 1997
McMonnies	12	McMonnies and Ho 1986
OSDI	12	Schiffman et al. 2000
SPEED	12	Korb et al. 2005
CANDEES	13	Doughty et al. 1997
DEQ	21	Begley et al. 2002
NEI-VFQ	25	
OCULAR COMFORT INDEX	31	Johnson Murphy 2007
IDEEL	57	Rajagopalan et al. 2005

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 corrlns with individual CKs.

Symptom sources in dry eye



- **Hyperosmolarity**
  - **Diffuse:** meniscus sample
  - **Focal:** tear film break up

    [Ocular Protection Index - BUT/Blink inter
- **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**

  [Alterred nerve excitability [3]; neuropathic firing [4]]

1. Ousler et al. 2008 2. Korb et al 2005 3. dePaiva and Pflugfelder 2004 4. Belmonte, Gallar 2011.

Symptom sources

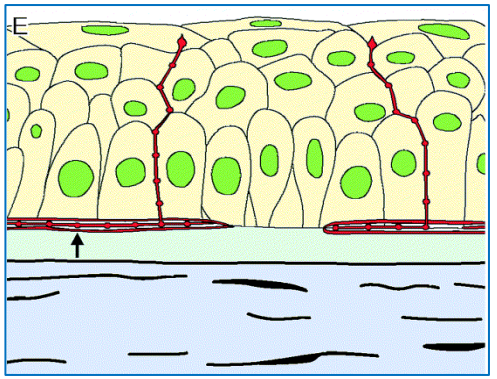
- are dependent on-

Corneal sensory fibres

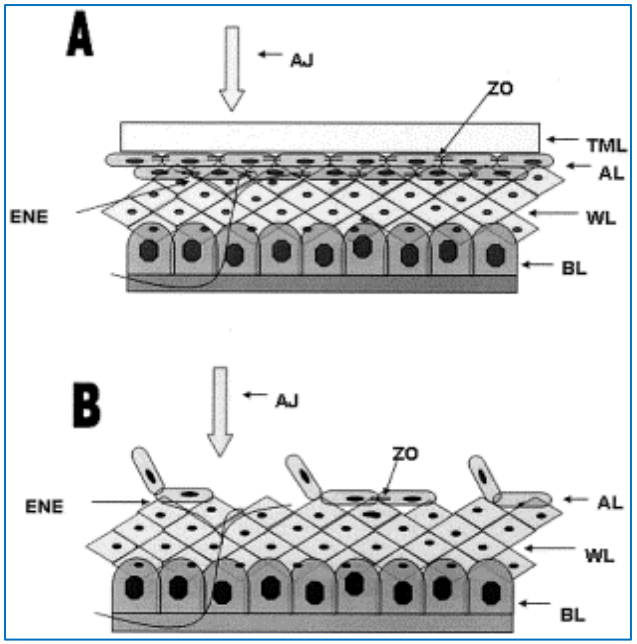
- Polymodal nociceptors
- Cold fibres[1]

- Physiological
  - Surface stress - increased stimuli
  - increased excitability
- Neuropathic firing
  - cold fibres[1]

Lid margin sensory fibres?



