Role of Hyperosmolarity in the Pathogenesis and Management of Dry Eye Disease: Proceedings of the *OCEAN* Group Meeting☆

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ABSTRACT **Dry eye disease (DED), a multifactorial disease of the tears and ocular surface, is common and has a significant impact on quality of life. Reduced aqueous tear flow and/or increased evaporation of the aqueous tear phase leads to tear hyperosmolarity, a key step in the vicious circle of DED pathology. Tear hyperosmolarity gives rise to morphological changes such as apoptosis of cells of the conjunctiva and cornea, and triggers inflammatory cascades that contribute to further cell death, including loss of mucin-producing goblet cells. This exacerbates tear film instability and drives the cycle of events that perpetuate the condition. Traditional approaches to counteracting tear hyperosmolarity in DED include use of hypotonic tear substitutes, which have relatively short persistence in the eye. More recent attempts to counteract tear hyperosmolarity in DED have included osmoprotectants, small organic molecules that are used in many cell types throughout the natural world to restore cell volume and stabilize protein function, allowing adaptation to hyperosmolarity. There is now an expanding pool of clinical data on the efficacy of DED therapies that include osmoprotectants such as erythritol, taurine, trehalose and L-carnitine. Osmoprotectants in DED may directly protect cells against hyperosmolarity and thereby promote exit from the vicious circle of DED physiopathology.**

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OUTLINE

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I. INTRODUCTION

Dry eye disease (**DED**) is defined in the International Dry Eye Workshop (DEWS) report as "*a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.*"[1] It is characterized by symptoms of eye irritation, blurred and fluctuating vision, tear film instability, increased tear osmolarity and impairment of ocular surface epithelia.[2] Epidemiological studies conducted in the general population have demonstrated that DED is a relatively common condition, with a prevalence of 5-34%, depending on the criteria for DED applied, the population studied, and geographic location.[3-9] DED has a significant negative impact on quality of life due to chronic irritation and pain,[10] which can have a negative impact on visual performance and ability to perform daily tasks (eg, reading, driving).[11] These detrimental effects on functioning may lead to anxiety and depression.[12]

The ocular surface is highly exposed, and efficient tear production and turnover is essential for its continued health. The tear film, lacrimal glands (main and accessory lacrimal glands, meibomian glands, goblet cells, and all ocular surface secretory cells), lacrimal outflow pathways, and corneal and conjunctival epithelia work together as a lacrimal functional unit (**LFU**) to maintain the tear film and protect the transparency of the cornea and the integrity of the ocular surface.[13,14] The LFU is not an isolated system and is affected by many factors, such as nerve connections and hormones. Disease or damage to any component of the LFU (eg, the afferent sensory nerves, the efferent autonomic and motor nerves, and the tear-secreting glands) can destabilize the tear film and lead to ocular surface disease that expresses itself as DED. It is therefore not simply a lack of tears, but a complex ocular surface disease in which the tear film is unbalanced and no longer provides sufficient nourishment or protection to the ocular surface (reviewed in 2007 Report of the International Dry Eye Workshop [DEWS][1]). This can lead to an imbalance in electrolytes, proteins and mucins and permanent damage to the corneal and conjunctival epithelial cells and the corneal nerve fibers that trigger secretion.

Figure 1 illustrates the concept of the vicious circle that may contribute to the pathophysiology of DED.[15] Risk factors or causative factors are shown on the outside of the circle; internal pathologic mechanisms are on the inside. The external causative factors are independent or interacting processes that may lead to entry into the circle; any form of DED can interact with and exacerbate other forms. The internal pathologic mechanisms also interact, as activity in one area exacerbates another process.

At the top of the figure, tear film instability/imbalance refers to an abnormally rapid breakup of the tear film after blinking, caused when interactions of the stabilizing tear film constituents are compromised by decreased tear secretion, delayed clearance, or altered tear composition.[1] This leads to local drying and hyperosmolarity of the exposed surface, surface epithelial damage, and disturbance of the glycocalyx and goblet cell mucins. Tear hyperosmolarity is one of the central events in the vicious circle of DED and refers to a state in which the osmolarity of the tear exceeds that of the epithelial cell, leading to reduced cell volume and increased concentration of solutes. As seen in Figure 1, tear hyperosmolarity stimulates death of the epithelial surface cells and a cascade of inflammatory events, which lead to loss of mucin-producing goblet cells. This exacerbates the tear film instability and contributes to the circle of events that perpetuate DED.

DED may be regarded as an inability of the eye to adapt to a challenging environment.[14] Epithelial cells exposed to tear hyperosmolarity face similar challenges to many different cell types in the natural world that must survive in hyperosmotic environments. Many cells are able to withstand such hyperosmotic stresses through osmoregulation. It is possible that examining osmoregulation in other cell types may enhance our understanding and treatment of DED. Indeed, osmoprotective topical treatments designed specifically to address hyperosmolarity are now emerging. The purpose of this review is to discuss osmoregulation in nature, explore the role of hyperosmolarity in DED, and consider the role of osmoprotective therapies in counteracting and protecting against hyperosmolarity.

