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Progress in the Development of Informative Biomarkers for Dry Eye Disease

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ABSTRACT

Dry Eye Disease (DED), one of the most common reasons for patients to seek ophthalmic care, remains a diagnostic challenge for clinicians and researchers. A pressing need exists for the identification of an informative biomarker for both diagnostic and staging purposes in this condition. Herein, an explanation for how the complex pathophysiology of DED hampers biomarker development is given. A review of previous efforts to identify suitable biomarkers using standard clinical measures, imaging modalities and molecular techniques is provided. Based on current understanding, it is likely that a suitable biomarker will need to incorporate information from multiple parameters using a variety of scientific approaches. Identification of an informative biomarker will dramatically alter the diagnostic landscape for this is disease and will greatly improve the quality of patient care and research studies for this condition.

INTRODUCTION

Dry Eye Disease (DED), a prevalent disease with diverse phenotypes, remains a challenging diagnostic entity for both clinicians and researchers in part due to the lack of informative diagnostic (and also predictive) biomarkers. The compelling need for a biomarker stems from the high prevalence of DED and its clinical impact. Both internists and eye care professionals would be greatly assisted by the development of such a test [1]. DED is endemic with a prevalence between 15 and 50% of the world's population [2-5]. DED is associated with a staggering financial impact on society [6,7] and an even greater health burden [4]. Although the majority of DED is mild or moderate, up to 10% can be severe [7]. In addition, DED can adversely affect the treatment and outcomes of other vision threatening diseases such as glaucoma [8-10], refractive surgery, and cataract surgery [11,12]. In this context, the impact of DED takes on an even greater public health importance.

Here, we highlight the pressing need for the development of DED biomarkers. After describing the characteristics of an ideal biomarker, obstacles for development of DED biomarkers are reviewed. Finally, a brief summary of the many avenues that have been pursued in search of such biomarkers is given.

IDEAL ATTRIBUTES OF A DED BIOMARKER

Biomarkers and surrogate endpoints have long been used in medicine to make rational clinical decisions. Biomarkers and surrogate measures are used because they are often cheaper and easier to measure than 'true' endpoints. A biomarker is "-a characteristic that is objectively measured and evaluated as an indication of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention-", while a surrogate endpoint is defined as "-a biomarker intended to substitute for a characteristic or variable that reflects how a patient feels, functions, or survives-" [13]. Characteristics of a biomarker that are more likely to make it useful have been described and are listed in Table 1 [14]. A useful or ideal biomarker is one through which the disease comes about or through which an intervention alters the disease [14]. For ocular diseases, an ideal biomarker should be easy to measure and collected from the target tissue of interest rather than from blood or urine.

Table 1: Biomarker characteristics that increase likelihood of a causative association

Characteristic	Attributes of characteristic
Strength	A strong association between marker and outcome is present, or between the effects of a treatment on both marker and outcome
Consistency	The association remains regardless of different person, place, time or circumstance
Specificity	The marker is associated with a specific entity or disease
Temporality	Changes in the marker and disease occur in parallel and with the same time course
Biological gradient (dose-responsiveness)	Increasing effects on both the marker and the disease occur with increasing interventions
Plausibility	A reasonable mechanism between the marker, disease pathogenesis, and the mode of action of the intervention exists
Coherence	The natural history of disease and changes in the marker are consistent
Experimental evidence	An intervention produces results consistent with the association
Analogy	A similar relation between disease and marker can be cited

In DED, both immune and non-immune mediated causes trigger inflammatory events in the ocular surface tissues. As DED pathophysiology manifests on the ocular surface, where tissues can be directly visualized and easily accessed for testing, it would be a reasonable expectation that a biomarker capturing the summative immunopathology within these tissues irrespective of the initial trigger could be determined. However, despite this apparent suitability, a well-accepted, informative biomarker has yet to be identified.

The inability to identify an ideal biomarker for DED is explicable when the complexity of the disease pathophysiology is considered. Ophthalmologic conditions such as cataract and glaucoma better lend themselves to defining relevant and useful biomarkers. In the case of cataract, a single tissue (lens), secluded from the external environment, develops a slowly progressive opacity. Vision serves as an informative biomarker because in the absence of other ocular pathology, a decrease in vision can be reproducibly measured and is directly related to the degree of lens opacity. Although decreased vision may not be specific for cataract, reliable measures to rule out other causes of vision loss are readily available. Glaucoma represents a more complex situation because tissue pathology may exist in both the anterior and posterior segment of the eye (trabecular meshwork and optic nerve). Glaucoma may have multiple etiologies, and disease progression can be influenced by external factors such as medication compliance. However, despite this additional complexity, all forms of glaucoma share the common final pathway of Retinal Nerve Fiber Layer (RNFL) loss that results in glaucomatous visual field defects. Regardless of the etiology or external factors that impact this disease, a single clinical parameter (intra-ocular pressure) imparts the predominant risk for progression. In addition, structural assessments for the affected tissues including the ganglion cell layer and optic nerve can be measured with great precision and reproducibility using optical Coherence Tomography (OCT). Functional assessments of vision are also possible with perimetry. The consistency and reproducibility of these measures make them good indicators of disease status.