De Paiva Pflugfeldedr 2004

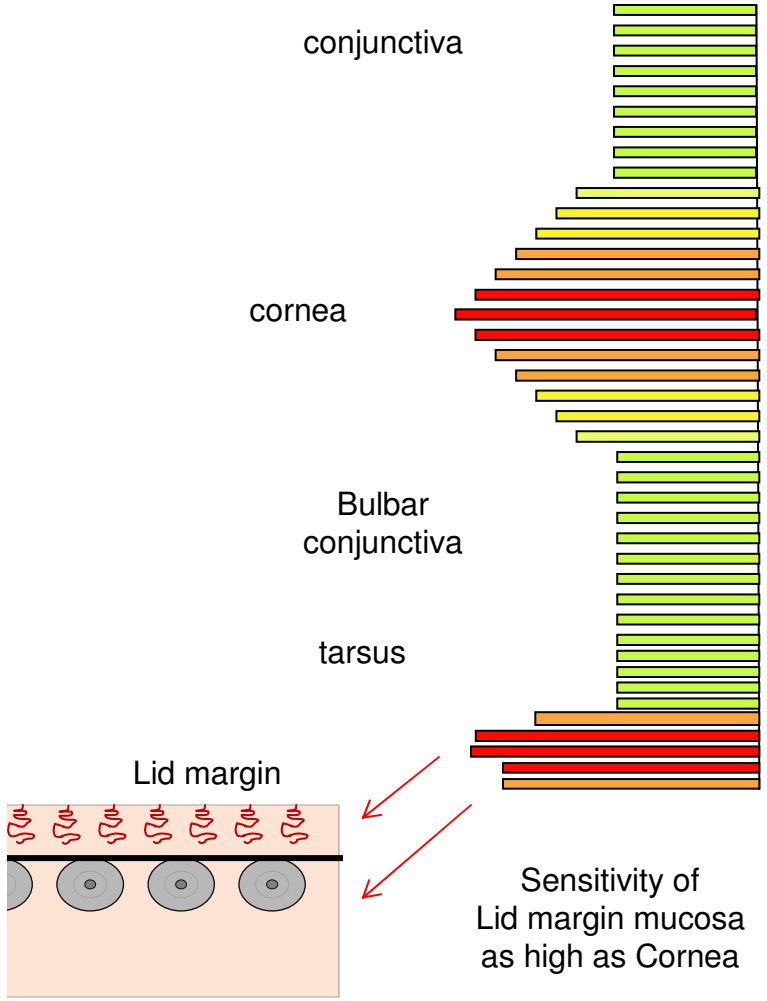


1. Belmonte Gallar IOVS 2011, Vol. 52, 3888

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

Innervation of the Eye
Sensitivity varies over the lids and ocular surface



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

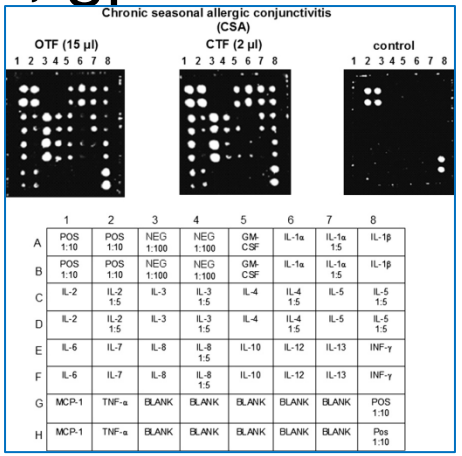
- Bioinformatics – protein networks.

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;
- E-,L-,P- selectin,
- **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



	1	2	3	4	5	6	7	8
A	POS 1:10	POS 1:10	NEG 1:100	NEG 1:100	GM-CSF	IL-1α	IL-1α 1:5	IL-1β
B	POS 1:10	POS 1:10	NEG 1:100	NEG 1:100	GM-CSF	IL-1α	IL-1α 1:5	IL-1β
C	IL-2	IL-2 1:5	IL-3	IL-3 1:5	IL-4	IL-4 1:5	IL-5	IL-5 1:5
D	IL-2	IL-2 1:5	IL-3	IL-3 1:5	IL-4	IL-4 1:5	IL-5	IL-5 1:5
E	IL-6	IL-7	IL-8	IL-8 1:5	IL-10	IL-12	IL-13	INF-γ
F	IL-6	IL-7	IL-8	IL-8 1:5	IL-10	IL-12	IL-13	INF-γ
G	MCP-1	TNF-α	BLANK	BLANK	BLANK	BLANK	BLANK	POS 1:10
H	MCP-1	TNF-α	BLANK	BLANK	BLANK	BLANK	BLANK	Pos 1:10

Candidates: Chemokines in Dry Eye:
Th-1 -dependent inflammation

Yoon IOVS 51 643 2010

Chemokine type

CXC [α] **CC [β]** **C [γ]** **CXXXC [δ]**

ELR+ **ELR-**

IFNγ

CXCL9 [MIG]
CXCL10 [IP-10]
CXCL11 [I-TAC]