A meeting was held in Nice, France, on 14-15 December 2012 by the OCEAN group to discuss the role of hyperosmolarity in the pathogenesis of dry eye disease. The name OCEAN was chosen because of its theme of salinity, reflecting the panel's focus on hyperosmolarity as a key

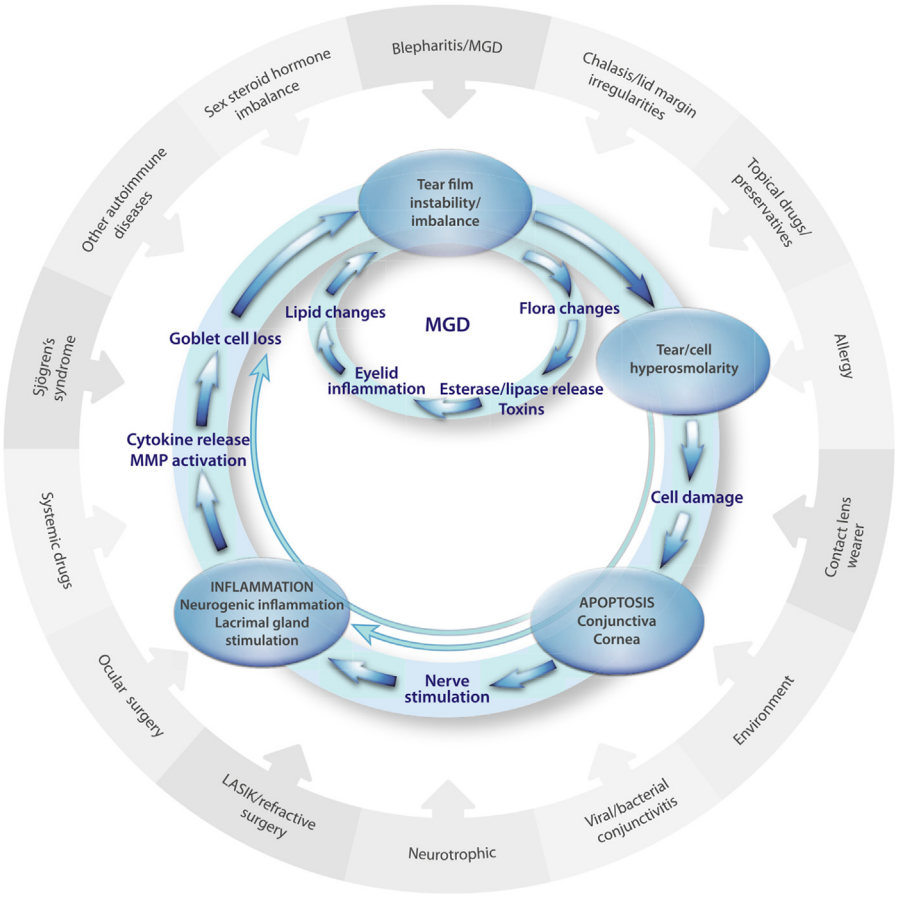

Figure 1. Proposed schema of the vicious circle theory for DED pathology, adapted from Baudouin.[15] MMP: matrix metalloproteinase. LPS: lipopolysaccharide. MGD: meibomian gland dysfunction.

pathogenic mechanism in DED, and osmoprotection as a potential therapeutic strategy. A consensus was reached by the expert committee and the findings are presented in this review.

II. OSMOREGULATION AS A PHYSIOLOGICAL SYSTEM

As mentioned above, hyperosmolarity leads to reduced cell volume and increased concentration of solutes. This in turn causes oxidative stress and disruption of DNA repair systems and thereby causes DNA damage and cell cycle arrest.[16] If the cell is unable to protect itself, pro-apoptotic signaling is upregulated and the cell exhibits classical features of apoptosis, including DNA condensation and mitochondrial dysfunction.[17,18] Cell volume decrease is a characteristic early event in apoptosis, even in cells not subject to hyperosmotic stress.

Cellular processes, such as metabolism, protein folding, and intracellular transport, require the cell to maintain a relatively stable osmotic pressure. This stability may be challenged by the cell's environment: indeed, life can be seen as "a thing of macromolecular cohesion in salty water."[19] This "salty water" may be the ocean (for marine invertebrates), a salt lake (for the brine shrimp), the extracellular fluid (for cells of the kidney medulla or brain) or the tears (for ocular epithelial cells).[20] In many cases, the cell can protect itself through homeostatic mechanisms.

In most cells, the first response to hyperosmolarity is regulatory volume increase, in which electrolytes (inorganic ions) and water are taken up. Although this inhibits expression of many genes and protein synthesis, DNA damage response proteins (eg, p53 and heat shock proteins) are activated and will promote survival.[20] Increased concentrations of inorganic ions, however, can only be a temporary measure, as they can disrupt protein structure and function. For example, in bluefin tuna, high levels of KCl and NaCl negatively affect participation of nicotinamide adenine dinucleotide (**NADH**) in the lactate dehydrogenase (**LDH**) reaction.[22]

In many cell types, a later stage of adaptation to hyperosmolarity occurs, and this can be referred to as *osmoprotection.* No specific mammalian osmosensor has yet been identified. It is possible that none exists and, instead, the cellular response to changes such as the decrease in cell volume, macromolecular crowding, and increased concentration of inorganic ions is achieved by means of pathway signals conserved throughout evolution.[21] Research suggests that a focal adhesion protein called tensin-1 responds to changes in cell shape[23] and activates a signaling cascade mediated by a heteromeric protein complex that includes Rho-type small G-proteins and protein kinases, including mitogen-activated protein kinase (**MAPK**)14 (reviewed by Brocker et al, 2012).[24] These signaling cascades activate tonicity-responsive element binding protein (**TonEBP**) or

NFAT5, a transcription factor that upregulates genes associated with antioxidant defense, molecular chaperones and synthesis and transport of "osmoprotectants" — small molecules known as "organic osmolytes" or "compatible solutes." This uptake is accompanied by a decreasing concentration of intracellular inorganic salts.[25]

Compatible solutes can be classified as amino acids (eg, glycine, betaine, proline, taurine), polyols (eg, glycerol, erythritol, inositols, sorbitol), small carbohydrates (eg, trehalose), methylamines/methylsulfonium solutes (eg, L-carnitine), or urea (Table 1).[26-31] They are all small molecules that do not perturb cellular macromolecules even at high concentrations. Many are zwitterionic, in that although they carry charged groups, the numbers of positive and negative charges are equal and the net charge is therefore zero. Others are weakly polar.

