Protocol: Omega-3 Fatty Acids for Prevention of Dry Eye Disease: VITAL-DryEye

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I. BACKGROUND AND SIGNIFICANCE

The tear film, ocular surface, and dry eye disease (DED).

The tear film is composed of 3 layers: an inner mucin layer, an intermediate aqueous layer, and an outer lipid layer. Mucins are produced by conjunctival goblet cells and by stratified squamous cells of the cornea and conjunctiva. The aqueous layer is produced by the main and accessory lacrimal glands, and contains water, electrolytes, antibacterial proteins and growth factors. The lipid layer is produced by the Meibomian glands, with a small contribution from the glands of Zeis.

The tear film functions to lubricate the ocular surface and eyelids; provide a regular optical surface for the eye; remove foreign material from the ocular surface; and supply nutrients to the ocular surface. The tear film also provides antibacterial substances, and promotes tissue maintenance and wound healing.

For many years, DED was considered to be due solely to a reduction of the aqueous layer of the tear film. More recently, DED is recognized as a complex, multifactorial disease of the ocular surface. The ocular surface is part of a functional system termed the Ocular Surface System.[3] This is defined as the wet-surfaced and glandular epithelia of the cornea, conjunctiva, and lacrimal gland, accessory lacrimal glands, nasolacrimal duct and Meibomian glands, and their apical and basal matrices, linked as a functional system by both continuity of epithelia, innervation, and the endocrine and immune systems. At least in theory, an alteration in any component can result in a change in the tear film composition and lead to desiccation and epithelial damage, triggering a release of inflammatory mediators that lead to the chronic damage seen in DED.

The definition of DED, based on a comprehensive review and interpretation by an international group of experts, reads: “Dry eye is 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.”[4]

Alterations of tear composition in DED involve a reduction in the amount of tears, increased tear osmolarity, reductions in specific protein content (e.g. lysozyme and lactoferrin), changes in matrix metallol proteinases and mucin concentration, and alterations in Meibomian lipid composition.[4, 5] DED can be divided in 2 non-mutually exclusive subclasses: aqueous tear deficient dry eye and evaporative dry eye.[4] Aqueous deficient DED is due to inadequate lacrimal tear secretion, causing tear hyperosmolarity and a cascade of inflammatory events releasing inflammatory cytokines and matrix metalloproteinases. Evaporative DED is due to excessive water loss in the presence of a normal lacrimal secretory function. Meibomian gland dysfunction is by far the most common underlying cause of evaporative dry eye.[4]

Public Health Importance of Dry Eye Disease (DED)

DED is characterized by an unstable tear film, subsequent ocular surface damage and increased risk of ocular surface infections. Symptoms of DED are bothersome and include chronic ocular irritation, dryness, and fatigue. People with DED also often complain of blurred and/or fluctuating vision, which can occur even in the presence of apparently normal visual acuity.[4] DED interferes with activities such as reading, computer work, driving a car, navigating stairs, recognizing friends, reading road signs, watching TV, and cooking.[6-8]

Costs associated with DED are substantial. A recent report estimates that $3.84 billion is spent in total annual direct costs to care for patients with DED; costs which may grow with increased awareness, increased medical care coverage, and better patient education. Indirect costs of DED

e.g. from lost productivity at work were $55.4 billion annually, including 14.2 days of lost work and 128 equivalent lost work days because of affected performance per year for a patient who is severely affected with DED.

Risk factors for DED include older age, female sex, reduced androgen levels, exogenous estrogen use in women, benign prostatic hypertrophy in men, and the use of certain classes of medications such as some antihypertensive medications (e.g. beta-blockers), antidepressant medications, and antihistamines.[6, 9-12]

Omega-3 Fatty Acids and DED -

In the past decade, we've discovered that inflammation in the lacrimal gland, Meibomian gland, and ocular surface plays a significant role in DED. Patients with DED have an increased concentration of inflammatory cytokines in the tear film, such as interleukin-1, interleukin-6, and tumor necrosis factor alpha. At the same time, research shows that dietary intake of omega-3 FAs and the ratio of omega-6 to omega-3 FA affects the overall level of inflammatory activity in the body. Early evidence suggests a possible protective role of omega-3 FA supplementation in treatment of DED, with a few small clinical trials suggesting benefit.