Receptors

CXCR 1 and 2 **CXCR 3 & 5** **CCR** 3 & 4

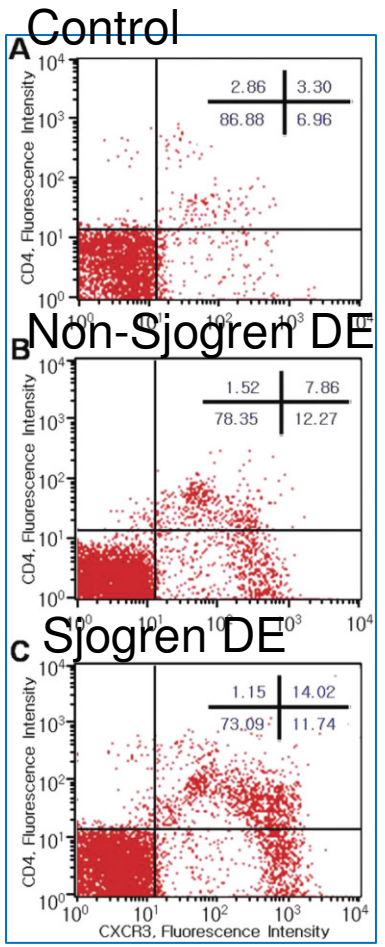
Recruits

PMNS

T-cells*
NK cells

Th-1 related inflammation

Th-2 related inflammation



Candidates: Chemokines in Dry Eye

Th-1 -dependent inflammation

Yoon IOVS 51 643 2010

- 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-epitheliopathy,
  - reduced goblet cell density.

Candidates: Cytokine profiles in Dysfunctional TS

Lam et al. 2008

- 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

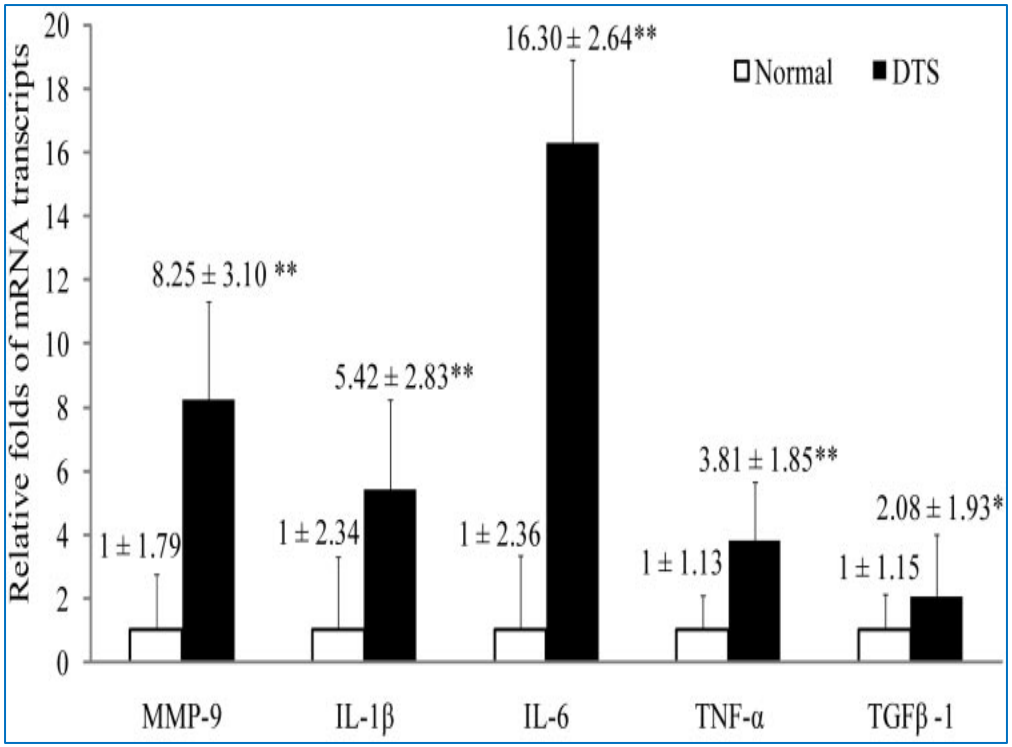
- Subjects: 30 DTS; 14 control
- 2-eye, pooled 0.5 µl tear capillary samples
- Luminex Bead array