Compatible solutes act as osmoprotectants in a variety of ways. Uncharged but weakly polar compatible solutes such as polyols act as osmoprotectants in yeasts and algae, restoring cell volume that may be lost through water loss to a hyperosmotic medium. Zwitterionic compatible solutes such as L-carnitine and betaine act as osmoprotectants in mammalian systems and bacteria,[32-35] and may stabilize protein surfaces and other osmo-sensitive macromolecules, thereby maintaining functionality of proteins despite external stresses.[33] Such stabilizing compatible solutes promote stability through an "osmophobic" effect — a repulsion between the solute and the protein's peptide backbone that encourages correct folding of the protein. Throughout the natural world, stabilizing compatible solutes such as trehalose (in insects) and methylamines promote protein stabilization despite heating, freezing, desiccation, and changes in pressure.[25] Compatible solutes have been shown to have many other cytoprotective effects beyond osmoregulation, including antioxidation, redox balancing in hypoxic conditions, sulphide detoxification, and Ca^{2+} modulation.[25]

There are many examples of compatible solutes acting in mammalian systems. The mammalian renal medullary cells,

Table 1. Examples of naturally occurring compatible solutes

Class	Osmoprotectant	Studied as osmoprotectant in	Other information
Methylamine	L-carnitine	Mammalian renal medulla Mammalian eye lens Mammalian mammary tissue	Thought to stabilize protein surfaces Present in ocular tear film
	Trimethylamine N-oxide (TMAO)	Sharks Mammalian renal medulla	Thought to stabilize protein surfaces Counteracts urea inhibition
Polyols	Erythritol		Present in ocular tear film
	Glycerol	Algae Salt-tolerant plants Insects	Protects against high levels of NaCl and KaCl Was the first to be termed 'compatible solute'
	Myo-inositols	Mammalian renal medulla Mammalian brain Hyperthermophilic archaea	
	Sorbitol	Mammalian renal medulla Mammalian brain Freeze-tolerant insects	
Amino acids	Taurine	Mammalian kidney medulla Mammalian brain Porcine arterial tissue Shallow water invertebrates	Present in ocular tear film Provides metabolic protection in many animals Widely distributed in animal tissues
	Betaine	Mammalian kidney medulla Mammalian brain Porcine arterial tissue Vascular plants Sharks	
Small carbohydrates	Trehalose	Insects Yeast	Can protect against freezing, overheating and desiccation
Urea		Sharks Mollusks Lungfishes Amphibians	

Information taken from references.[25-31]

for example, are frequently exposed to hyperosmotic stresses for long periods and adapt to these conditions by accumulating sorbitol, betaine, inositol, taurine, and glycerophosphocholine (**GPC**).[36] L-carnitine may also play a role in kidney osmoregulation.[34] In the brain, adaptation to *hypo*-smolarity is achieved by loss of amino acids, polyalcohols, and methylamines.[37] Brain cells adapt to hyperosmotic stress by activating distinct osmoprotective genes, mediated by tonEBP.[38] These genes code for aldose reductase (which catalyzes production of sorbitol from glucose) and three transporters of osmoprotectants: sodium-dependent myo-inositol transporter, betaine/GABA transporter (**BGT1**) and taurine transporter (**TauT**). Porcine pulmonary arterial endothelial cells accumulate myo-inositol, taurine, and betaine when adapting to hyperosmotic stress.[39]

Osmoregulation is also a feature of different parts of the eye. Human and bovine cultured lens epithelial cells respond to hyperosmotic stress by raising taurine transport activity,[40] and human corneal epithelial cells show a 4.1-fold increase in uptake of taurine in response to exposure to a 450 Osm/L medium.[41] Similarly, low levels of carnitine have been observed in the lens in experimental models of diabetes, and it has been suggested that this is in response to osmotic stress: carnitine may promote homeostasis by preventing biochemical modifications of lens proteins and protecting the cell during extracellular osmotic fluctuations.[34]

III. HYPEROSMOLARITY IN DRY EYE DISEASE

Tear hyperosmolarity results from reduced aqueous tear flow (aqueous-deficient dry eye) and/or increased evaporation of the aqueous tear phase from the exposed ocular surface (evaporative dry eye).[42] Evaporative dry eye predominates and most cases are a mix of aqueous-deficient and evaporative dry eye (caused by lipid deficiency or compromised lipid quality).[43] Patients with DED, with or without tear volume reduction, have higher evaporation rates than controls, and this results in tear hypertonicity.[44] In the healthy state, the osmolarity of blood is 285−295 mOsm/L,[45] and the osmolarity of the tear film is in homeostasis with this, with recorded measurements being 296−302 mOsm/L.[46-48] In patients with DED, however, this value is generally 316−360 mOsm/L.[47,49,50] Spikes in tear film osmolarity of 800−900 mOsm/L are thought to occur over the central cornea,[51] but not in the meniscus, where samples are collected in clinical practice. An analysis of data from 16 studies published 1978−2004 found substantial overlap between osmolarity values in "normal" eyes and those of patients with DED (Figure 2).[47] The utility of osmolarity in diagnosis is discussed below.