Of even greater interest for prevention, there is evidence that omega-3 FA may protect against DED development. Data from the WHS indicate that women with higher intake of omega-3 FA have a lower risk of DED, including a 68% reduction for ≥5-6 4 oz servings/wk compared to ≤1 serving/wk of tuna, one of the largest single contributors of omega-3 FA in US diets. In addition, a high ratio of omega-6 to omega-3 FA (>15:1) was associated with a >2-fold higher risk of DED.[13] There is also laboratory evidence showing protection from DED in a rat model when omega-3 FA supplementation was started 2 months earlier.[14]

Evidence from observational studies - In addition to our prior work linking omega-3 FA to DED in the WHS,[13] other observational studies have suggested a link between omega-3 FA and DED. In a cross-sectional study of 41 patients with primary Sjogren's syndrome,[15] omega-3 and omega-6 FA levels within erythrocyte phospholipids, plasma phospholipids, plasma triglycerides and plasma cholesterol esters were investigated for associations with immunopathological and clinical disease parameters. Docosahexanoic acid (DHA; omega-3) was inversely correlated with the clinical DED status. In a separate study, 68 women with Sjogren's syndrome were found to have a lower dietary intake of omega-3 FA compared to age-matched controls.[16]

Evidence from small randomized controlled trials (RCTs) -

Table 1. Summary of Small RCTs of Omega-3 FA for Treatment of DED

RCT	Macsai MS; 2008[17]	Wojtowicz JC, et al; 2011[18]	Pinheiro MN, et al; 2007[19]
Dose of Omega-3 FA Tested	1) 6 g flaxseed oil per day for a total daily dose ~3.3 mg ALA 2) Placebo	1) 450 mg EPA + 300 mg DHA + 1000 mg flaxseed oil 2) Placebo	1) 1 g flaxseed oil per day 2) 2 g flaxseed oil per day 3) placebo
Patient Population	US Patients with Meibomian gland dysfunction and blepharitis	US Patients with DED	Brazilian female patients with Sjogren's syndrome and associated rheumatoid arthritis or systemic lupus erythematosus
N	38	36	38
Length of Follow-up	1 year	90 days	180 days
Major Study Findings	ALA supplemented subjects had significantly 52% higher plasma levels and 40% higher RBC levels of omega-3 FA, whereas levels were unchanged in the placebo group. The ratio of omega-6 to omega-3 FA also decreased in both plasma (from 14 to 8.8, or 36%) and RBCs (from 6.2 to 4.3, or 31%) among subjects in the active treatment arm.	70% of subjects on omega-3 FA were asymptomatic versus 7% on placebo. Increases in tear production as measured by the Schirmer test (from 8.3 mm to 12.3 mm in omega-3 FA group versus 8.0 mm to 10.5 mm in placebo group) Increases in tear flow tests, and tear volume (increase from 0.86 uL to 0.98 uL in omega-3 FA group versus decrease from 1.44 uL to 0.62 uL in placebo group)	Significant improvements in DED symptoms, ocular surface inflammation by conjunctival impression cytology, tear production by Schirmer scores, and tear film break-up times in both active treatment arms versus placebo

	Active treatment was associated with a significant improvement in dry eye symptoms, and non-significant improvements in tear break-up time and the quality of Meibomian gland secretions.	No changes in the ratio of the major detected compounds in Meibomian lipid samples. LA and ALA were not visible as individual peaks.[20]	

