Diploma Thesis

Effect of a topical Cyclosporine A therapy on the meibomian glands in patients with Sjogren Syndrome

A retrospective data analysis

Submitted by

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Graz, 15.11.2021

Affidavit

I hereby affirm that this Thesis represents my own written work and that I have used no sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are properly cited and attributed. The thesis was not submitted in the same or in a substantially similar version, not even partially, to another examination board and was not published elsewhere.

Graz, 15.11.2021 Wintersteller Paul eh

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Acronyms

(ADDE) – Aqueous tear deficient dry eye

(AECG) – American-European Consensus Group

(CREST) – Calcinosis-Raynaud phenomenon-esophageal involvement-sclerodactyly-telangiectasia syndrome

(CsA) – Cyclosporin A

(DED) – Dry eye disease

(EBV) – Epstein-Barr Virus

(EDE) – Evaporative dry eye

(HLA) – Human leucocyte antigen

(IL) – interleukin

(KCS) – Keratoconjunctivitis sicca

(M3R) muscarinic type 3 receptor

(MG) – Meibomian glands

(MGD) – Meibomian gland dysfunction

(MHC) – Main histo-compatibility complex

(MMP) – matrix metalloproteases

(NO) – nitric oxide

(OSS) – ocular staining score

(pSS) – primary Sjögren Syndrome

(RA) – rheumatoid arthritis

(SLE) – systemic lupus erythematosus

(SS) – Sjögren Syndrome

(SSDE) Sjögren Syndrome dry eye

(sSS) – secondary Sjögren Syndrome

(vBS) – van Bijsterveld Score

(TNF) – Tumor necrosis factor

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Kurzfassung

Das Sjögren-Syndrom ist eine Autoimmunerkrankung, die primär mit der Zerstörung der Speichel- und Tränen-Drüsen einher geht. Typische Symptome sind ein trockener Mund, sowie trockene Augen. In weiterer Folge ist auch die subjektive Lebensqualität der Patient*innen reduziert. Der Symptomkomplex schließt in vielen Fällen auch die Ausbildung einer Meibom-Drüsen-Dysfunktion ein. Je nach Schwere werden Tränenersatzmittel, Salben, Gele, Lidrandmassagen oder auch das immun-modulierende Cyclosporin A angewendet.

Eine Erhebung der subjektiven Beschwerden erfolgte über spezielle Patient*innen-Fragebögen. Die objektiven Parameter wurden mit der Spaltlampe oder anderen Geräten, wie etwa dem Keratographen 5-M kontrolliert. Ein besonderer Schwerpunkt der Studie lag in der Bewertung der Meibom-Drüsen, diese erfolgte sowohl über deren Verlauf am Lidrand, der Exprimierbarkeit und Qualität ihres Sekrets als auch in der Beurteilung der Morphologie mittels Meibographie.

In unserem retrospektiven Studiendesign, wurde Cyclosporin A über einen Mindestzeitraum von einem Jahr verabreicht. 14 Patient*innen mit primärem oder sekundärem Sjögren-Syndrom der Ambulanz für Benetzungsstörungen der Universitäts-Augenklinik Graz konnten aufgrund der Einschluss-/Ausschluss-Kriterien in diese Auswertung inkludiert werden. Über den Studienzeitraum zeigte sich eine Stabilisierung der objektiven sowie subjektiven Parameter des Trockenen Auges. In weiterer Folge konnte auch eine signifikante Verbesserung der Exprimierbarkeit der Meibom-Drüsen nach der Cyclosporin A Therapie ($p=0,025$) festgestellt werden.

Unsere Studienergebnisse legen nahe, dass eine kontinuierliche Cyclosporin A Gabe in Kombination mit Tränenersatzmitteln und einer regelmäßigen Lidrandmassage zu einer Stabilisierung der Erkrankung an der Augenoberfläche und der Meibom-Drüsen-Dysfunktion führen kann. Daher empfehlen wir eine kontinuierliche Cyclosporin A Therapie, zusammen mit den bereits genannten Therapieformen, bei Patient*innen mit moderaten, bis schweren Ausprägungen des Sjögren-Syndroms. Zusätzlich sollte die regelmäßige Durchführung einer

Meibographie zur Erfassung der morphologischen Veränderungen der Meibom-Drüsen und Feststellung einer Progression Teil des Follow-ups sein.

Abstract

The Sjögren Syndrome is an autoimmune disease, which is primary associated with the damage of salivary and/or lacrimary glands. Typical symptoms are a dry mouth and dry eyes, as well as the reduction of the quality of life. Furthermore Sjögren Syndrome induced meibomian gland dysfunction is also diagnosed in many cases. The mainstay therapy are artificial tears, ointments, gels, regularly applied eye lid massage and the application of cyclosporine A.

The evaluation of subjective discomfort is analyzed via patient-questionnaires, objective parameters are evaluated with the slit-lamp or other devices, like the Keratograph 5-M. A focus of this study was the evaluation and grading of the meibomian glands. This was achieved by the examination of their morphology with the meibography, and the evaluation of the quantity and quality of meibum secretion.

In our retrospective design, Cyclosporine A was applied for minimum one year. 14 patients with primary or secondary Sjögren Syndrome of the dry eye unit of the Department of Ophthalmology, Medical University of Graz met the inclusion and exclusion criteria. Analyzed patient data showed a stabilization of objective and subjective patient/dry eye parameters. Furthermore, a significant improvement in the expression of meibum after the continuous application of Cyclosporine A ($p=0,025$) was observed.

Our results suggest that a continuous longtime application of Cyclosporine A, in combination with artificial tears and regularly applied eye lid massage, can lead to a stabilization of the disease and a deceleration of the progression of Sjögren Syndrome induced meibomian gland dysfunction. This led to the recommendation that Cyclosporine A, complemented with the mentioned therapies, should be prescribed in moderate to severe cases of Sjögren Syndrome. Additionally, a meibography should be part of the follow up process to enable a better comparability of the patient history.

I. Anatomy/Histology/Physiology of the eye

The eye is one of our primary sensory organs and is located in the orbital cavity, an osseous structure within the cranium. Stabilization of the ocular bulbus is ensured due to fixation to retrobulbar fat and the insertion of our straight and oblique eye muscles (lateral/medial/superior/inferior rectus muscles and the superior/inferior oblique muscles), also called the extraocular muscles.

I.I The eyeball

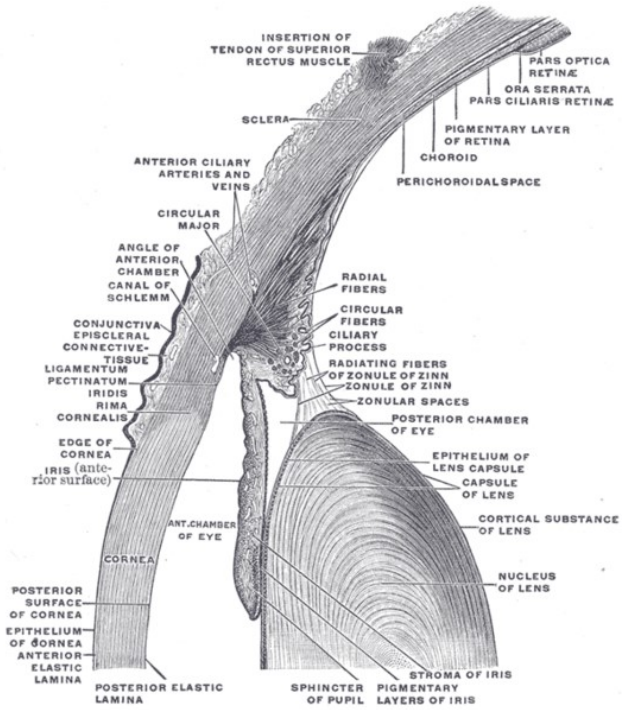

Figure 1 - Anatomy of the eyeball - https://commons.wikimedia.org/wiki/File:Gray883

The bulbus of the eye consists of three layers:

The retina, the innermost layer, is converting visual stimulation of the rods and cones into electric nerve signals, which are then forwarded via the optical nerve and the optical tract to our brain for processing. The macula is located in the central area of the retina and contains the fovea centralis, the location with the highest density of cones, which plays an important role in enabling sharp central vision.