DED presents additional challenges to the development of an informative biomarker due to an even higher level of disease complexity. Unlike cataract, DED is not just one disease but "-a multi-factorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles-" [15].

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Unlike cataract and glaucoma, DED presents a conundrum because its clinical signs can vary widely, and its symptoms (e.g., burning, aching) often do not correlate with tear and ocular surface findings (e.g., decreased tear production, decreased tear break-up or abnormal corneal epithelium staining) [16]. This apparent disconnect between signs and symptoms is common in systemic diseases associated with DED including fibromyalgia, migraine, and traumatic brain injury [17]. In fact, contrary to what the name DED suggests, most individuals with DED do not have insufficient tear production. Only 10-20% suffer this sub-type of DED (i.e., aqueous deficiency) which is commonly encountered in individuals with Sjögren's syndrome or graft versus host disease [5,18]. Another major challenge to identifying a DED biomarker is that the clinical phenotype is affected by the multiple tissues of the Lacrimal Functional Unit (LFU). The LFU includes the main and accessory lacrimal glands, cornea, conjunctiva, meibomian glands, eyelids, and all the sensory and motor nerves that connect them [19,20]. All components of the LFU work in concert as an integrated system. When this well-orchestrated system is functioning in normal equilibrium, the end result is a healthy ocular surface. Although disease of any single LFU component will disrupt this equilibrium to cause DED, all of the remaining healthy LFU tissues can compensate for the malfunctioning component resulting in a more diverse range of physiologic responses and disease phenotypes than seen with other conditions such as glaucoma. This may also help to explain why clinical signs and symptoms of DED do not correlate well in the clinical setting [21]. Finally, and perhaps most importantly, exogenous factors play an important role in disease phenotype. Environmental conditions such as ambient temperature, humidity and wind play a role. Patient related behaviors (use of video monitors, reading etc.) and systemic / topical medication use also can impact disease severity. In addition, many of these variables are difficult or impossible to control or modify (unlike intraocular pressure for glaucoma). Because DED has so many etiologies, exacerbating factors, and possible compensatory mechanisms, the total number of variables that can impact the clinical presentation of DED is far greater than for glaucoma or cataract. The end result is that the complexity of DED pathophysiology has made it difficult to develop a single informative test or biomarker of disease.

Nevertheless, DED does possess attributes which hold promise for development of a biomarker. The following paragraphs highlight prior efforts to identify a biomarker for DED.

DED BIOMARKERS IN CLINICAL PRACTICE AND THE FUTURE AHEAD

Standard clinical assessments of DED that measure tear production and function have been well described and reviewed elsewhere [22]. Some tests such as the Schirmer Tear Test (STT) have been performed for over a century [22]. However, no individual assay can capture the entire clinical spectrum of DED. Furthermore, the most commonly performed clinical tests which include Tear Osmolarity (TOsm), STT, Tear Break Up Time, and vital dye staining of the ocular surface have major limitations including high variability, inadequate sensitivity and/or specificity, and problems inherent in the determination of their normal values including spectrum and observer bias [23]. Many of the procedures clinically used to diagnose and monitor dry eye syndromes demonstrate large variability such that they have been described as largely unrepeatable [24]. For example, since a single TOsm measurement may be normal in patients with DED, the average of repeated TOsm determinations rather than a single assay may have better diagnostic value [25]. Increased variability of TOsm has also been shown to be indicative of DED severity [26]. Although STT is one of the most commonly used tests to assess DED it can have limited reproducibility or inaccurate results due to reflex tear secretion [27]. In a recent study, our laboratory documented the inadequacy of STT to grade aqueous deficient DED in an optimized animal model [28]. Fluorescein, routinely used in the assessment of clinical TBUT, can destabilize the tear film and thus affect its results [29]. These shortcomings explain why some clinicians routinely opt not to use any of these tests for their evaluation of DED patients, basing instead their clinical decisions on symptoms alone.