- IFNγ / IL-13 ratio ↑ in DTS
- IFNγ 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:

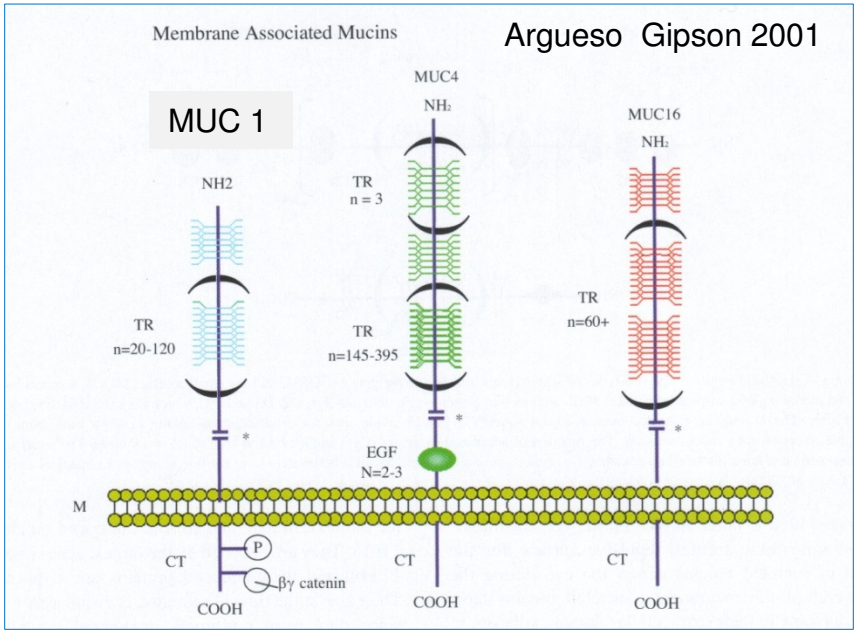
- Surface stain; confocal epithel. score; surface irregularity; low contrast sensitivity.
- No corrln with BUT.

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]

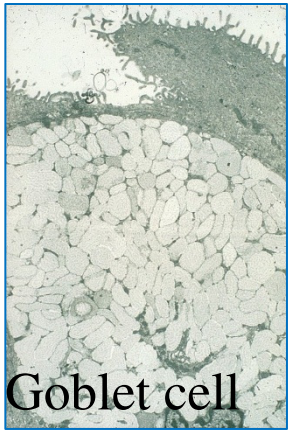
Candidates: tear and membrane bound MUC1