The middle layer, the vascular tunic or uvea, is comprised of the choroid, the ciliary body and the iris. The main function of the choroid is nourishment of the more remote areas of the retina (via branches of the short posterior ciliary arteries),

whereas the ciliary body is producing an aqueous intraocular fluid to maintain a specific intraocular pressure and for nourishing more anterior parts of the eye, like the cornea. Furthermore, the ciliary body also includes the ciliary muscles for accommodation. The iris is the most anterior part of the uvea and physically acts as an aperture for the eye.

The outermost layers are the cornea, the conjunctiva and the sclera. The cornea is dome shaped and consists of the epithelium layer, the Bowman´s layer, the stroma, the Descemet membrane and the endothelium. The average diameter of a healthy cornea is about 11.04–12.50 mm in males and 10.7–12.58 mm in females [1].

The epithelium layer is organized by stratified non-cornified squamous epithelium cells and functions as a protective barrier, as well as improving the refracting capacities of the eye. The main function of the Bowman´s membrane is to assist in sustaining the dome shaped structure of the cornea. The stroma is vital for the transparency, the mechanical resistance and acts as the main refracting lens of the cornea. The Descemet`s membrane is important for the fixation of the endothelium and the passage of nutrients from the endothelium to the stroma. The endothelium removes excess fluid of the stroma and therefore, enables corneal clarity. [1]

Despite its thickness of 0.5 mm the cornea contributes 43 diopters out of a total of 60 diopters. This concludes that an irregular surface of the cornea, like in keratoconjunctivitis sicca, goes along with a visual impairment [2]. Due to a high regeneration rate of basal cells at the corneal limbus (the border between the cornea and the sclera), damages to the epithelium usually heal within a few days [3]. The corneal surface is permanently covered with lacrimal fluid to obtain optimal working conditions. The nutrition of the cornea is a process which is partitioned into three parts, anterior the cornea is nourished by the tear film and posterior by intraocular fluid and the more peripheral areas are covered by small arteries located near the limbus, whereas the cornea itself features no blood vessels. In addition to this, the count for free corneal nerve endings is among the highest nerve-ending/cell ratios in the human body. The corneal innervation is estimated to be 300 to 600 times higher than the innervation of the skin and even the tooth bulb contains less free nerve endings [4].

The corneal epithelium continues into the conjunctiva, which is for this reason also a non-cornified squamous epithelium with its main purpose to ensure protection against germs, due to its richness of mast cells and immune cells. An infection of the conjunctiva is usually associated with its reddening, in some cases the same applies to keratoconjunctivitis sicca and the symptom complex of dry eye disease (DED). Furthermore, the conjunctiva with its goblet cells provide the basic for smooth eye movements.

The sclera (from the Greek term “scleros”: hard) is below the surface of the conjunctiva and stretches to all over the bulbus, with exceptions for the cornea and the exit of the second cranial nerve, the optical nerve (the optical disc) where it merges with its coating of the dura (bulbar sheat/capsule of Tenon). The sclera works as counterpressure layer against the intraocular pressure and therefore is determining the form of the bulbus. Further it determines the length of the optical axis, if there is any abnormal deviation it results in myopia or hyperopia.

I.II The palpebrae

The eyelids, palpebrae or tarsi are positioned anterosuperior and anteroinferior to the optical bulbus. The upper and lower eyelid are histologically built quite similar.

Beneath the cutis is a circular muscle, the orbicularis oculi muscle, it could be divided into three parts, the orbital-, the palpebral- and the lacrimal- division. The orbital part originates from the medial palpebral ligament and osseous structures (frontal process of the maxilla and the nasal part of the frontal bone), runs around the orbital opening and inserts into its own fibers on the lateral site. The Riolan muscle is also located in this part, this muscle is of uttermost relevance for the secretion of meibum (described in chapter I.III The glands). The palpebral part stretches from the medial palpebral ligament and inserts into the lateral palpebral ligament. The lacrimal division originates from the lacrimal bone and inserts into the lacrimal sac. Innervation is delivered by the temporal branch of the fascial nerve (CN VII). The m. orbicularis oculi is the most important muscle involved in the act of blinking, it possesses the ability to close our eye voluntary and involuntary. [5]

The tarsal plates are located at a deeper level of the eyelids and are build out of dense connective tissue. As mentioned in the paragraph above, the upper and lower tarsi feature the same histological structure, but they differ in their shape and their size. The superior tarsus is shaped in a semilunar-like form and has an average diameter of approximately 10 mm, whereas the inferior tarsus is shaped elliptical and usually features a diameter of 5 mm.

The inner surface of the eyelids is covered with a stratified squamous epithelium called the palpebral conjunctiva, which possesses a large number of goblet cells [4]. These are responsible for the production of the mucin component of the tear film (described in the chapter: I.IV The tear film). Other glands, like the meibomian glands are also located on the inner surface and will be described later (chapter: I.III The glands).

The Marx line is also referred as the mucocutaneous junction, as it marks the borderline between the mucosal and cutaneous part of the eyelid and is usually situated posterior to the meibomian glands and determines the borderline between the epidermal and mucosal part of the eyelids. The exact position is of the utmost importance as it could be also used as a criterion in the diagnosis of meibomian gland disfunction. Furthermore, it also correlates with the position of the tear meniscus, which is also linked with the clinical expression and clinical severity especially in patients with diagnosed Sjögren Syndrome. [6]

Eyelashes are small hairs that are growing on the edges of the eyelids. Protection is their main function; they prevent particles and other foreign bodies or liquids from getting direct contact with the ocular surface. In case of malposition, irritation of the ocular surface can occur (trichiasis) and lead to severe damage. Patients diagnosed with dry eye disease tend to present with crusted eyelashes.

The act of blinking is necessary for clear vision and maintaining an optimal function of the eye. Due to the closure of the eyelids the tear film is spread over the cornea and the conjunctiva, this provides removal of foreign bodies and an optimum moistening of the outer layers of the bulbus.

If the closure is dysfunctional, like in a periphery paresis of the facial nerve or due to post-surgical scarring etc., the superficial structures of the bulbus (the cornea and

the conjunctiva) are on a higher exposition for developing an inflammatory process or a keratitis. One of the main reasons for this is the evaporation of the tear film. This and the process for developing an inflammation of the ocular surface are later described in the chapter II.IIa.

I.III The glands

Several glands are located in the eyelids, these glands are the sebaceous glands and the accessory lacrimal glands. The sebaceous glands are the Meibomian glands (MG) and the glands of Zeis. The accessory lacrimal glands are the Krause glands and the Wolfring glands. Perspiratory-gland like glands are called Moll glands or ciliary glands. The main lacrimal gland will also be covered in this chapter. It is located in the supratemporal area of the ophthalmic cavity. All of the glands have an exocrine function.

The glands of Zeis are located in the dermis, farthest away from the ocular surface compared to the other glands. Furthermore, they are part of the human's integumentary system and are holocrine glands. This means the mature cell dies due to a rupture of its plasma membrane, in order to secrete their sebaceous content. The sebum consists of triglycerides, wax esters, free fatty acids and squalene (a biochemical precursor of steroids) and is necessary for preventing dehydration of the anterior part of the eyelids.