Table 2. Summary of Small RCTs of Combined Omega-3 & Omega-6 FA Supplements for Treatment of DED			
RCT	Creuzot et al; 2006[21]	Larmo et al; 2010[22]	Brignole-Baudouin et al; 2011[23]
Daily dose of Omega-3 & Omega-6 FA Tested	1) 392 mg EPA + 28 mg EPA + 82 mg GLA + 126 mg LA 2) Placebo	1) 149 mg ALA, 245 mg LA and antioxidants (carotenoids, alpha tocopherol, and gamma tocopherol) 2) Placebo	1)1283 mg EPA + 855 mg DHA, borage oil (45 mg omega-6 FA including GLA), and antioxidants (vitamin C, zinc, vitamin E, and vitamins B6, and B12) 2) Placebo
Patient Population	Patients with mild to moderate DED	Patients with dry eye based on self-reported symptoms	Patients with DED, 43% had Sjogren's syndrome
N	71	100	138
Length of Follow-up	6 months	3 months	3 months
Findings	Improvements in Schirmer scores, tear break-up time, and ocular surface staining, but differences were not statistically significant compared with placebo. Improvement of patient symptoms were of borderline statistical significance	Significantly lesser increase in tear film osmolarity, Significant reduction in ocular redness. In the subgroup who wore contact lenses, there was also a significant reduction in the number of days of dry eye symptoms. Analysis of tear film samples failed to identify significant differences, but there was a non-significant reduction in the level of omega-6 FA in the active group (omega-3 FA levels were not measured)	The primary end point of the study was expression of the inflammatory marker human leucocyte antigen-DR (HLA-DR) in the conjunctival epithelium Significant decrease in the percentage of HLA-DR-positive cells. Expression of HLA-DR was also significantly reduced. No significant differences were observed between treatment groups for DED symptoms or clinical signs, although the active treatment group had non-significant reductions in symptoms and lissamine green staining

Identification of safe, effective strategies to prevent DED will change the individual and societal perspective on the disease. The ultimate goal of ***VITAL-DryEye*** is to accelerate the identification and availability of such a strategy. We propose to take advantage of the unique opportunity offered by VITAL, and if our hypotheses are correct, we expect a reduction in the incidence and progression of DED among participants assigned to omega-3 FA. Given the randomized design of the study, such a finding would be unlikely to be the result of chance or due to confounding or bias. Given the sound biological rationale for these effects, a causal interpretation would be plausible. Thus, positive findings from ***VITAL-DryEye*** would support a recommendation for omega-3 FA supplementation to prevent DED in older men and women. Any such treatment recommendation would of course need to be approached in light of the specific clinical situation of each individual, but in this regard the main findings of VITAL will provide insight into the effects of long-term omega-3 FA supplementation on risk of major diseases, and myriad other health outcomes, to allow for decisions based on a weighing of risks and benefits. If omega-3 FA were found effective for DED prevention and/or improvement of its natural history and quality of life for patients, this would be a major public health benefit.

II. SPECIFIC AIMS

Primary Aims:

1. **To test in a RCT if omega-3 FA supplementation reduces the incidence of DED.**
2. **To test in a RCT if omega-3 FA supplementation improves the natural history of DED.**

Secondary Aims:

3. To estimate the incidence of DED in a large population of US men and women, and identify risk factors.
4. To study the natural history of DED in terms of symptoms, clinical findings, and quality of life.
5. To examine if the effect of omega-3 FA supplementation varies by (a) age, (b) sex, (c) race, (d) dietary intake of omega-3 FAs, (e) presence of major medical comorbidities (e.g. diabetes, hypertension, rheumatoid arthritis), (f) use of certain medications (e.g. antihistamines, antidepressants, beta-blockers).

Tertiary Aims:

6. To examine whether the presence of DED at baseline increases risk of incident and recurrent depression among all participants in the VITAL trial, and whether omega-3 FAs modify any association.
7. To examine if the effect of omega-3 FA supplementation is modified by baseline levels of omega-3 FAs.
8. To test in a RCT whether vitamin D_3 supplementation (2000 IU/d) reduces the incidence and/or progression of DED.
9. To examine whether there are synergistic or antagonistic effects of omega-3 FAs and vitamin D_3 supplementation on the primary and secondary DED endpoints.

III. SUBJECT SELECTION/ENROLLMENT

VITAL-DryEye is an ancillary study of the NIH-funded ***VITAL*** trial. All persons randomized in ***VITAL*** are included in this ancillary study.