Tear hyperosmolarity is a principal step in the vicious circle of DED pathology (Figure 1). In a recent study of patients with a relapsing type of infectious keratitis, onset of DED was characterized by hyperosmolarity before changes were evident in clinical measures such as tear film breakup time [**TFBUT**], Schirmer test results, and corneal sensitivity.[52] Tear hyperosmolarity leads to morphological changes such as apoptosis of cells of both the conjunctiva and cornea. It is also associated with inflammatory events, which lead to further cell death and loss of mucin-secreting goblet cells. Hyperosmolarity will ultimately result in breakdown of the corneal epithelial barrier function, which corresponds to fluorescein permeation on examination (reviewed in Ubels et al, 1994).[53]

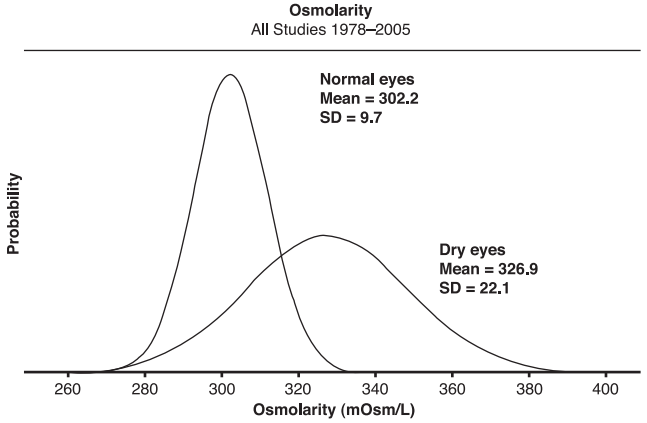

Figure 2. Normal distribution of tear osmolarity in normal eyes and in those from patients with DED. A cutoff value for DED obtained from the intercept of the curves was defined as 315.6 mOsmol/L.[47] (SD = standard deviation).

A. Hyperosmolarity and Inflammation

In vitro and in vivo experiments have linked hyperosmolarity to inflammatory changes in DED. In vivo models are complicated by confounding factors that may interact with osmoregulation and have indirect protective effects; in vitro assays have the advantage of providing a simplified, almost pure system to assess the effects of products, but it is difficult to ensure that the results are relevant in vivo. Systems frequently used in in vitro experiments are murine (mouse), leporine (rabbit), and human cultured cells. Most animal and in vitro studies of hyperosmolarity in DED have been performed at elevated osmolarity (400−600 mOsm/L) in order to demonstrate an inflammatory response via activation of the MAPK pathway and cytokine production.[2,54,55] In the human eye, such high levels are hypothesized to be reached and exceeded over the central cornea.[51,56] However, it is questionable whether exposure to elevated osmolarity in an experimental model reflects exposure in vivo, as each blink may transiently alleviate hyperosmolar stress.

Water loss and changes to cell shape may stimulate specific ion channels, such as transient receptor potential vanilloid (TRPV) channels.[57] This may enhance NFkB signals, inducing the expression of proinflammatory cytokines, chemokines, and adhesion molecules. Chronic production of these molecules stimulates and maintains the inflammatory response. In many experiments with cultured human corneal epithelial cells, exposure to hyperosmotic stress resulted in increased expression and production of proinflammatory cytokines and chemokines, including matrix metalloproteinases (**MMPs**),[58] interleukin (**IL**)-1,[54,59] IL-8,[54] and tumor necrosis factor (**TNF**)-alpha.[54] This

appears to be mediated by two MAPK pathways — c-Jun N-terminal kinase (**JNK**) and extracellular-regulated kinase (**ERK**) pathways. JNK- and ERK-mediated increases in expression of proinflammatory products such as IL-1beta, TNF-alpha and MMP-9 are also observed in murine corneal and conjunctival epithelia exposed to hyperosmotic stress.[60,61] In vitro and in vivo experiments on human conjunctival epithelia from DED patients demonstrated that extracellular hyperosmolarity induces overexpression of human leukocyte antigen (**HLA**)-DR, a glycoprotein presented by antigen-presenting cells (**APC**s) to helper T-cells in the initial stages of the immune response.[62]

The subclinical inflammation observed in DED leads to apoptosis of conjunctival, corneal, and lacrimal gland epithelial cells through a cytochrome c-mediated death pathway that may be controlled by MAPK and other signaling pathways,[55] as well as neuronal disturbance of lacrimal gland secretion.[63]

B. Hyperosmolarity and Morphological Changes

In vitro experiments with rabbit corneal epithelial cells and two rabbit models of DED showed that hyperosmolarity was associated with a decrease in corneal epithelial glycogen and conjunctival goblet cell apoptosis.[64,65] Hyperosmolarity in murine models of DED was also associated with apoptosis of cells in the central and peripheral corneal epithelia, and bulbar and tarsal conjunctival epithelia.[66]

These morphological changes are evident in patients with DED. Hyperosmolarity has been shown to be responsible for reduced goblet cell density in the interpalpebral bulbar conjunctiva,[67] and loss of goblet cells may be responsible for an unstable tear film[51,64] and a reduction of mucin.[64] A combination of in vitro experiments in bovine corneal epithelial cells and in vivo experiments in human patients showed a link between hyperosmolarity and tear film instability.[51]

IV. USING A MEASURE OF OSMOTIC STRESS IN CLINICAL PRACTICE

As hyperosmolarity is a key event in the pathology of DED, there is the possibility that it could be used as a sign of the disease and that measurement of tear film osmolarity could become an important test in its diagnosis and follow-up.[47] Methods of measuring osmolarity include using freezing point depression, vapor pressure, or electrical impedance. However, tear film osmolarity is difficult to measure with conventional laboratory techniques, because it is difficult to harvest an adequate volume of tears without activating reflex secretion. One osmometer that uses a freezing point depression method (Clifton Technical Physics, Hartford, NY, USA) can perform measurements on nanoliter samples of tear, in theory avoiding reflex tearing.[68] However, this equipment requires continual maintenance, the user needs to have significant expertise, and the accuracy can be diminished by evaporation of the test sample. A portable in situ osmometer (TearLab™, OcuSense, TearLab Corp, San Diego, CA, USA) is available that measures electrical impedance of nanoliter volumes of tear fluid directly from the eye. This may avoid some of the problems with earlier techniques, and its availability has led to significant interest in whether osmolarity could be measured in the clinic to provide a global marker of DED.