The glands of Moll are in close proximity to the glands of Zeis. Compared to the previous mentioned type, they are apocrine. Secretion occurs as release of extracellular membrane-bound vesicles. The exact function is not completely known yet, but due to the presence of several components belonging to the immune system (lysozyme, membrane associated mucin-1 and immoglobulin-A) it is estiminated that they are part of immunological responses [7].

On the inner surface of the eyelids located are the glands of Wolfring and Krause. They are classified as accessory lacrimal glands. There are approximately 30 glands of Krause located in the conjunctiva of the upper fornix and six to eight in the lower fornix, whereas the glands of Wolfring only exist in a smaller number [8].

Together they contribute up to 5% of the total amount of lacrimal fluid, but more importantly they are responsible for the basal production rate of lacrimal fluid.

With about 95%, *the lacrimal glands* are the main contributors in the production of the tear film aqueous layer. The main gland is situated in the supratemporal corner of the ophthalmic cavity and can be divided into a palpebral part and a larger orbital part [5] which contains most of the interlobular ducts. A bunch of the interlobular ducts forms a secretory duct, which then secretes his content via the excretory ducts to the ocular surface. Innervation is achieved by the lacrimal nerve, which derives from the ophthalmic nerve, a branch of the trigeminal nerve (BN V). The gland is supplied by parasympathetic and sympathetic stimuli. As later described in chapter: III. Sjögren Syndrome, the gland can be a target of inflammatory autoimmune processes and for this reason be restricted in function.

The meibomian glands (MG) or tarsal glands are holocrine, tubulo-alveolar glands and are situated in the tarsus. As described first by Meibom and later by Leeson in 1963 up to 40 MGs can be found in the upper eyelid, compared to approximately 20 to 30 MGs in the lower eyelid [9,10]. As Butovich I. A. stated in his multi-article review, Meibum is an oily substance which is mainly comprised of wax esters, cholesteryl esters, free cholesterol and triacylglycerols in combination with small amounts of polar substances, phospholipids, free fatty acids etc [11]. It contributes to the layered structure of the tear film (described in I.IV The tearfilm). The orifices of the MGs can be found on the posterior edge of the ridge of the eyelid. Secretion of meibum is triggered by a contraction of the muscle of Riolan (the most superficial part of the orbicularis oculi muscle). Furthermore, increased secretion can also be obtained by manual mechanical compression of the eyelid. Proper functioning of the tarsal glands is linked with an optimal

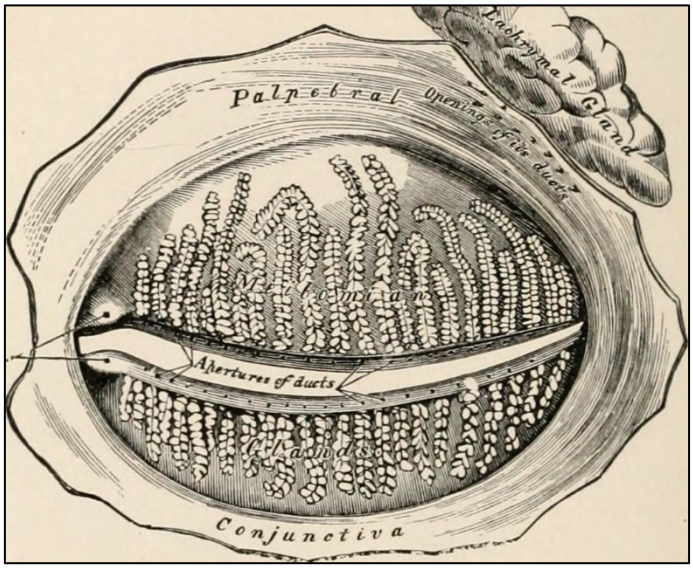

Figure 2 - Image from page 57 of "A treatise on diseases of the eye" (1910) by John Elmer Weeks

stability of the tear film. Restriction in their function like in meibomian gland dysfunction due to the reduction of lipids goes along with a desiccation of the ocular surface.

I.IV The tear film

The tear film consists of three layers: The external located lipid layer, the central aqueous layer, and the inner mucus layer.

Lipid layer: The outermost layer consists mainly of lipids and is therefore referred to as the tear film lipid layer. With its thickness of 0,015-0,160 µm it provides a hydrophobic border providing a reduced surface tension and a reduced level of evaporation [12]. The main part of this layer consists of meibum, a lipid substance which is produced in meibomian glands. Due to its relative low melting point between 19° to 40°, it is typically in a fluid state [13]. This physical state enables superficial floating of the meibum and dissemination over the aqueous layer to prevent evaporation.

Mucus layer: As the name indicates, this layer primary consists of mucins. Therefore, despite its thickness of ~2,5 to 5,0 µm it plays a role of uttermost importance in re-spreading and anchoring the tear film [12].

The production of those mucins takes place in conjunctival goblet cells and epithelial cells. Other containing substances are immunoglobulins, glucose, cellular debris, urea and enzymes. Its main functions are the lubrication of the corneal tissue and the creation of a protecting barrier to resist mechanical forces during the act of blinking and bacterial infestation. The mucus layer helps to spread the tear fluid evenly distributed across the ocular surface and create a floating base for the aqueous phase. Important mucins are the gel-forming mucins (MUC5AC) and several membrane-bound mucins (MUC-1, -4 and -16). [14,15]

Aqueous layer: Between the two layers mentioned above the aqueous layer is located, nourishing the corneal tissue and removing foreign bodies and cellular material. It also enables smooth movements of the eyeball. Furthermore, it acts as

a protective layer for the corneal surface and it is also preventing desiccation of the cornea. The basal production rate of the aqueous tear fluid is ~ 1.2µm/minute. Albumin and prealbumin, lactoferrin, transferrin, lysozyme and immunoglobulins (like secretory IgA and IgG) can be found within this layer. Reduced production maybe an indicator for an impairment in the function of the lacrimal glands. [14]

There is also the hypothesis that the tear film consists rather of two, than three layers. Gipson and Argüeso state that in addition to the superficial meibomian layer, there is a mixed aqueous-mucins gradient which consists of an aqueous phase with dispersed mucins within [15].

Due to blinking the tear film is spread over the ocular surface and provides nourishment and protection. Superfluous tear fluid is transported via blinking to the lacrimal puncta, which are located on the nasal margins of the superior and the inferior eye lid. Their function is the collection of tears, their transport through the lacrimal canaliculi and to retain them in the lacrimal sac, where they are stored as a reservoir. During blinking tears are drained due to the contraction of the orbicularis oculi muscle. Furthermore, the lacrimal duct shows a linkage to the nasal cavity via the nasolacrimal duct [5].

II. Pathological conditions

II.I Meibomian gland dysfunction (MGD)

MGD is a disease, which affects the function of MGs and therefore has an impact on the ocular surface and the composition of the tear film. Thus, MGD plays a rather big part in the development of DED. The term includes congenital- (Turner Syndrome...) and acquired causes (environmental, dietary, unintended pharmaceutic adverse effects…) and is usually linked with a decreased tear film stability, an inflammation as well as damage of the ocular surface [2].

a. Pathophysiology

Under physiological conditions, the meibomian glands produce meibum (described in chapter I.III The glands). Disruption in the production is caused by a combination of multiple processes and factors.