Caffery 2010

- 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.*

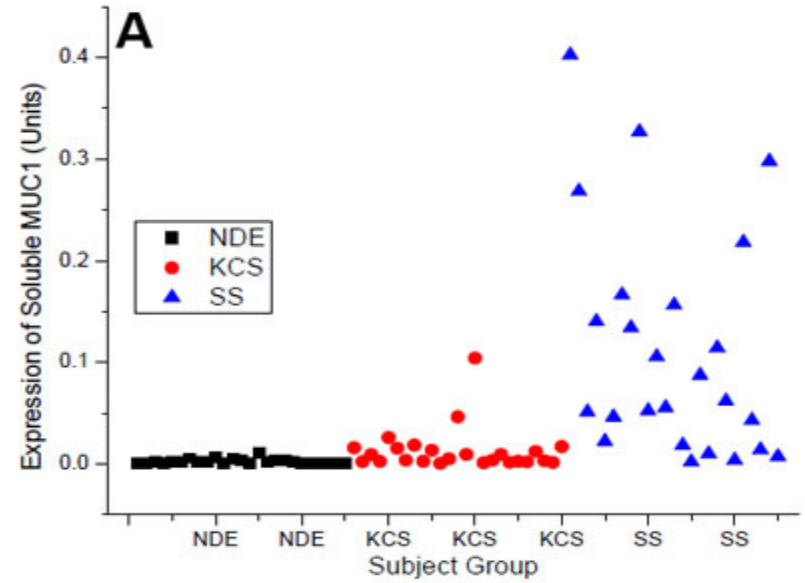


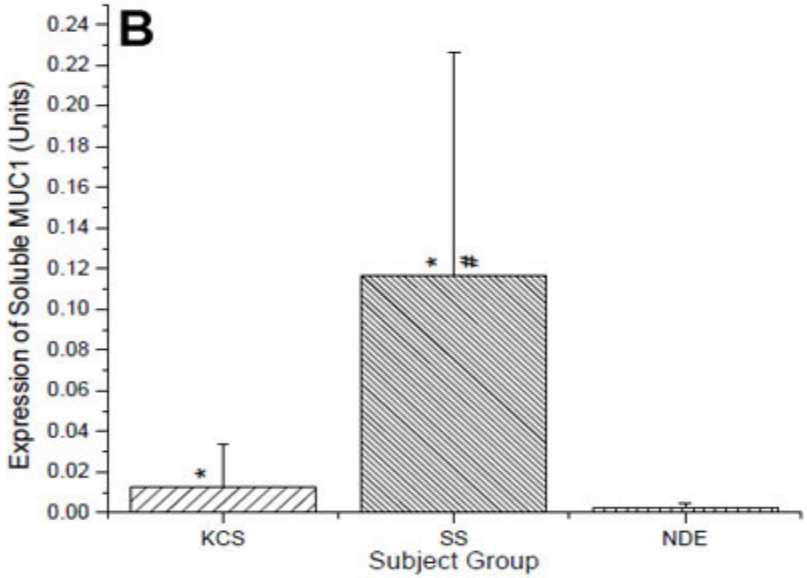
Candidates: tear and membrane bound MUC1

Caffery 2010

- 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

Corrales 2011

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

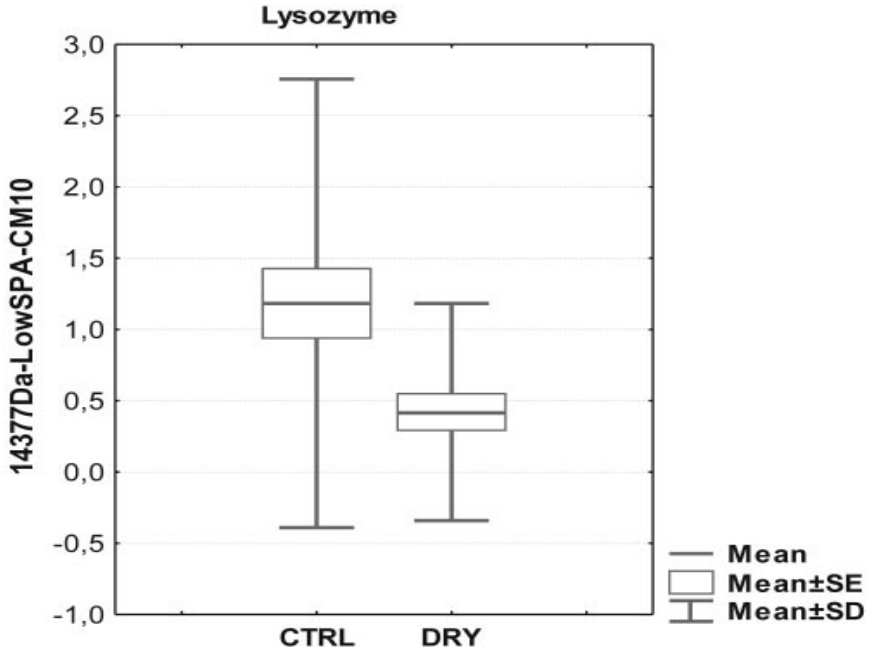
- Expression of MUC 1, 2, 4 and 5AC lower in NSDE
- Using MUC1 expression in dry eye diagnosis:

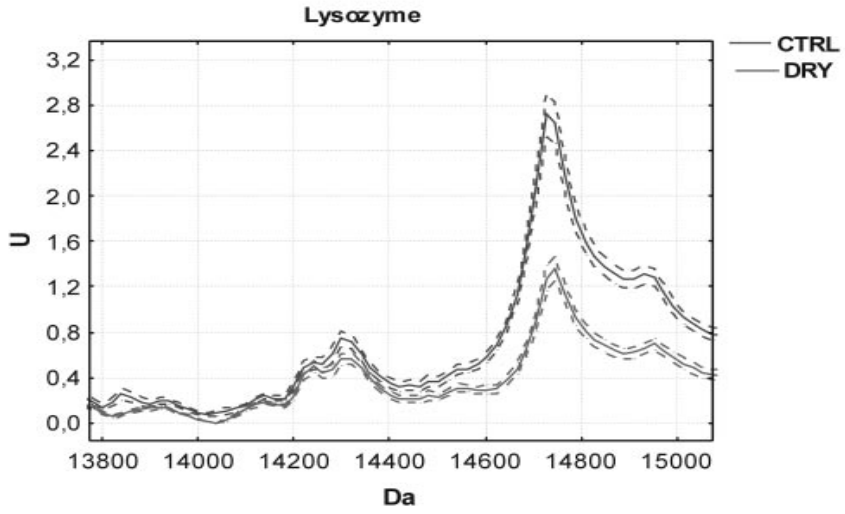
$$DR_{12.5} = 83.3\ \%$$

- Validated in additional control and DE groups.