A. Utility of Osmolarity in Diagnosis of Dry Eye Disease

There is poor correlation between individual tests for DED, and each provides distinct information about the ocular surface.[69] The diagnosis of DED therefore typically relies on information from multiple tests and symptom questionnaires. For example, DEWS recommends basing diagnosis on clinical history, symptom questionnaire, TFBUT, ocular surface staining, Schirmer test, lid and meibomian morphology, and meibomian expression.[1] Studies to assess the value of osmolarity in diagnosis have focused on its ability to identify DED patients diagnosed by a battery of tests and/or its correlation with severity. These studies have investigated whether osmolarity is a global marker of DED that captures the totality of the disease, rather than whether osmolarity provides useful information independent of other tests. The in situ impedance-based osmometer is currently approved to aid in diagnosis in conjunction with other clinical tests, but its optimal use in the diagnostic process has yet to be established.

Because there is significant overlap between the distributions of osmolarity values in healthy subjects and DED patients (Figure 2),[47] the choice of diagnostic cut-off value in DED is necessarily a trade-off between sensitivity and specificity (or false positives vs false negatives). Among 25 healthy subjects and 77 patients with DED, a cut-off of 316 mOsm/L identified DED more accurately than other single tests, including the Schirmer test, rose bengal staining, and lactate levels,[47] and was comparable overall with the use of a combination of tests. While the overall sensitivity for

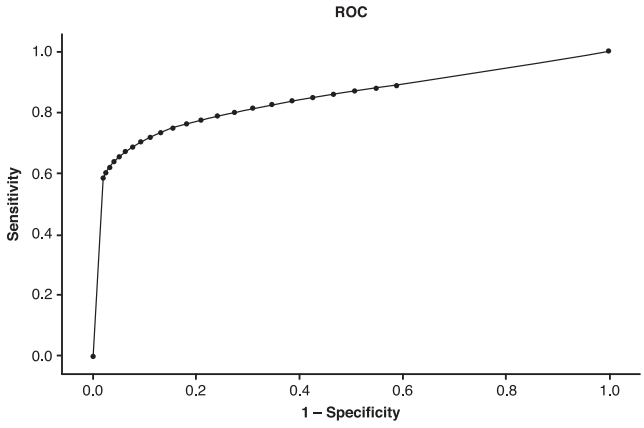

Figure 3. ROC (receiver operator characteristic) curves for sensitivity and specificity of data from a meta-analysis of studies of tear osmolarity between 1978 and 2004 were derived. The curve is shown for osmolarities between 300 and 322 mOsmol/L, a cut-off that maximised the sensitivity and specificity in differentiating DED patients from normal individuals.[47]

detecting DED was only 59%, specificity was 94% and predictive accuracy was 89% (Figure 3). A cut-off of 316 mOsm/L was also supported by Jacobi et al in a study of 133 patients with moderate/severe DED.[70]

Using an in situ impedance-based osmometer, Lemp et al found that a cut-off of >311 mOsm/L correctly identified 92%, 64%, and 89% of normal, mild/moderate, and severe DED subjects, respectively.[46] Sensitivity was 73% and specificity was 92%. In comparison, corneal staining, conjunctival staining, and meibomian gland grading were less sensitive, and TFBUT and Schirmer test were less specific. The manufacturer of this osmometer recommends a cut-off of >308 mOsm/L,[71] which in the same study, identified 81%, 73%, and 90% of normal, mild/moderate, and severe DED subjects, respectively.[46]

In a study of 25 normal subjects and 105 DED patients with a range of severities, Versura et al selected >305 mOsml/L as the cut-off value for dry eye, >309 mOsm/L for moderate dry eye, and >318 mOsm/L for severe dry eye.[48] Osmolarity correlated with disease severity better than other clinical tests. Similarly, in a retrospective review of 314 patients, osmolarity had the strongest correlation with disease severity, as determined using a continuous composite severity index ($r^2 = 0.55$), compared with other DED