Age or gender related hormonal changes and environmental factors can either lead directly to a hyperkeratinisation of the glandular system or indirectly via the reduced inhibition of ductal cornealisation and therefore to changes of the quantity and quality of meibum. Inflammatory processes can also change the characteristics of meibum and trigger keratinisation. The hyperkeratinisation in combination with the altered meibum viscosity are the main driving factors in the progress of obstruction of the meibomian glands. The obstruction leads to a reduction of dispersed meibum, as meibum is blocked from reaching the ocular surface. Further a stasis of the secretion can be described. The intraglandular pressure increases and the ductal system dilates, resulting in an atrophy of the secretory acini of the MGs and their dropout. The use of contact lenses and the ongoing ageing process also play an increased role in ductal dropout and are therefore important factors in the vicious cycle of the pathogenesis of MGD. Furthermore, chronical inflammation and auto-immune processes can directly lead to an obstruction of the meibomian orifices. [16]

b. Pathological morphology

As described in chapter I.III, up to 40 meibomian glands can be found in the upper eyelid and 20-30 in the lower eyelid. In physiological condition these glands are straight and arranged parallel. The orifices typically lie on the posterior edge of the eyelid. Within pathological changed glands this layout is no longer intact. In earlier stages of the disease the glands appear thicker due to hyperkeratinization, the obstruction of the duct system and the associated accumulation of non-secreted meibum. In some cases, seborrhea and an inflammation of the glands and the surrounding structures can also be observed. In later stages of the disease the

glands are atrophic or show degenerated coiled ducts (described as tortuosity in the methodic chapter), with severely restricted functions.

Patients diagnosed with MGD suffer from a quantitative and qualitative meibum disbalance, further negative impacts are irritation of the eye, clinical apparent inflammation and ocular surface disease [16]. MGD is also a high-risk factor for developing an EDE.

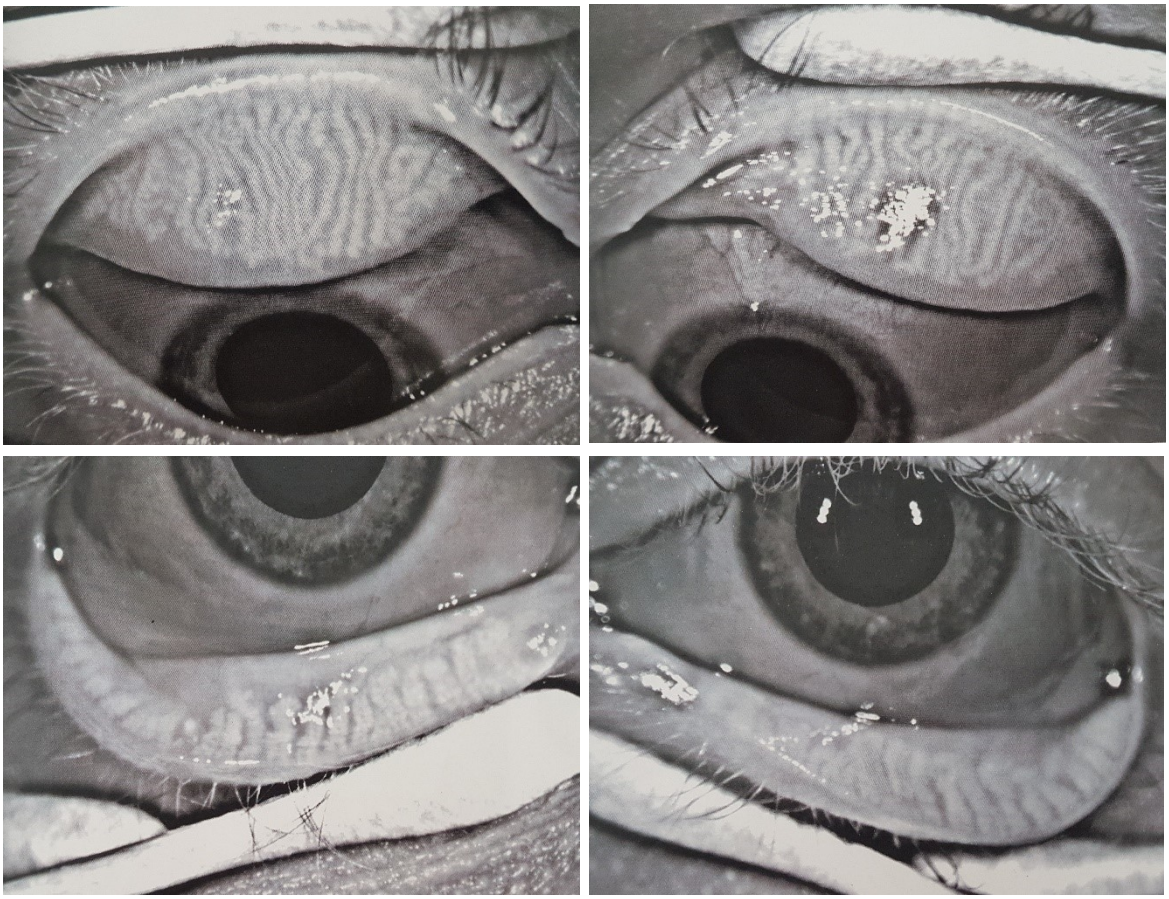

Figure 3 - Meibography (Infrared picture) of the eyelids – upper right, upper left, lower right, lower left. Mild dropout conditions can be seen in all pictures

II.II Keratoconjunctivitis sicca (KCS)

Keratoconjunctivitis sicca (kerato- as a reference for the cornea, conjunctiva- for the conjunctiva and the suffix –"itis" for an inflammation of the named structures), is also well known as dry eye disease (DED). It is a disease of the ocular surface and can feature damaged lacrimal glands, a dysfunctional composition of the tear film and an irritated conjunctiva or cornea, followed by a variety of ocular symptoms [2].

These are burning, stinging, itching and red dry eyes. Swollen eye lids, a higher rate of infections and blurred vision are also mentioned. Furthermore, patients describe the feeling of a foreign body and loss of lacrimal secretion, all of them go along with subjective discomfort, leading to a reduced quality of life, with a negative affection of their daily routines and their lifestyle [2]. Higher sensitivity exposed to light, called photophobia, is also described, as well as a reduction of functional vision, which leads to a significantly longer reaction time [17].

In 2017 J.P. Craig et al. stated that the latest definition should be unified in word, diction and meaning, to create a new unified international understanding of the DED [2]. These changes have led to the international point of view, that DED should be considered as a disease of multifactorial pathogenesis, meaning that there is no single factor which could represent the clinical picture on its own [2]. Reasons for developing keratoconjunctivitis sicca are hormonal imbalances, meibomian gland dysfunction (MGD) and other lid related issues, overuse or incompatibility of contact lenses, autoimmune disorders, allergens and congenital defects in the anatomy of the eye [18].

KCS can be divided into two major domains of pathophysiological genesis: Evaporative dry eye (EDE) and aqueous tear-deficient dry eye (ADDE), although they are seen as two different etiologies, they can also occur together.

a. Evaporative dry eye (EDE):

In EDE the protection of the lipid layer is disturbed, and the aqueous layer vaporizes faster than normal. In an eye, which is functioning normally, the lacrimal glands can replace the loss of tears due to higher secretion, while in EDE the loss of tears is too fast to be compensated [19]. Even then several factors are needed for developing KCS, as other authors once again stated that KCS is a multifactorial disease [19]. As mentioned in the DEWS II pathophysiology section, EDE may develop due to lid related factors (age related meibomian gland changes, sexual hormone influences, MGD with high and low meibum delivery states), disorders of lid aperture and dynamics, other blink-related disorders, ocular surface-related disorders such as allergic diseases, auto immune diseases, vitamin A deficiency, and induced by

topical agents [2]. In this context it should be mentioned that also Sjögren Syndrome plays a role in the development of an EDE, more information can be obtained in chapter III. Sjögren Syndrome.