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

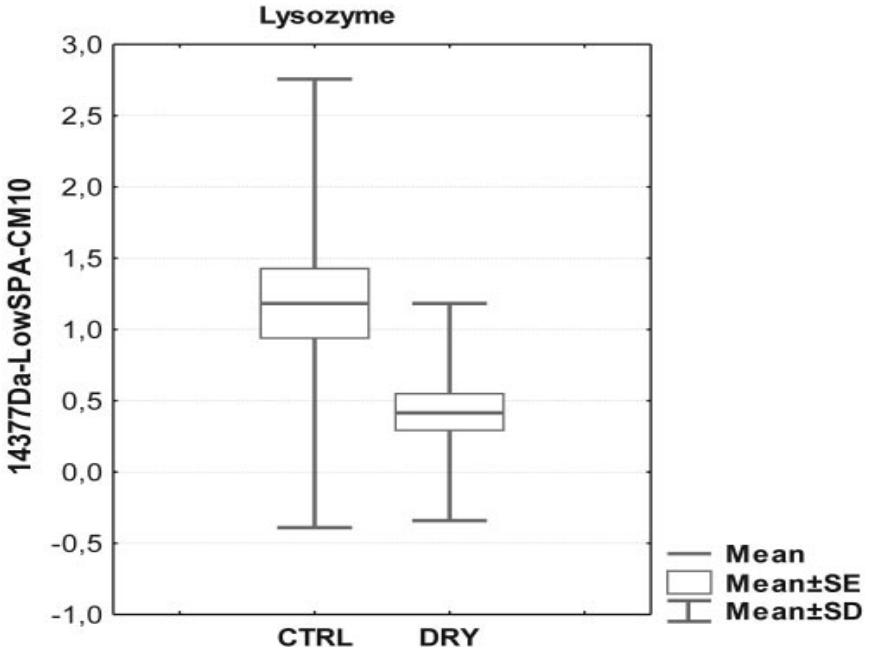


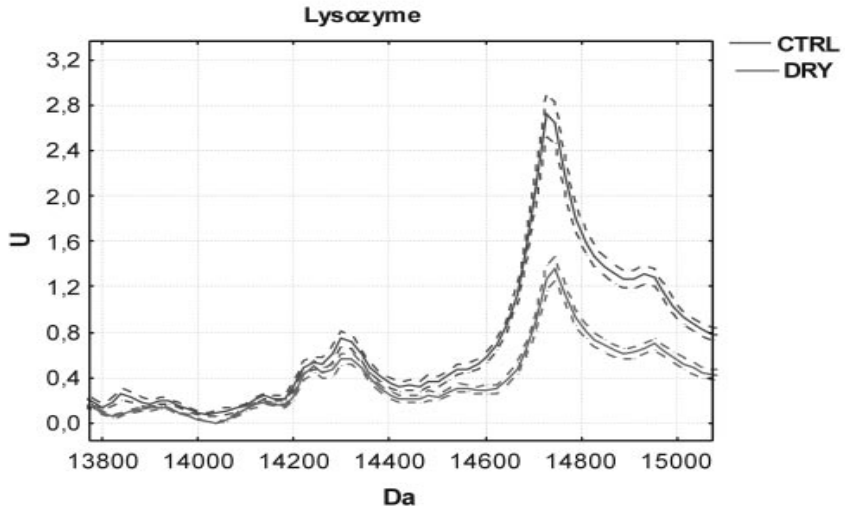


Grus 2005

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





Grus 2005

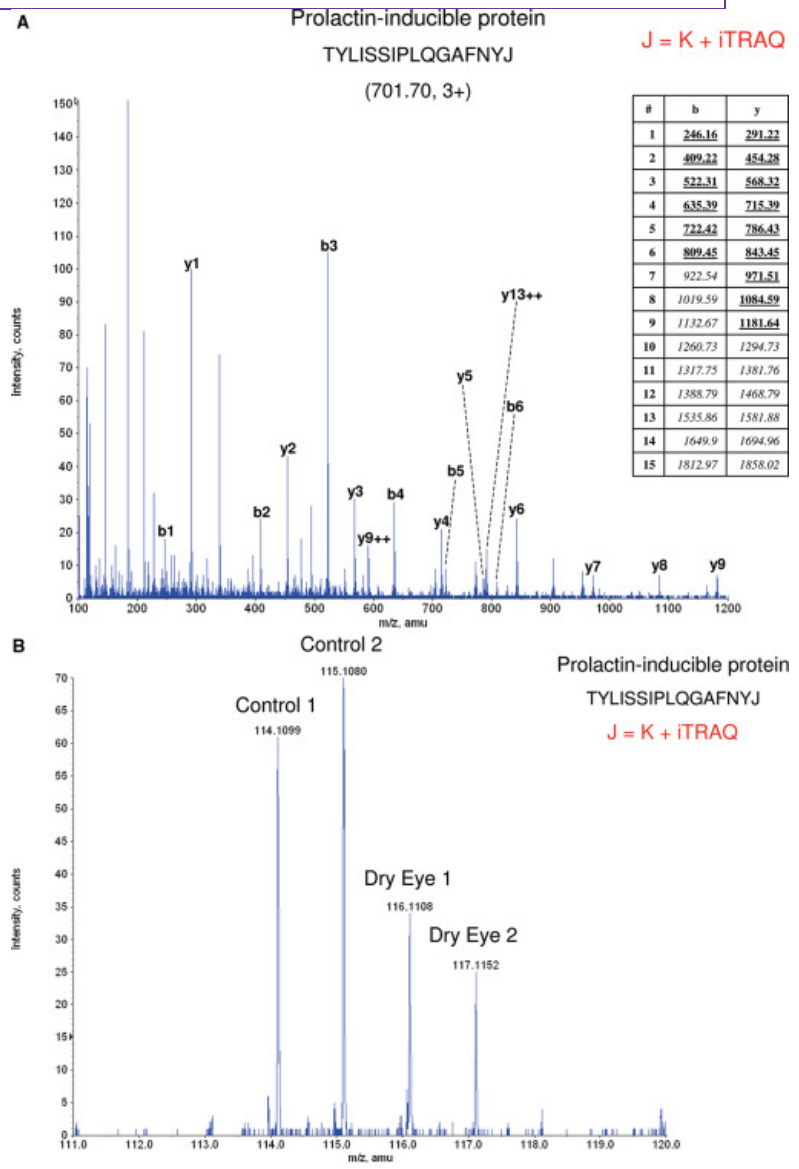
iTRAQ technology with 2D-nanoLC-nano-ESI-MS/MS

Zhou

Proteome Res 2009

- Subjects: 56 DE: Symptoms+; Sch ≤10 mm; FBUT ≤ 10s; Cr Stain >2 Oxf
- 40 control
- 10 mm Schirmer strip sample