Due to the limitations of individual tests, combining the results of more than one clinical measure to produce a more informative biomarker has been suggested as a way to better characterize DED [23,30]. Our lab has recently reported on 2 novel rabbit models of DED [31,32]. In both of these aqueous deficient models, DED status was assessed using standard clinical measures including tear osmolarity, Schirmer test, Tear Break

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Up Time, and corneal staining. Using these models, we demonstrated that a mathematically derived metric for DED can be generated using principal component analysis. This metric is superior to any individual clinical measure and overcomes many of the problems of selection and spectrum bias [28]. Interestingly, this metric possesses all the features of an ideal biomarker as listed by Hill [14]. Although not yet demonstrated in humans, the results validate the use of commonly employed standard clinical tests for the evaluation of DED, supports the hypothesis that more than one metric will be needed to characterize this complex disease, and highlights the need for more innovative approaches to interpret clinical data. Identification of an analogous parameter for humans (if one exists), could markedly improve the current diagnostic landscape for patients with DED.

Recent technological advances have improved capabilities to image the ocular surface as well as the architecture and function of the tear film. Highly specialized equipment including Optical Coherence Tomography (OCT), interferometers, infrared light cameras and specialized keratographers can now objectively and non-invasively measure the architecture and function of the tear film overcoming observer bias inherent in clinical measures and may even differentiate between various types of DED better than standard clinical measures [19,24]. Tear meniscus height is a common measurement shown to correlate with DED and the traditional measurements for DED that can be measured with infrared light cameras or OCT [33-35]. Tear meniscus cross-sectional area and tear meniscus depth can also be calculated from OCT images, although some observer bias may be present given the need for users to specify the borders of the tear film [36]. The thickness of the tear film lipid layer, which plays an important role in maintaining tear film stability [37-39], is now directly measurable on some devices. Abnormalities of the lipid layer thickness can be observed in DED [40], more specifically in meibomian gland disease [41,42], but results are not always as expected [43]. In addition to tear film structure, advances in imaging also allow for the function of the tear film to be assessed objectively and non-invasively. Non-invasive Tear Break Up Time (NITBUT) correlates well with DED and can provide statistically significant differences between DED and normal states in addition to having good intra-examiner repeatability and inter-examiner reproducibility [44,45]. However, there is appreciable variability when comparing NITBUT with the traditional FBUT [46]. Meibography, a recent technique using infrared light, laser confocal microscopy or OCT allows for evaluation of meibomian gland anatomy including morphological abnormalities and quantification of meibomian gland loss [47]. Although meibography alone cannot be used to diagnose or stage DED it is a useful clinical tool that can reinforce the diagnosis of evaporative DED [48]. Additional efforts using imaging to provide biomarkers for DED have included grading of bulbar redness due to conjunctival congestion [49] as well as efforts to evaluate corneal innervation [50]; however neither of these methods has provided a viable biomarker yet. Quantitative and qualitative analysis of the superficial corneal epithelium in dry eye syndromes using in vivo confocal microscopy (in addition to histologic assessment) has been done. Studies with these techniques demonstrated similar morphological alterations in both Sjogren and non-Sjogren dry eye syndromes recapitulating the notion that regardless of the initiating inflammatory events, significantly reduced epithelial cell density across all layers of the epithelium results. These changes may be due to enhanced desquamation, inflammatory mediated apoptosis and / or impaired epithelial regeneration. Table 2 summarizes imaging modality metrics that have been investigated as possible biomarkers for DED.

Table 2: Imaging tests to quantify the tear film and ocular surface in dry eye disease.

	Parameter measured	Device(s)	Aspect measured
Tear Film Architecture	Tear meniscus height	Infrared camera, OCT	Structure
	Tear meniscus area	OCT	Structure
	Tear meniscus depth	OCT	Structure
	Lipid layer thickness	Interferometer	Structure
Tear Film Function	Non-invasive keratographic breakup time	Keratometer	Function
Ocular surface / lids	Meibography	Infrared camera, confocal scanning laser, OCT	Structure
	Bulbar redness; conjunctival congestion	Camera	Structure
	Corneal nerves	Confocal scanning laser	Structure

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Table 3: Molecules and mediators investigated in Dry Eye Disease.