Age relation is usually linked to glandular atrophy and changes in the acini. These changes typically occur in meibomian glands after the ages of 50 and 60 years, affecting the lipid composition of the meibum [20]. Another reason is a deficiency of sex hormones, especially in androgens. It is known that a significant decrease of androgens can be observed in the climacteric period, which also occurs around the age of 50. Androgen acts as a gland inducing hormone. The correct function of meibomian glands relies on sufficient hormone levels, in case of decreased levels an adequate function is not guaranteed. Other risk factors for EDE are lid aperture disorders such as ectropion, lagophthalmos and blink related disorders leading to an insufficient closure of the eyelids. This manifests in a reduced break-up time (time between the last blink and the first occurrence of dry spots on the ocular surface, (later described in the methodological chapter). [2].

This starts a vicious circle, leading from a reduced break-up time to an increased evaporation, which results in a hyperosmolarity of the tear film and finally to an inflammation which once again affects the break-up time.

b. Aqueous tear-deficient dry eye (ADDE):

Whereas EDE is based on dysfunctional layers of the tear film, ADDE occurs if there is a malfunction in production or secretion of the tear fluid in the lacrimal glands. Less tears are produced and the concentration of sodium, glucose and other substances in the tear film remain unchanged, leading also to hyperosmolarity with a cascade of inflammatory reactions as consequences of the vicious circle [2]. The main acting cytokines are:

Interleukin (IL) -1α and 1β, are produced by endothelial cells, neutrophils, epithelial cells and macrophages and play a major role in the activation of the immune system. Tumor necrosis factor (TNF α), produced by macrophages and monocytes, is also involved in immune processes and stimulates

necrosis and apoptosis. Further enzymes in this cascade are Matrix metalloproteases such as MMP-9. It is involved in physiological events, as well as tissue remodeling and disease processes. MMP-9 is also linked with the destruction of collagen and therefore to the loosening of conjunctival tissue.

ADDE can further be divided into non-Sjögren Syndrome dry eye (non-SSDE) and Sjögren Syndrome dry eye (SSDE)

non-SSDE

Unlike SSDE, non-SSDE is not an immunological disease. This means the absence of autoimmune reactions and auto-antibodies (Ro, La, MC3AK and Anti-CA6). Risk factors for developing a non-SSDE are age, a reduction of the glandular innervation (reflex sensory block or reflex motor block) or lacrimal gland infiltration (Sarcoidosis, Lymphoma, etc.) [21]. To summarize, in non-SSDE no autoantibodies can be detected and the criteria for SS have to be negative. This enables the discrimination between SS and non-SS dry eye.

SSDE

SSDE is an autoimmune induced disease. This subdivision of ADDE (including primary and secondary SS) is of great importance as there are specified characteristics in the pathogenesis, as well as the diagnosis. SSDE can be part of the Sjögren Syndrome complex and features T-cell infiltrated lacrimal glands and the production of auto-antibodies, resulting in less secretion due to the autoantibody induced damage of tubes and acini. A secondary result is the development of a MGD and furthermore, an imbalance of the lipid components of the tear film, as well as a mucin deficiency. Therefore, SSDE can have a contributing effect in the development of EDE. [2]

III. Sjögren Syndrome (SS)

Sjögren Syndrome, named after the Swedish ophthalmologist Henrik Sjögren (1930), is a multisystem autoimmune disease, which primary affects various systems in the human body, most of the afflicted cells are located in glands. This leads to its characteristic symptoms: dry eye (KCS) and dry mouth.

Furthermore, there are two types of SS. Primary Sjögren Syndrome (pSS), showing symptoms of dry mouth (oral symptoms) and dry eyes with histopathology changes in the salivary and lacrimal glands. Primary Sjögren Syndrome develops due to multifactorial reasons, which are described in the next chapters.

Secondary Sjögren Syndrome (sSS), on the other hand is linked with the occurrence of other connective tissue diseases, such as systemic lupus erythematosus or rheumatoid arthritis [21].

According to the ICD-10 classification, M.35 is also specified as Sicca syndrome. Mentioned symptoms are keratoconjunctivitis, lung involvement, myopathy and renal tubulo-interstitial disorders. ICD-10 subsections include mixed connective tissue problems, Mb. Behcet, polymyalgia rheumatica, diffuse eosinophilic fasciitis, multifocal fibrosclerosis, relapsing panniculitis, hypomobility syndrome and an increased risk of suffering from other autoimmune diseases. However, it seems that this classification is outdated. SS is a specific symptom complex, and the addition of the term sicca seems inappropriate, as sicca also includes a range of SS unspecific etiologies (Mb. Behcet e.g.). This will be changed in the next revision of ICD in October 2021.

III.I Diagnostic criteria and epidemiology

AECG-Classification

The classification from the American-European Consensus Group was published in 2002. This classification has 6 separate criterions, each weight 1 point.

Table 1 - AEG-Classification for pSS *- The points of the positive criteria are summed, ≥4 points and a positive tested criterion number 5 or number 6 are required for the diagnosis of pSS.*

1	Ocular symptoms	Dry eyes for more than 3 months, foreign-body sensation, use of tear substitutes more than 3 times daily
2	Oral symptoms	Feeling of dry mouth, recurrently swollen salivary glands, frequent use of liquids to aid swallowing
3	Ocular signs	Schirmer test performed without anesthesia (≤5 mm in 5 min), positive vital dye staining results
4	Oral signs	Abnormal salivary scintigraphy findings, abnormal parotid sialography findings, abnormal sialometry findings (unstimulated salivary flow ≤1.5 mL in 15 min)
5	Biopsy	Positive minor salivary gland biopsy finding
6	Antibody results	Positive anti–SSA or anti–SSB antibody results

[22].

For the diagnose of sSS the following criteria must be given: oral dryness, ocular dryness and a connective-tissue disorder must be tested positive, as well positive criteria 3,4 or 5 (the diagnosis of a collagenosis in addition to subjective symptoms and 2 out of 3 objective symptoms).

ACR/EULAR-Classification

State of the art is the more recent updated classification criteria (2016) from the American College of Rheumatology ACR/European League Against Rheumatism EULAR for primary Sjögren's Syndrome: pSS is tested positive if the patient scores

an overall score ≥4 points and either oral or ocular dryness is reported. The points of all criteria are summed:

Table 2 - ACR/EULAR-Classification for pSS *- The points of all positive criteria are summed, ≥4 points represent a positive diagnosis of SS.*

Points	Criterion
3	Labial salivary gland biopsy showing focal lymphocytic sialadenitis and focus score of ≥1 foci/4mm^2
3	Anti-SSA/Ro antibodies tested positive
1	Ocular Staining Score ≥5 (or van Bijsterveld-score of ≥4) in at least one eye
1	Schirmer's test ≤5 mm/5 min in at least one eye
1	Unstimulated whole saliva flowrate ≤0.1 mL/min

[23].

In the ACR/EULAR classification sSS does not have separate classification criteria.

Epidemiology

Due to these criteria, the American-European Consensus Group (AECG) has calculated an overall yearly incidence of 4/100.000 and a prevalence of 0.06% in pSS, the male/female ratio can be up to the range of 1:20. The median onset is expected to be about 40 to 50 years of age. [24,25]

The prevalence of sSS varies between 4% and 19% [24], this is due to the application of different criteria and the predominant autoimmune diseases in the investigated areas. Specific incidences for sSS are only available in combination with the predominant autoimmune disease, an overall incidence cannot be found. Despite this missing numbers, the prevalence data alone sufficiently showcase the epidemiological differences of pSS and sSS. Also, they implicate that sSS is the more common form.

III.II Genetics

In the last decade the genetics behind Sjögren Syndrome have been analyzed in varies studies, the most ambitious projects are Genome-Wide Association Studies, with the aim of retrieving further SS associated genetic locations.