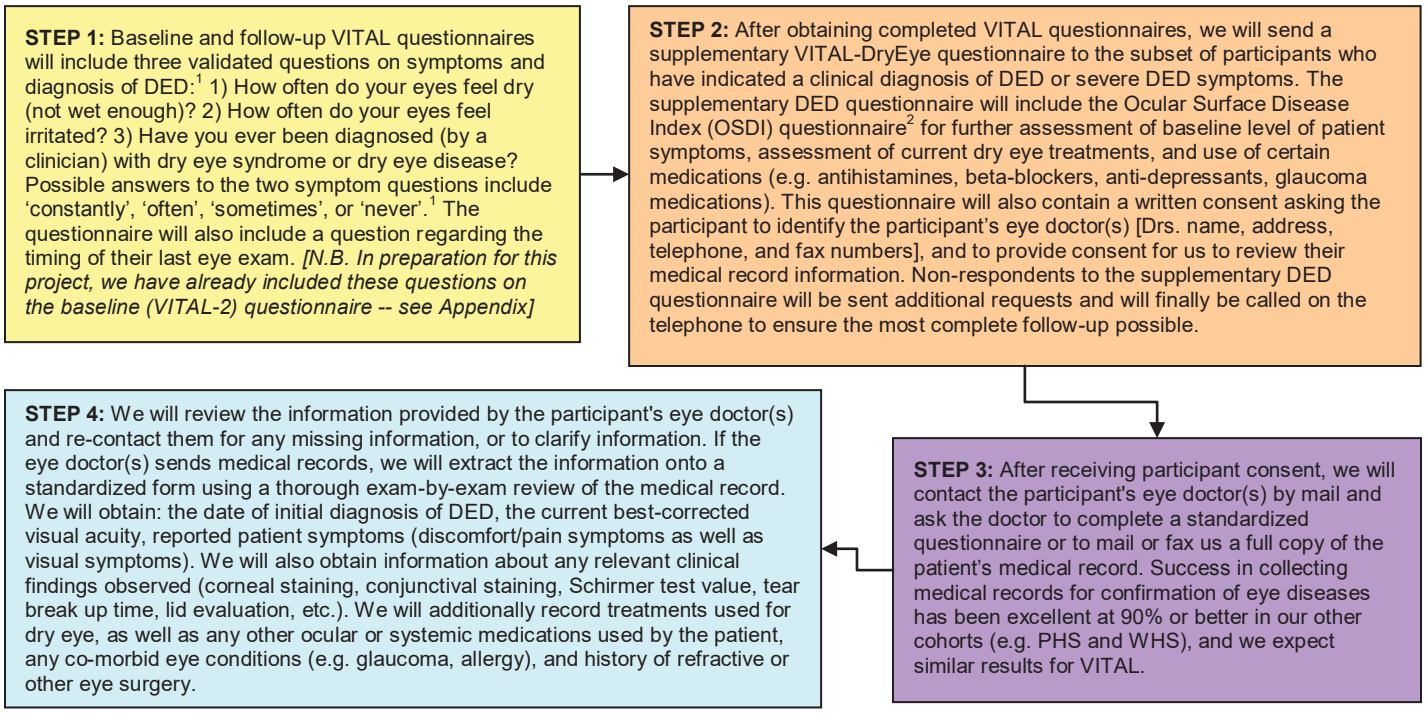
Individuals are eligible for ***VITAL*** trial participation if they meet the following criteria: (1) are age ≥60 (men) or ≥65 (women); (2) have at least a high school education (to complete mail-based questionnaires); (3) have no history of cancer (except non-melanoma skin cancer), MI, stroke, TIA, angina pectoris, CABG, or PCI; (4) have none of the following safety exclusions: history of kidney stones, renal failure or dialysis, hypercalcemia, hypo-or hyperparathyroidism, severe liver disease (cirrhosis), or sarcoidosis or other granulomatous diseases such as active chronic tuberculosis or Wegener's granulomatosis; (5) have no allergy to fish (for EPA+DHA); (6) have no other serious illness that would preclude participation; (7) are consuming no more than 800 IU of vitamin D from all supplemental sources combined (individual vitamin D supplements, calcium+vitamin D supplements, medications with vitamin D [e.g., Fosamax Plus D], and multivitamins), or, if taking, willing to decrease or forego such use during the trial (to ensure the overall dose is well below the no-observed-adverse effect level (NOAEL) of 4000 IU specified by the European Commission Scientific Committee on Food (ECSCF); (8) are consuming no more than 1200 mg/d of calcium (the RDA) from all supplemental sources combined, or, if taking, willing to decrease or forego such use during the trial; (9) are not taking fish oil supplements, or, if taking, willing to forego their use during the trial; and (10) are willing to participate, as evidenced by signing the informed consent form.