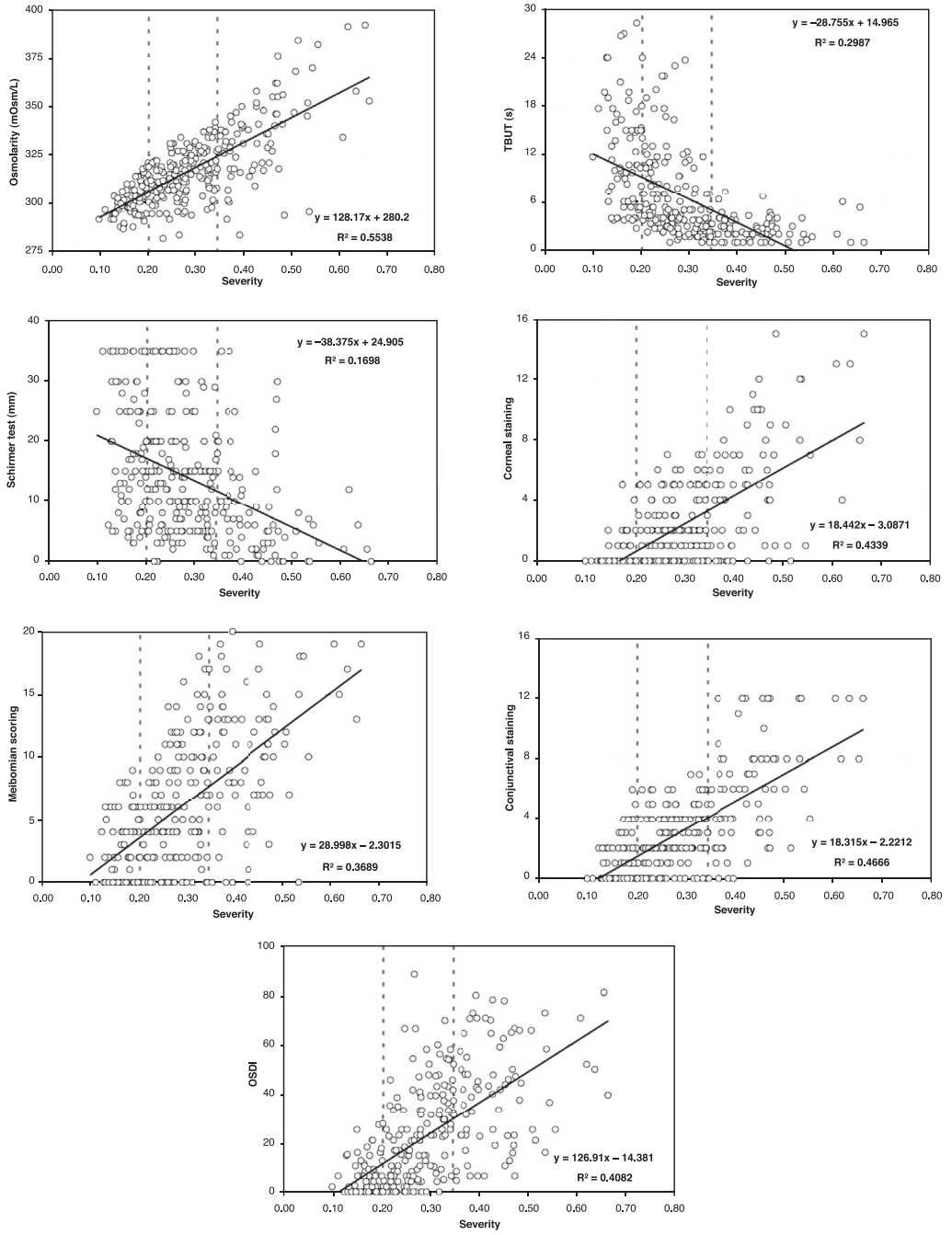

Figure 4. Relationship between individual clinical signs and the composite disease severity index. Raw clinical data for each sign is plotted on the *y*-axis against disease severity on the *x*-axis. Vertical dashed lines indicate the three quartile-derived groups of normal, mild/moderate and severe. Correlation coefficients of each clinical sign (R^2) are given. Only osmolarity showed significant correlation to disease severity within the normal to moderate cohort. Most clinical signs performed well only for patients with severe disease.[50]

tests such as conjunctival staining, Ocular Surface Disease Index© (**OSDI**), TFBUT, and Schirmer test (Figure 4).[50]

However, studies of osmolarity as a diagnostic tool in DED have not been uniformly positive. In a study by Messmer et al, tear film osmolarity as measured with an in situ osmometer was 308.9 ± 14.0 mOsm/L in 129 patients with 3-6 signs or symptoms of DED and 307.1 ± 11.3 mOsm/L in 71 patients with ≤2 signs or symptoms.[72] Osmolarity correlated with age, but not with any of the six individual signs/symptoms of DED examined, and more surprisingly, there was also no significant difference in osmolarity between patients who were positive for all six signs/symptoms compared with those positive for two or fewer. While there is generally poor correlation between individual tests in DED, with each providing independent information, these findings raise the question of whether tear osmolarity provides further diagnostic information independent of other tests, about which there is little evidence. Szalai et al found that while Schirmer test, corneal staining, TFBUT and meibomian gland status were significantly different in DED patients compared with participants without DED, there was no significant difference in mean tear osmolarity: 296.77 ± 16.48 mOsm/L in non-Sjögren syndrome DED, vs 303.36 ± 17.22 mOsm/L in Sjögren syndrome DED, and 303.52 ± 12.92 mOsm/L in those without DED.[73]

B. Variability in Osmolarity Measurements

Osmolarity measurements are subject to variability arising from both natural variations in osmolarity in the meniscus and measurement error. While measurement of natural variations might be clinically useful, it can be difficult to distinguish the two sources of variation. Variability in examiner technique is also a concern.

Turning first to short-term variability (minutes or hours), repeatability describes the ability of a test to measure a quantity accurately in the same laboratory, with the same operator, with the same tools, and with a short time between tests. There are some uncertainties regarding the repeatability of in situ impedance-based osmometer measurements, and to what extent this is influenced by sampling technique. With control solutions, the standard deviation is ± 4-7 mOsm/L,[74] but studies have reported variable estimates of repeatability in patients. Khanal et al reported that consecutive measurements in an individual varied by up to 35 mOsm/L,[75] Eperjesi et al reported a coefficient of repeatability of 33 mOsm/L,[76] and Gokhale reported a coefficient of repeatability of 9.4 mOsm/L.[77] Some authors have reported that multiple measurements may sometimes be necessary to achieve accurate readings.[74,75,78]

However, while these variations in results between groups suggest that differences in measuring technique or calibration may be important, short-term variations in osmolarity might also correspond to the tear film instability that characterizes much DED. In a study in which four measurements were taken every 15 min followed by four every 1 min, greater variability was observed in 10 DED patients than in 10 healthy controls (±11.3 mOsm/L vs ± 9.8 mOsm/L for 15 min intervals, and ±11.3 mOsm/L vs ±6.2 mOsm/L for 1 min intervals.[79] Osmolarity may also vary between eyes in the same individual;[46] this intereye difference may be an indicator of short-term variability in a given patient, and correlate with disease severity ($r^2 = 0.32$, $P < 0.0001$).[46] The intereye difference was 6.9 mOsm/L in normal patients, close to the 4−7 mOsm/L variability recorded with control solutions, but 11.7 mOsm/L in patients with mild/moderate DED and 26.5 mOsm/L in those with severe DED.