The HLA system are glycoproteins, which are located in the cell membrane and take part in the regulation of the immune system. It encodes the main-histocompatibility complex (MHC) and is a highly adaptable system. HLA is involved in the proper function of the adaptive immune system via multiplication of T-helper cells and the attraction of T-killer cells, as well as in the innate immune system. Furthermore, organ transplant failures can be traced back to HLA mismatches in patients and for over 50 years a connection between HLA mutations and autoimmune diseases is established [26]. HLA can be divided into class I, II and III. Class I is used for coding and transcription, whereas class III contains complement factor genes, in class II are genes with an established immune function [27]. The gen loci for class II are specified as HLA-DP, -DQ and -DR regions, these regions are also linked with other auto immune diseases.

Up to date several HLA types have identified, variations depend on the patient ethnical affiliation, these types are located in HLA class II regions. In most recent studies American Caucasians usually demonstrate differences in HLA-DRB1*0301, HLA-DRB3*0101, HLA DQA1*0501 and HLA DQB1*0201, whereas DRB1*0803-DQA1*0103-DQB1*0601 are found in the Chinese population, Norwegian Caucasians mainly show HLA DRB1*0301, HLA DQB1*02 and DQA1*0501. A Spanish study marks HLA-Cw7, HLA-DR3 and HLA-DR11 and British colleagues detected DR-3 & DRw52. Despite the fact of these given geographic differences, meta-analyses show that HLA mutations usually stick with the HLA regions DRB1, DR3, DQA1 and DQB1. [28]

Other non-HLA-related genes are interferon regulatory factor 5 (IRF5), signal transducer and activator of transcription 4 (STAT4), interleukin 12A (IL-12A), tumor necrosis factor receptor-1 (TNFR-1), genes within the Family with sequence similarity 167 member A–B lymphoid tyrosine kinase (FAM167A-BLK) and genes which are coding for Serotonin transporters ((5-HTT or SLC6A4) [28].

III.III Etiology of primary Sjögren Syndrome

Due to the multifactorial etiology, genetics are not the only factor, which should be considered in the pathogenesis of pSS. Sjögren Syndrome also has some environmental and external factors, these range from viral infections, bacteria to lifestyle factors e.g. smoking, alcohol intake, stress, exposure to silicones or the median vitamin D intake and hormonal imbalances [29].

a. Hormones

Remarkable studies in this field are Daniels and Whitchers "Association of patients of labial salivary gland inflammation with keratoconjunctivitis sicca" which suggested the important role of estrogens in pSS in 1994 and Ahmeds et. al. "Gender and risk of autoimmune diseases: possible role of estrogenic compounds" from 1999 [30,31]. These studies show the correlation of the level of estrogen with autoimmune diseases, esp. with pSS. Estrogen has an impact on T- and B-cells, T-cells show a state of decreased activity due to increased estrogen explosion, whereas B-cells are stimulated to produce more antibodies [29].

In 2003 Sullivan et. all published that woman diagnosed with SS also showed a significant decrease in the androgen levels, compared to a matched control group [32]. In 2008 Pauliina Porolas study group confirmed these results. A malfunction in the Dehydroepiandrosteronacetat-metabolism, a precursor of both androgen and estrogen, lead to a significant androgen deficiency in women with pSS and sSS [33].

In recently published study from June 2019, Sara S. McCoy et. al investigated the possible association of pSS with a reduced lifetime exposure of sex hormones. The results showed that patients with diagnosed pSS also had significantly decreased levels of estrogens throughout their life, whereas higher estrogen levels act as a protective factor for the manifestation of pSS [34]. This primary applies for women, as the study only interacts with female sex hormone levels. However, studies show that both androgens and estrogens play a crucial role in the pathophysiology of SS.

The decreased quantity in sex hormones also has a negative impact on the meibomian glands and is therefore a risk factor for developing MGD. Meibomian glands produce meibum depending on the exposed hormone levels, especially the level of sex hormones. Quantitative shifts of androgen, progesterone, and especially estrogen lead to altered secretions of meibum, these changes can be permanently altered after ovariectomy or in the menopause) [29]. As mentioned, women in the menopause can experience particular changes in function of the meibomian glands, this may also lead to the development of MGD.

According to Palacios et al. the menopause has a median onset of 50.1 to 52.8 years of age for the female European population [35]. This also shows a strong correlation with the average onset of pSS with about 40 to 50 years of age [24].

b. Viral infections, bacteria, and environmental factors

Most evidence about viral infections are stated about Epstein-Barr Virus (EBV). EBV is known to show an extremely high prevalence. An infection with the virus is either asymptomatic or leads to typical symptoms such as hepatosplenomegaly, fever, lymphadenopathy, and sore throat (also called infectious mononucleosis). The symptoms last about up to 2 months in most cases, the virus itself persists within the human body and can be reactivated due to immunological disbalances. An EBV infection also results in an induction of B-cell growth and differentiation, these modified B-cells are reduced over time, but never completely eliminated [34]. The modified B-cells in combination with EBV-encoded proteins trigger pathophysiological pathways, which result in a similar B-cell hyperactivity seen in patients with pSS [37]. A study from 2014 also established an association of past EBV infections and the detection of autoimmune disease specific antibodies: anti-Ro/SSA and anti-La/SSB [38].

Connections between pSS and cases of Cytomegalovirus-, Hepatitis C-, Coxsackievirus- and Human T-lymphotropic virus type-I infections have also been investigated in various studies. Although there may be a connection, the conducted studies have not shown sufficient scientific evidence [29].

The point of lacking scientific evidence applies to many other factors. Björk et. al investigated in a meta-study the associations between pSS and bacteria (e.g., helicobacter pylori, mycobacteria tuberculosis, and the oral and gut microbiome), organic chemical factors (smoking, alcohol intake and stress), or inorganic chemical factors (exposure to silicones). The reviewed studies show promising directions, all of those factors could potentially be risk factors, however there is not enough evidence for this conclusion and further studies in this field are needed [39].

III.IV Etiology of Secondary Sjögren Syndrome

The etiology of sSS is linked with the emergence of other autoimmune diseases. Described in Chapter III.II Genetics, the development of SS is linked with mutations within the HLA system, especially in class II. Mutations in class two are also responsible for multiple other autoimmune diseases, e.g., SLE, rheumatoid arthritis (RA), multiple sclerosis, diabetes mellitus type 1, celiac disease etc. [39]. Yao Q´s et al. meta-analysis shows that the prevalence of sSS in SLE can be as high as 17.8%, out of 2489 patients with SLE, 444 were also diagnosed with SS [40]. In 2003, a Spanish study tested 788 patients diagnosed with RA, out of those 17% were classified as sSS patients [41]. In 2011, McGuire et al investigated the presence of a special variation of T-helper (TCCR9) cells in Sjögren Syndrome and type 1 diabetes. Results highlighted an increased number of TCCR9 cells in both sSS and type 1 diabetes mellitus and furthermore, a possible connection between the two diseases [42]. Further prevalence of sSS in other autoimmune diseases are stated in the 2014 published "*The epidemiology of Sjögren's Syndrome*", these data showed the strong crosslink in the occurrence of autoimmune diseases and sSS [24].

III.V Pathogenesis

As SS itself is a multifactorial disease, the pathogenesis also has multiple ongoing processes. As already mentioned, hormonal imbalances, environmental factors and genetics lead to a cascade autoimmune reaction. These range from a pre-

immune phase with the expression of certain HLA loci, to an inflammatory/immune phase, with increased antibodies. Results are lymphocytic infiltrations, an increased acinar degeneration and reduced secretion. Ultimately, this leads to its characteristics, exocrine dysfunction and sicca symptoms. [29]

a. Antibodies

Ro- (SSA) and La- (SSB) are known antibodies in association with SS and other autoimmune diseases like RA or SLE and are identified to occur with the development of SS in many cases [43,44]. The production of those antibodies is induced by a cascade of immune dysregulations. Ro can be classified in Ro-52 and Ro-60 antibodies, Ro-52 antibody production can also be induced by IFN-γ [45].