IV. STUDY PROCEDURES

Methods to Study DED in VITAL - We have developed and validated a methodology for mail-based ascertainment and confirmation of study outcomes (Figure 1). We plan to confirm baseline and incident cases of DED by a standardized approach based on a questionnaire to ascertain diagnoses of DED and DED symptoms, followed by a supplementary questionnaire to obtain

additional information from participants who report symptoms or a diagnosis of DED, and then a review of the medical records of participants who report a diagnosis of DED. This method of an initial questionnaire-based ascertainment of disease followed by confirmation through medical record review is also being used in VITAL for confirmation of the primary cancer and cardiovascular disease endpoints; and we have used this approach previously in our research on other eye diseases such as macular degeneration, as well as for our prior work on the epidemiology of DED.[9-11, 13, 24, 25]

Figure 1. Schematic of Procedures for Ascertainment and Confirmation of Prevalent and Incident Dry Eye Disease.



VITAL-DryEye Procedures for Studying the Natural History of DED. Participants with diagnosed DED will receive an annual supplementary DED questionnaire including the OSDI,[2] other symptom and quality of life questions, assessment of current dry eye treatments, and use of certain medications (e.g. antihistamines, beta-blockers, anti-depressants, glaucoma meds), and a written consent asking the participant to identify the participant's eye doctor(s) [Drs. name, address, telephone, and fax numbers] and to provide consent for us to review their medical record information. Medical record information will be reviewed once every 2 years to obtain information on corneal staining and any other clinical test values (e.g. Schirmer scores, TBUT values).

V. BIOSTATISTICAL ANALYSIS

VITAL-DryEye will have ≥80% power to detect reductions of ≥15% in incident diagnosed DED in the omega-3 FA group versus placebo. Power is higher at ≥91% detection of a ≥15% reduction in risk of the composite endpoint of incident diagnosed DED or development of severe symptoms of the disease. Power was estimated at ≥80% for a sample size of N=1050 or greater to detect even very modest improvements in symptom levels over time with omega-3 FA. Power to detect larger improvements was estimated at 99% or greater. Given the estimated prevalence of DED based on our experience in prior studies, we should therefore have high power to detect long-term improvements in symptoms in the omega-3 FA group versus placebo.

With the large sample size, randomization should assure an equal distribution of known and unknown confounders between treatment groups, but we will control in analysis for any chance imbalances that occur for DED risk factors or conditions that could increase the likelihood of DED diagnosis. The primary aims will be addressed in analyses involving all VITAL participants without a confirmed diagnosis of DED at baseline (N~18,837). The primary endpoint for Aim 1 will be incident clinically diagnosed DED. Secondary endpoints will be assessed from the population free of that endpoint at baseline. For Aim 2, we will examine the effect of omega-3 FA on DED symptom progression, other quality of life measures, and use of dry eye therapies. For Aim 2, the primary focus will be on all cases with a confirmed diagnosis of DED at baseline, but we will also examine the impact of randomized treatment natural history outcomes among confirmed incident cases and test whether there is evidence for differential effects among these two subgroups. We will estimate the main treatment effects for all specific aims using the intention-to-treat principle based on randomized treatment assignment. Initial analyses of primary and secondary DED endpoints will include contingency tables in which the rate of each endpoint (number of events per person-year of observation) among participants allocated to active treatment will be compared with the rate among those allocated to its placebo; controlling by stratification for the other treatment assignment. In addition, Kaplan-Meier survival estimates, the logrank test, and proportional hazards regression models[26] will be used to determine whether there is a difference in time to an DED event. Because an extended exposure to the study agents may be required to observe an effect on disease prevention, we will also conduct analyses that exclude DED events that occur during the early years after randomization. To address the issue of DED ascertainment, we will also test associations in models where the at-risk population during each 1 y follow-up period is restricted to those who report having had an eye exam during that year. We will examine effect modification by the other randomized intervention and by other risk factors by including an interaction term in proportional hazards models.