Osmolarity is also subject to longer-term variability, over weeks or months. Over a 3-month period, osmolarity showed significantly less variability than corneal staining, conjunctival staining, or meibomian grading, and similar variability to Schirmer test, OSDI and TFBUT.[46] Again, variability was more marked in patients with severe disease than in those with mild disease (10% vs 5.9%).

The manufacturer of an in situ osmometer recommends testing both eyes and taking the higher value as an indicator of disease severity, with the difference in values reflecting variation in osmolarity. While such variations in osmolarity could potentially be diagnostically valuable, the problem remains of distinguishing it from measurement errors or variations in technique. The clinical significance of a given level of variability is also uncertain, particularly when osmolarity appears within the normal range, and the role of assessment of variability in the diagnostic approach thus remains to be determined.

C. Use of Osmolarity Measurement in Assessing Therapeutic Efficacy

If osmolarity could be used to predict symptomatic response, it could be particularly useful in clinical follow-up. Various DED therapies can reduce hyperosmolarity significantly. For example, in a study of 18 DED patients and 19 controls, an artificial tear containing carboxyl methylcellulose (CMC), osmoprotectants and a lipid reduced osmolarity from 326 mOsm/L to 302 mOsm/L over 2 weeks.[80] Several trials have reported associations between different therapies and improved osmolarity values, while other tests have shown no difference.[81,82] In a longitudinal observational case series study followed by an interventional study in a subset of subjects treated with cyclosporine A, osmolarity was subject to less variability than conjunctival staining, corneal staining, or meibomian gland grading over a 3-month period, with greater improvements following treatment with cyclosporine than with sodium hyaluronate artificial tears.[83]

It is hoped that new markers of osmotic stress at the ocular surface will be identified and implemented in diagnosis and monitoring. In the future, compound measures incorporating, for example, tear evaporation, tear production, and osmolarity may have better diagnostic and monitoring capability than single measures. Sullivan et al used a continuous composite severity index based on tear osmolarity, Schirmer tests, TFBUT, corneal staining, meibomian

score, conjunctival staining, and OSDI.[50] This approach may reduce "noise" and create a better consensus of severity.

In conclusion, osmolarity measurement is likely to have a role in diagnosis and follow-up of DED, but is probably best used alongside standard tests and symptom questionnaires to provide a complete clinical picture. No single measurement has yet been robustly established as sufficient for diagnosis and follow-up in DED.

V. PROTECTING AGAINST HYPEROSMOLARITY

The goal of treatment of DED is to improve the patient's quality of life, symptoms and visual function by reestablishing homeostasis and integrity of the ocular system. Essentially, one should aim to prevent the patient from entering the vicious circle (Figure 1) or promote exit from it. Preventing entry into the circle refers to addressing causative factors such as systemic disease, contact lens use, environmental factors, surgery, infection, etc. Reducing exposure to preservatives (eg, benzalkonium chloride [**BAK**]) in ophthalmological preparations may be useful in patients with moderate-to-severe DED, but preserved eyedrops are usually well tolerated in patients without DED or with mild DED if usage does not exceed 4−6 times per day.[1] Promoting exit from the vicious circle involves addressing the central mechanisms such as tear film instability, inflammation of the LFU, and tear hyperosmolarity.

A. Hypotonic Solutions

Given the role of tear hyperosmolarity in the pathogenesis of DED, it has been suggested that hypotonic tear substitutes could correct the hyperosmolarity and encourage the establishment of regular osmotic balance between the epithelial cells and their environment.[49] While it has been shown that hypotonic tear substitutes can be helpful in reducing ocular surface damage in DED, it appears that the level of hypotonicity is important.[84,85] In one study (n = 40), a hypotonic formulation of sodium hyaluronate (150 mOsm/L) was more effective than an isotonic formulation of sodium hyaluronate in terms of changes in symptoms and markers of epithelial damage.[84] In a separate trial (n = 158), a less hypotonic formulation (215 mOsm/L) was associated with outcomes similar to those of its isotonic equivalent (305 mOsm/L).[85]

The disadvantages of excessive hypotonic lubrication with very hypotonic surface lubricants are evident from observation of patients who have undergone transplantation of the autologous submandibular gland (**SMG**).[86] Salivary tears resulting from this provide excessive, hypotonic lubrication, equivalent to an eyebath of 160−170 mOsm/L, and can be associated with microcystic corneal edema.

Hypotonic tear substitutes generally have limited persistence in the eye, and this means that after instillation, osmolarity returns to a hyperosmolar range within approximately 1−2 minutes.[87] Now, with greater understanding of hyperosmolarity and osmoprotection, other means of protection are being developed. DED treatments may be improved by inclusion of osmoprotectant compatible solutes, as discussed below.

B. Osmoprotectant Compatible Solutes

One way to counteract the effects of hyperosmolarity may be to use osmoprotectant compatible solutes to protect ocular epithelial cells. If this is successful, one would expect this to reduce inflammation and additional cell damage. There are a number of osmoprotectants that have been included in DED therapies. Osmoprotection appears to be one way by which the vicious circle of DED pathology can be broken, allowing improvement of signs and symptoms.