> A detailed distribution can predict a higher affinity for specific illnesses. The majority of patients with predominant Ro-52 antibodies suffer from a wider field of autoimmune diseases compared to predominant Ro-60 or mixed Ro-52/60 subtypes. Furthermore, a higher prevalence of inflammatory myositis and inflammatory rheumatitis can be found in this patient group. Isolated Ro-60 is linked with increased diagnoses of SLE, as well as elevated levels of antiphospholipid-antibodies. Levels of increased Ro-52 and Ro-60 tend to be noticeable related with pSS. However, there are a lot of discrepancies, as some other studies are not necessarily matching this result. [46]
>
> On the other side an increased level of anti-SSA antibodies is detected in only about 33-74% of patients with SS [24]. This means that measured SSA antibodies cannot be taken as a guaranteed indicator for the diagnosis of SS. This has also led to a change in the official SS-criteria guidelines. La-antibodies are therefore no longer required for the confirmation of a pSS diagnosis.

However, the presence of SSA and SSB antibodies is still associated with the presentation of increased lymphocytic infiltrates in minor salivary glands, increased glandular dysfunction, a younger age at the diagnosis of SS, etc. [47].

Other antibodies which should be mentioned are anti-muscarinic type 3 receptor autoantibodies (anti-M3R) and anti-CA6 (antibodies to carbonic anhydrase 6). Anti-M3R is associated with the dysfunction of the saliva production and the inhibition of its secretion. Therefore, anti-M3R could play an important role in future diagnostics of primary Sjögren Syndrome, as well as in its pathogenesis. Anti-CA6 can also be measured in both primary and secondary Sjögren Syndrome and could therefore provide a diagnostical value. However, their full way of function and pathogenesis are not scientifically clarified yet. [48,49]

b. The T-cell population

TH1 cells are responsible for the cell mediated response and the IFN-gamma production. In SS however the Th1 cell mediated response is increased via an elevated IL-7 expression and larger amounts of IFN-gamma are produced [50]. IFN-gamma in combination with IL-12 activates the differentiation of CD4+ cells into Th1 lymphocytes [29], IFN-gamma also activates macrophages and gathers immune cells at the inflammatory location [51]. As follows, an increased quantity of Th1 cells can be measured, this correlates with the progress of the immunopathological lesion of SS [52].

Th17 cells are a subset of CD4+ cells and responsible for the production of IL-17, a proinflammatory cytokine, which plays a huge role in SS [29]. Furthermore, Th17 cells are also linked with an elevated quantity of IL-6 and IL-23 [53]. IL-6 is also a proinflammatory cytokine and stimulates the growth and differentiation of B-cells [54]. IL-23 plays an important role in the pre-immune phase of SS [55], furthermore, it is linked with IL-22 in the TH17 pathway [56]. IL-22, in combination with IL-17 is pivotal in the development of inflammatory reactions in SS [57]. IL-21 should also be mentioned in this context, it is also associated with TH17 cells and follicular T-helper cells and likewise IL-6 it has an influence on the B-cell population [29].

T cells also feature STIM1 and STIM2 proteins, these are key components of calcium release-activated calcium (CRAC) channels. CRAC channels play a center part in the T-cell pathway and malfunction of these proteins are linked with increased lymphocytic infiltrates and progressive destruction of salivary glands. Mice studies

show a significantly decrease of STIM1 and STIM2 levels in SS positive mice. Furthermore, these studies have also shown that STIM1/2 knockouts can lead to the development of spontaneous and severe SS as well as the production of antibodies against La and Ro. [58]

c. The B-cell population

In addition to T-cells, B-cells are also included in the pathogenesis of SS. An altered proliferation and differentiation of B-cells, as well as germinal center formations, hypergammaglobulinemia, antibody production and increased cryoglobulins are key elements in the pathophysiology and the continuous driving of SS [29].

Germinal centers are regions within lymph nodes and other secondary lymphoid structures and are responsible for the proliferation and differentiation of B-cells [57]. Functional formations and neogenesis of germinal centers play a major role in the production of antibodies (Ro and La) and the progression of the disease, furthermore they are apoptotic centers in SS affected organs [60].

B-cells are attracted via the B-cell attracting chemokine-1 to exocrine glands, e.g. the salivary glands, this leads to the common lymphoid infiltrations in glandular biopsies [29].

Another cytokine which should be mentioned is BAFF (B-cell activating factor). BAFF is a member of the tumor necrosis factor ligand family. An elevated expression of BAFF by antigen-presenting cells, neutrophils, epithelial cells or activated T lymphocytes can lead to immunoglobulin isotype switching, hyperactivity, maturation and proliferation of the B-cell population [29]. An increased production of antibodies is also described [61]. One factor that should highlight the association of BAFF and SS is that apart from T-cells, B-cells and macrophages, BAFF is also expressed in enlarged quantities in salivary gland epithelial cells which are highly affected by SS [62].

d. The pre-immune phase

In Pulukool Sandhyas et al. "*Update on Pathogenesis of Sjögren's syndrome*" there is also mentioned a pre-immune phase, which takes place before the onset of the immune phase [29]. This phase could be the trigger event to cause the dysfunction of glands later on in the disease. Mice studies show that NOD mice, a model for SS patients, have an altered glandular homeostasis and increased protease activity [63].

Elevated protease activity can lead to an increased production of corrupted protein with immunogenic functions [63]. Mice with an autoimmune predisposition show a lower acinar cell proliferation rate, an elevated level of metalloproteinase activity and an unusual organogenesis of the submandibular glands, which can act as the fundaments of future autoimmune reactions [64]. Furthermore, patients with SS exhibit structural changes in tight junctions (especially ZO-1, occludin, claudin-3 and claudin-4) due to cytokine exposure [29], leading to problems in the structure and polarity of cells, as well as a facilitated expansion of inflammation and autoimmune processes.

Another process in the pre-immune phase is the functional disturbance of calcium channels. These channels are of utmost importance for any secretion process and are highly affectable by increased nitric oxide (NO) levels. Elevated NO levels are directly linked with the hyposecretion in salivary glands and are therefore common in SS patients. Furthermore, high NO levels do not only affect the calcium pathway, they also result in an intensified oxidative stress situation, a condition which is common is SS patients. An oxidative stress situation also leads to an increased cellular vulnerability. [29]

e. The immune phase

The immune phase is a result of lymphocytic infiltration, in combination with an increased production of antibodies and cytokines. It is also the phase of inflammatory processes, in which damage of the salivary glands occurs.

As mentioned above, several cell types (TH1-, TH2-, TH17-, B-cell etc.) are involved, as well as the production of cytokines. Cytokines stimulate these cells to produce more cytokines and antibodies against cellular structures. The result is an autoimmune induced inflammation. IL-1 and TNF-alpha also affect the function of glands and lead to glandular dysfunction in later stages of the disease [29], a characteristic feature of SS.

Autoantibodies against the muscarinic acetylcholine receptor M3 (M3R), could contribute to the inflammation within the glandular tissue, the malfunction of calcium channels and a decreased glandular function [29]. The inflammation and a damaged extracellular matrix can lead to changes in cell polarity and this in turn favors the pathological process, creating a vicious circle.

IV. Cyclosporine A

After the discovery of beta-lactam antibiotics, big pharma companies started a program for further investigation of fungal-derived antibiotics. In 1971 Sandoz (today known as Novartis) envisaged the soil fungus Tolypocladium Inflatum and isolated ciclosporin, an 11 amino acid peptide [65]. It appeared that it was not an antibiotic. After several years of animal- and human studies, ciclosporin got licensed in 1983 by the Food and Drug Administration (FDA) for the use in organ transplantations.