VI. RISKS AND DISCOMFORTS

The only possible risk in this study involves the social/psychological risk that could result from inadvertent disclosure of confidential information from the questionnaires or blood tests. However, we have many safeguards in place to avoid this possibility, and we have never had an inadvertent breach of confidentiality in any of our trials. Confidentiality of participants is secured via locked file cabinets, use of participant ID numbers, and restrictions on access to computerized records and use or release of both individual and aggregate ***VITAL*** participant data, including randomized treatment assignments, in publications and presentations. Access to the ***VITAL*** databases and certain network files is restricted to essential staff only, and is protected by passwords and restricted access accounts. All blood specimens sent to the laboratories for analysis will be labeled with an ID number only, so that no individual identifying information will be made available in any form to these sites. In addition, employees involved with the proposed study will be asked to sign a form agreeing not to disclose any information to which they might have access, regardless of their personal perception about its confidentiality.

There is no additional risk to participation in ***VITAL-DryEye*** beyond that incurred by participants as part of their regular activities in ***VITAL***. All participants will have agreed by signing their ***VITAL*** consent form that their blood and other relevant data will be stored for future analyses and that they would not learn of the results of those analyses.

VII. POTENTIAL BENEFITS

For the majority of participants, there will be few direct benefits from participating in this primary prevention study other than the awareness of being involved in a large endeavor to answer relevant and timely questions regarding the possible benefits of vitamin D and marine omega-3 fatty acids. The potential benefits to society relate to the increasing use of both vitamin D and fish oil for many health purposes, with data not yet clearly indicating either clear benefit or harm. This study will provide a wealth of data on the effects of these supplements on risk and natural history of DED, and will help guide individual decisions, clinical recommendations, and public health guidelines.

VIII. MONITORING AND QUALITY ASSURANCE

Dr. Debra Schaumberg will be responsible for monitoring data collection and assuring the validity and integrity of the data and adherence to the IRB-approved protocol.

A ***VITAL*** Data and Safety Monitoring Board (DSMB) has been created as an independent body charged with ensuring that the safety of participants is protected and that the scientific goals of the study are being met. The ***VITAL*** DSMB is charged with monitoring differences by treatment agent of ancillary study outcomes, including AMD, and will be empowered to terminate the trial based on evidence of substantial harm or benefit. To support those purposes, the DSMB will review any proposed amendments to the study protocol, examine the progress of the trial and the unblinded data on study endpoints, perform expedited review of all serious adverse events (i.e., events meeting the FDA definition of Serious Adverse Events, such as any fatal event including suicide, immediately life-threatening event, or permanently or substantially disabling event), perform ongoing monitoring of drop-outs and non-serious adverse events, determine whether study procedures should be changed or the study should be halted for reasons related to the safety of participants, and perform periodic review of the completeness and validity of data to be used for analysis of safety and efficacy. The DSMB will also ensure participant privacy and research data confidentiality. The DSMB will employ monitoring rules[27, 28] that will serve solely as guidelines in decisions regarding continuation or stopping of treatment arms. While these rules are intended for the primary endpoints of ***VITAL*** (primary prevention of cancer and CVD), the DSMB will also consider secondary and ancillary ***VITAL*** endpoints (including DED) in assessing the overall balance of benefits and risks of the two agents. All decisions must be made after examining the totality of evidence, including other trial data, on these agents. All decisions will be made after examining the totality of evidence, including other trial data, on these agents.

IX. REFERENCES

1. Gulati A, Sullivan R, Buring JE, et al. Validation and repeatability of a short questionnaire for dry eye syndrome. Am J Ophthalmol 2006;142(1):125-31.
2. Schiffman RM, Christianson MD, Jacobsen G, et al. Reliability and validity of the Ocular Surface Disease Index. Arch Ophthalmol 2000;118(5):615-21.
3. Gipson IK. The ocular surface: the challenge to enable and protect vision: the Friedenwald lecture. Invest Ophthalmol Vis Sci 2007;48(10):4390; 1-8.
4. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 2007;5(2):75-92.