Type	Name	Tissue source	
Inflammatory Cytokines	IFN-γ	Tears, conjunctiva	
	TNF-α	Tears, conjunctiva	
	IL-1 α, IL-1β	Tears, conjunctiva	
	IL-6	Tears, conjunctiva	
	IL-8	conjunctiva	
	IL-3	conjunctiva	
	IL-17A, IL-17F, IL-22		
	TGF- β1		
Chemokines	IL-8 / CXCL8	Tears	
	MIP-1 α / CCL3, MIP-1β/CCL4, RANTES/CCL5, Fractalkine, CX3CL1, CXCL9, CXCL10, CXCL11, MCP-1/CCL2	Tears	
	CCL2		
	CCL12		
	CCR2		
	CXCR4		
Protein	Lactoferin	Tears	
	MMP-9	Tears	
	EGF	Tears	
	LPRR4, LPRR3	Tears	
	α-1 antitrypsin	Tears	
	LCN-1	Tears	
	α -enolase	Tears	
	S100A8/Calgranulin A,	Tears, conjunctiva	
	S100A9/Calgranulin B, S100A4, S100A11	Tears, conjunctiva	
	S100A6	conjunctiva	
	Annexin A1	Tears, conjunctiva	
	Anexnin A11	Tears	
	Muc5AC	Tears, conjunctiva	
	Muc16	conjunctiva	
	Epidermal Fatty Acid-Binding Protein	Tears, saliva, serum	
Neuromediators			
	Substance P	Tears	
	NGF	Tears	
	VIP	Tears	
	CGRP	Tears	
Other			
	HLA-DR	conjunctiva	
	ICAM-1	conjunctiva	
	Goblet cells	conjunctiva	
	Galectin-3	conjunctiva	
	CD4+, CD8+		
	HEL		
	4-HNE		
	MDA		

Table 4: Inflammatory cytokines in various anterior segment pathologies.

Condition	IL-1	IL-4	IL-5	IL-6	IL-8	IL-13	IL-17	IL-21 (T_H2)	IL-22 (T_H2)	IFN-γ (T_H1)	TNF-α
Dry Eye Disease	+	+	+		+	+	+	+	+	+	+
Medicamentosa			+	+	+	+	+				+
Allergic Conjunctivitis	+	+	+	+		+				+	+
Blepharitis				+			+				
Ocular cicatrial pemphigoid				+	+		+				
Scleritis	+	+	+	+			+		+	+	+

Table 5: Inflammatory mediators and molecular markers in anterior segment pathologies.

	HLA-DR	CCR4 (T_H2)	CCR5 (T_H1)	CD11a, CD11b	CD45RO	vimentin	ICAM-1	MMP-9
Glaucoma Medications	+	+	+	+	+	+	+	+
Allergic Conjunctivitis	+	+					+	+
Dry Eye Disease	+		+				+	+
Blepharitis								+
Ocular cicatrial pemphigoid								+

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Recent advances in impression cytology sampling and advanced molecular techniques have allowed for the pathophysiology of DED to be defined. DED has an inflammatory basis with activation of both innate and adaptive immunity pathways. Activation of the inflammatory cascades results in changes in expression of many proteins and other molecules at the ocular surface. Table 3 provides a list of molecules and mediators that have been investigated in hopes clarifying the pathophysiology of DED. Immense efforts have been made to determine if alterations in the levels of such molecules and mediators could better define DED. Unfortunately, to date, this body of work has not identified an ideal molecular biomarker. Most of the potential biomarkers investigated to date suffer at least one or two of the shortcomings listed by Hill [51,52]. For example, MMP-9, although readily available as a commercial test for DED, suffers from the fact that it is not specific for DED. Tables 4 and 5 illustrate the lack of specificity of various cytokines and inflammatory mediators for DED which greatly limits their usefulness to serve as biomarkers. As illustrated, altered levels of MMP-9 as well as other molecules that have been associated with DED, are also seen in numerous other anterior segment pathologies. Although none of the markers in the tables are specific for DED, they do confirm the inflammatory basis of this disease. Identification of new molecules, such as Epidermal Fatty Acid-Binding Protein [53], may ultimately reveal a more informative and predictive biomarker for this disease. That changes in these molecules are seen in multiple anterior segment pathologies suggests the ocular surface may employ a limited number of molecular pathways to respond to external stimuli and that their variable involvement may account for the variable clinical manifestations of DED. If true, this would present both an opportunity and a challenge to develop a useful biomarker for DED. “Omics” or the combined use of genomics, proteomics, and lipidomics to generate a comprehensive profile of disease hold promise to clarify complex systemic diseases such as DED. These approaches have demonstrated a relationship between changes in metabolites, symptoms of dry eye syndrome, and age [54]. The use of “omics” to define a biomarker for DED also supports the hypothesis that more than one metric will likely be needed to characterize and define this complex entity.

CONCLUSION

In conclusion, the search for an informative and clinically useful biomarker for DED continues while the corresponding need for clinicians and researchers persists. The ideal biomarker has remained elusive as DED’s almost protean manifestations defy efforts to identify a single parameter as its marker. It appears increasingly likely that only a constellation of parameters may address the issue of DED biomarkers. Continued contributions and advances from all areas of ophthalmology research including more informative animal models, improved ocular surface imaging technologies to quantify tear anatomy and function, and further delineation of the molecular pathophysiology of DED will ultimately provide a suitable biomarker that can aid in the diagnosis and staging of this vexing disease.

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