- 93 tear proteins identified, 10 differentially expressed



iTRAQ technology with 2D-nanoLC-nano-ESI-MS/MS

Zhou

Proteome Res 2009

- **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

iTRAQ technology
MGD and Dry Eye

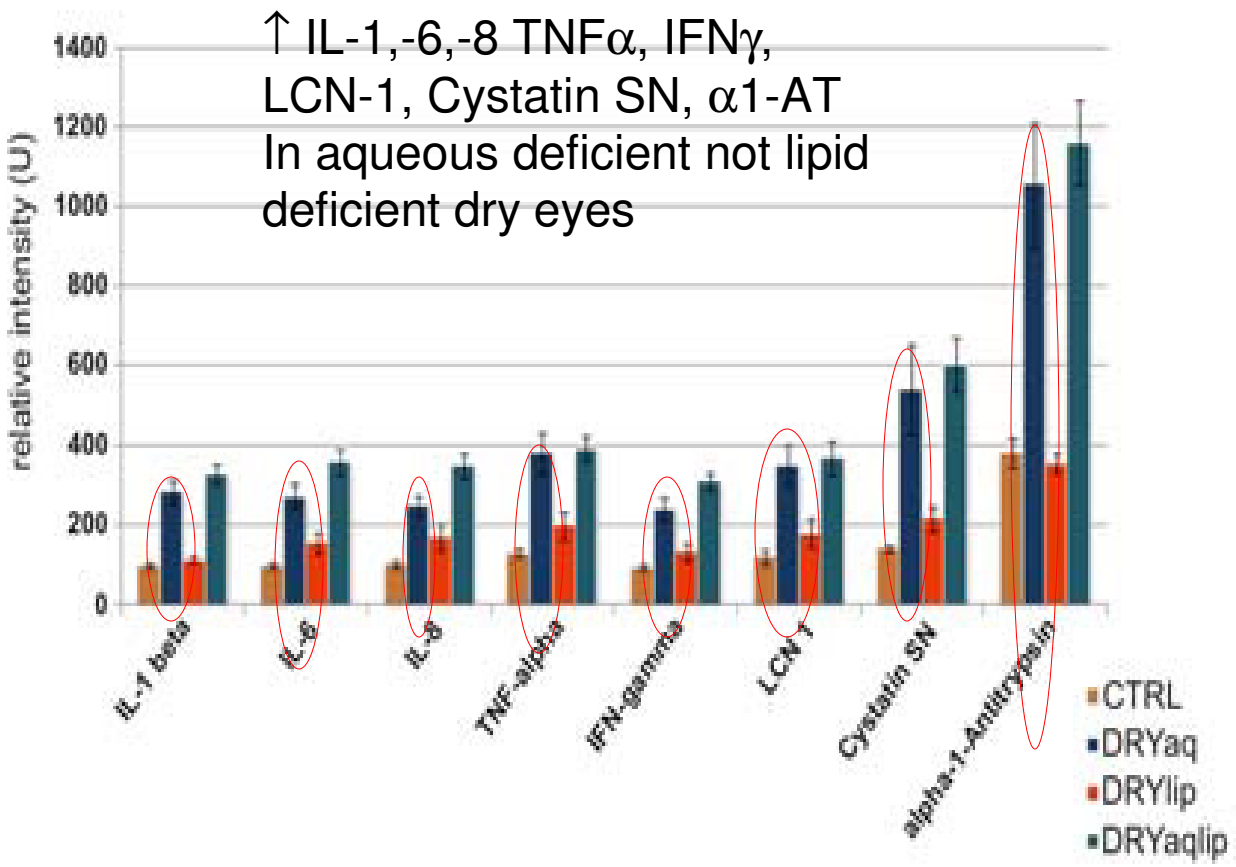
Tong et al. 2011

- 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".

Cytokines - Antibody Microarray
Aqueous- and lipid-deficient Dry Eye

- Subjects:
- 35 DRYaq;
- 36 DRYLip;
- 34 mixed
- 38 Controls.
- Eluted Schirmer strips
- Antibody microarray



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