5. Research in dry eye: report of the Research Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 2007;5(2):179-93.
6. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 2007;5(2):93-107.
7. Miljanovic B, Dana R, Sullivan DA, Schaumberg DA. Impact of dry eye syndrome on vision-related quality of life. Am J Ophthalmol 2007;In Press.
8. Tong L, Waduthantri S, Wong TY, et al. Impact of symptomatic dry eye on vision-related daily activities: the Singapore Malay Eye Study. Eye (Lond);24(9):1486-91.
9. Schaumberg DA, Buring JE, Sullivan DA, Dana MR. Hormone replacement therapy and dry eye syndrome. Jama 2001;286(17):2114-9.
10. Schaumberg DA, Dana R, Buring JE, Sullivan DA. Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies. Arch Ophthalmol 2009;127(6):763-8.
11. Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. Am J Ophthalmol 2003;136(2):318-26.
12. Schaumberg DA, Sullivan DA, Dana MR. Epidemiology of dry eye syndrome. Adv Exp Med Biol 2002;506(Pt B):989-98.
13. Miljanovic B, Trivedi KA, Dana MR, et al. Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. Am J Clin Nutr 2005;82(4):887-93.
14. Viau S, Maire MA, Pasquis B, et al. Efficacy of a 2-month dietary supplementation with polyunsaturated fatty acids in dry eye induced by scopolamine in a rat model. Graefes Arch Clin Exp Ophthalmol 2009;247(8):1039-50.
15. Oxholm P, Asmussen K, Wiik A, Horrobin DF. Essential fatty acid status in cell membranes and plasma of patients with primary Sjogren's syndrome. Correlations to clinical and immunologic variables using a new model for classification and assessment of disease manifestations. Prostaglandins Leukot Essent Fatty Acids 1998;59(4):239-45.
16. Cermak JM, Papas AS, Sullivan RM, et al. Nutrient intake in women with primary and secondary Sjogren's syndrome. Eur J Clin Nutr 2003;57(2):328-34.
17. Macsai MS. The role of omega-3 dietary supplementation in blepharitis and meibomian gland dysfunction (an AOS thesis). Trans Am Ophthalmol Soc 2008;106:336-56.
18. Wojtowicz JC, Butovich I, Uchiyama E, et al. Pilot, prospective, randomized, double-masked, placebo-controlled clinical trial of an omega-3 supplement for dry eye. Cornea;30(3):308-14.
19. Pinheiro MN, Jr., dos Santos PM, dos Santos RC, et al. [Oral flaxseed oil (Linum usitatissimum) in the treatment for dry-eye Sjogren's syndrome patients]. Arq Bras Oftalmol 2007;70(4):649-55.
20. Jarvinen RL, Larmo PS, Setala NL, et al. Effects of oral sea buckthorn oil on tear film Fatty acids in individuals with dry eye. Cornea 2011;30(9):1013-9.
21. Creuzot C, Passemard M, Viau S, et al. [Improvement of dry eye symptoms with polyunsaturated fatty acids]. J Fr Ophtalmol 2006;29(8):868-73.
22. Larmo PS, Jarvinen RL, Setala NL, et al. Oral sea buckthorn oil attenuates tear film osmolarity and symptoms in individuals with dry eye. J Nutr 2010;140(8):1462-8.
23. Brignole-Baudouin F, Baudouin C, Aragona P, et al. A multicentre, double-masked, randomized, controlled trial assessing the effect of oral supplementation of omega-3 and omega-6 fatty acids on a conjunctival inflammatory marker in dry eye patients. Acta Ophthalmol 2011.
24. Miljanovic B, Dana, R., Sullivan, D.A., Schaumberg, D.A. Impact Of Dry Eye Syndrome On Vision-related Quality Of Life Among Women. Association for Research in Vision and Ophthalmology (ARVO). Fort Laudrerdale, Florida: ARVO 2004, 2004.
25. Schaumberg DA, Li J. Gender differences in dry eye disease impact, management, patient satisfaction, and comorbid conditions. 6th International Conference on the Tear Film & Ocular Surface. Florence, Italy, 2010.

26. Cox DR. Regression models and life-tables. J Royal Statist Soc B 1972;34:187-220.
27. Haybittle JL. Repeated assessment of results in clinical trials of cancer treatment. Br J Radiol 1971;44(526):793-7.
28. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. I. Introduction and design. Br J Cancer 1976;34(6):585-612.