Erythritol, a natural polysaccharide small enough to penetrate the corneal epithelium is transported by aquaglyceroporin channels in the corneal epithelium.[88] It stabilizes proteins,[89] reduces MAPK-mediated signaling in corneal epithelial cells,[26] and has a positive effect on cellular function (as measured by transepithelial electrical resistance [**TEER**]) on leporine corneal epithelial cells exposed to hyperosmotic stress.[88]

Glycerol is a polyol and was the first osmolyte to be termed a "compatible solute." It has been extensively studied in algae, salt-tolerant plants and insects. It has also demonstrated osmoprotective effects in corneal epithelial cells in vitro.[88]

Trehalose is a small disaccharide that, in yeast and insects, stabilizes proteins and protects against extremes in temperature and desiccation.[25] Human primary fibroblasts transfected with genes for trehalose biosynthetic enzymes were able to survive desiccation for up to five days.[90] Corneal epithelial cells incubated with trehalose were also able to survive desiccation.[91] Trehalose protected against apoptosis of corneal and conjunctival cells (including goblet cells) in a murine model of DED[92] and against UV-induced oxidative damage in leporine corneal cells.[93] In a murine model of DED, trehalose reduced levels of inflammatory cytokines in the conjunctiva.[94]

Taurine is an amino acid-compatible solute that is widespread throughout the natural world and is compatible with intracellular structures.[95] It was also shown to protect leporine corneal epithelial cells exposed to hyperosmotic stress.[88] Furthermore, a contact lens solution with taurine was associated with a reduction in proteins associated with inflammation.[96]

L-carnitine, an amino acid found in food and synthesized by the liver, is actively transported via OCTN2 into cytosol.[97] It has been shown to protect retinal pigment epithelial cells from oxidative damage[98] and reduce activation of MAPK-mediated signaling in vitro[88] and in corneal epithelial cells cultured in a hyperosmotic medium.[26] It was shown to protect leporine corneal epithelial cells exposed to hyperosmotic stress[88] and inhibit apoptosis of cultured human corneal epithelial cells exposed to hyperosmotic stress.[99] L-carnitine has also been shown to delay cataract development in animal models.[100]

The osmoprotective effect depends on how much osmoprotectant the cell takes up and how long it is retained. Glycerol and erythritol are small polyols and can enter the

cell very quickly via the water channel. However, compared with erythritol, glycerol also leaves very quickly.[101] In contrast, L-carnitine is taken up via the amino acid transporters and is retained for longer periods. While glycerol may be important as a short-term osmoprotectant, clinical results may be improved if it is combined with a protectant that acts over a longer term. Consequently, combining several osmoprotectants that act with different kinetics into one formulation may work better to increase the overall protective effect.

C. Osmoprotective Solutions

There is now an expanding pool of clinical data on the role of osmoprotectants in patients with DED. A preparation of CMC and osmoprotectants (in most preparations: glycerol, erythritol and L-carnitine) has been studied in patients with DED. In a 1-month, randomized controlled trial (**RCT**) of 47 patients treated with either CMC plus osmoprotectants (CMC-OP, Optive®) or a hyaluronate-based product that did not contain osmoprotectants, conjunctival staining was reduced with CMC-OP and a greater percentage of patients receiving CMC-OP were reported to have no staining at 1 month.[102] In a 6-week comparison of CMC-OP vs an eye drop containing hydroxypropyl guar in 105 patients, both products were found to significantly reduce signs and symptoms of DED from baseline, and CMC-OP led to greater reductions in corneal and conjunctival staining.[103] In a large, uncontrolled observational study (n = 5277), CMC-OP use was associated with improvements in symptoms and signs of DED compared with baseline.[104]

In an RCT of 82 patients with DED, CMC-OP was noninferior to sodium hyaluronate in terms of osmolarity, Schirmer test scores, scores on the 12-item OSDI questionnaire, ocular staining, and patient preference.[105] Moreover, osmoprotection may provide some benefits over sodium hyaluronate: in a comparison of CMC-OP with sodium hyaluronate in patients with glaucoma using antiglaucomatous agents, CMC-OP significantly improved OSDI scores and Oxford Grading System scores compared with baseline, while sodium hyaluronate did not.[106] A small 3-month study of 19 patients with DED suggested that CMC-OP could be used adjunctively with cyclosporine 0.05%.[107] In this study, improvements from baseline were noted in conjunctival staining, TFBUT, OSDI scores, and patient-reported ocular discomfort.

Another approach has been to use eye drops containing trehalose (Thealoz®). In a 4-week trial, 34 patients were treated with eye drops containing different concentrations of trehalose six times daily. Measurements of TFBUT and ocular staining showed significant improvements from baseline with 100 mM trehalose solution.[108] In a 4-week randomized, double-masked, crossover study of 36 patients with moderate to severe DED, eye drops containing trehalose significantly improved ocular staining scores compared with DED therapies containing hyaluronan or hydroxyethylcellulose.[109]

VI. CONCLUSION

Tear film instability and tear hyperosmolarity play major roles in the vicious circle of DED pathology. Hyperosmolarity directly causes cell damage and nerve stimulation and triggers inflammatory cascades. These cascades then contribute to further cell damage, including loss of mucin-producing goblet cells. This exacerbates tear film instability and drives the circle further. Methods for measuring osmolarity in a clinical setting need further research in order to find a standard and reliable measure that is useful for diagnosis and follow-up.

Tackling causative factors such as systemic disease, contact lens use, environmental factors, surgery, infection, etc. may prevent the patient from entering the vicious circle of DED. In order to remove patients from the cycle of interactions that can amplify the severity of DED, central mechanisms such as tear hyperosmolarity must be addressed. Traditional approaches to correcting hyperosmolarity in DED include use of hypotonic tear substitutes, which have relatively short persistence in the eye and correct osmolarity for 1-2 minutes. DED treatments may benefit from inclusion of osmoprotectants, naturally occurring compatible solutes that are internalized by cells, restoring cell volume and stabilizing proteins. New formulations of artificial tears have been developed that include one or more osmoprotectants. Emerging clinical trial data suggest a beneficial role for osmoprotectant solutions in patients with DED, and further research into osmoregulation may enhance our understanding of DED pathology and provide new avenues for its treatment.

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