Cyclosporine A (CsA), also known as Ciclosporin is a calcineurin inhibitor, which is primary used for its immunosuppressive effect. The application of CsA lowers or prevents immune- and autoimmune reactions. This effect is fundamental in the treatment of autoimmune diseases, as well as organ transplantations.

IV.I Chemical composition

Wikimedia shows a picture of the chemical structure of CsA. The structure formula is $C_{62}H_{111}N_{11}O_{12}$ and the molecular weight is 1,202.61 g/mol. The solubility of CsA with Dimethyl sulfoxide is graded with +, with water with +/- and +++ with methanol [66].

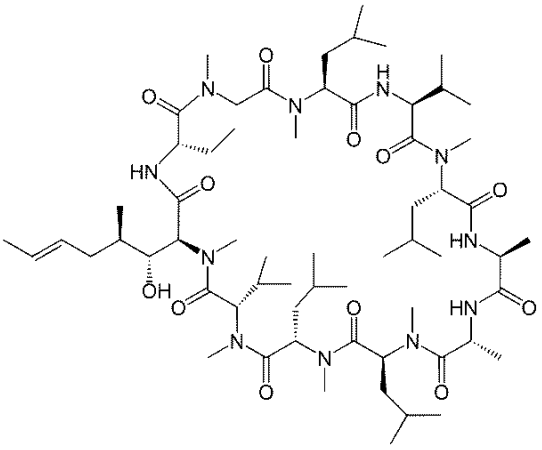

Figure 4 - Chemical structure of CsA - File:Ciclosporin.svg from Wikimedia 2008

(soluble at room temperature: +++, soluble on heating: ++, precipitation after cooling hot solution: +, partially soluble on heating: −, insoluble: −−). Dimethyl sulfoxide and methanol are solvents, which are often used in the field of pharmacology.

CsA is a cyclic peptide, which consists of 11 amino acids. One of those acids is a D-amino acid, meaning it possesses the D-configuration, which appearance is uncommon in nature. Although, D-amino acids reactions also occur in prokaryotic and eukaryotic cells, these reactions are not regulatory processes. Reactions, which involve D-amino acids can cause toxic effects (for instance renal toxicity) and

therefore need counteractions. The D-amino acid is also found to be one of the starting points in the biosynthesis of CsA [67].

IV.II Effect of CsA

CsA affects several cells within the human immune system. The main target is the T-cell population [68]. As already mentioned, CsA is categorized as a calcineurin inhibitor, therefore it plays an important role in the blockade of several mechanisms in the cascade of the immune response.

Foxwell and Ryffel state that CsA prevents the production of T-cell growth factor (also known as IL-2) in a relatively selective way [68]. The induction of IL-4 and IFN-gamma (proinflammatory cytokines, which takes a leading role in inflammatory- and autoimmune processes [51]), is also blocked via CsA [69]. It is also stated by Herold et. al that in addition to IL-2 and IL-4, the production of IL-3 is also limited under the course of CsA therapy [70]. CsA also disrupts the priming of T-cells, via a modification of the dendritic cells, which are involved in the process of priming [71].

In addition to its effect on T-cells, the population of B-cells is also greatly influenced. According to Thompson, there is an indirect inhibition of growth and differentiation of B-cells, due to the inhibition of CD4+ produced cytokines [72]. Growth and differentiation of B-cells are also key points in the pathogenesis of SS [29]. Thompson also stated that Ca^{2+} induced pathways in B-cells are downregulated due to CsA, this applies especially for IgM related processes, as they are typically dependent on Ca^{2+} channel signals [72]. As a result, this leads to a decreased immune answer and therefore also reduced autoimmune reactions.

Other cell types that are affected by CsA are direct acting cells of the innate immune system like macrophages and neutrophile granulocytes. These cell types show a decreased activity in the production of cytokines [71].

This interference in addition with the implications on the IL-system and the modified function of T-cells and B-cells attest CsA an enormous impact on both the innate and the adaptive immune system.

IV.III The usage of CsA in SS

Up to date, CsA is the therapy of choice to treat inflammation in SSDE. CsA can alleviate typical SS symptoms and most of all, it has a positive effect on ocular dryness [73].

Although, CsA is available in various application forms, the main usage in Sjögren Syndrome induced dry eye is via CsA eye drops. One big advantage is that topically CsA does not reach the systemic metabolism in extensive amounts and therefore does not affect it. Furthermore, no renal toxicity or general immune deficiency is mentioned [73]. This is extremely important, due to the fact that a systemic immune modulation could result in a low immunity and an increased risk for suffering related consequences.

Another big advantage of CsA towards cortical steroids is that the usage of CsA does not trigger the development of a cataract or show elevated intraocular pressure levels. Therefore, it can be used for a long-term application.

The major disadvantage is that some patients develop intolerance reactions towards CsA. Typical symptoms of this conditions are ocular burning, epiphora, mild to severe ocular pruritus and increased KCS symptoms, like foreign body sensation [73].

V. Non cyclosporine A therapy

Artificial tears are the mainstay therapy for DED, also for SSDE. Depending on the severity of SS, punctum plugs, lid margin massage, other anti-inflammatory therapies, and eyedrops of human origin are also therapeutic options. A continuous administration is needed. If used irregularly or the usage is suspended, typical DED symptoms can reoccur or intensify. It is also recommended to use a combination of treatment options to achieve the best possible results. The number of applications varies with the level of the symptoms and signs. [74]

V.I Artificial tears, gels, and ointments

Ophthalmological medical products and drugs are applied topically via drops, gels, or ointments. Due to their compared shorter duration drops have to be applied more often than gels or ointments, but with the advantage that the patient's vision is not impaired. Gels and ointments have a longer lasting effect with the disadvantage of reduced and blurred vision. Therefore, drops are usually used during the day, whereas gels and ointments are mainly used in the night. In severe cases gels and ointments also have to be administrated during the day.

Artificial tears provide additional wetting of the ocular surface with the goal to imitate the human tear fluid, providing a lubricant and protective layer. They are used to treat ocular dryness and prevent the resulting damage.

The main ingredient is a saline solution, the blending of additional hygroscopic polymers like hyaluronic acid and derivates of cellulose is also common. Additional substances vary from manufacturer to manufacturer. Some use conserving agents. These enable a prolonged period of usage without contamination, but the conserving agents can have a toxic effect. Therefore, the administration of artificial tears without conserving agents is recommended in the therapy of DED. [74]

V.II Topical corticosteroids

Like CsA, corticosteroid eye drops, or ointments also act as immune modulating substances and reduce auto-immune reactions and the levels of cytokines. The results are a reduction of symptoms and a stabilization or decline of the disease. Furthermore, an improvement of the ocular surface can be achieved. However, the frequent use of topical corticosteroids also has major disadvantages. Typical reactions are the development of cataract and increased levels of intraocular pressure. Although there are studies which suggest that loteprednol have a reduced risk for elevated intraocular pressure levels, its usage is not common. This results in the fact that a continuous application of corticosteroids is not recommended by the DEWS II Management and therapy report. Corticosteroids can be used for the initial approach of limiting the disease and alleviate the subjective and objective symptoms of the patient. [74]

V.III Punctum plugs

Punctum plugs can offer an efficient way to block the outlet of the lacrimal fluid – the lacrimal puncta or the canaliculi. The wetting of the ocular surface with tear fluid increases and the surface damages due to a inadequate tear film layer can be reduced.

Blocking can be classified in temporary or permanent and partial or total. This enables the possibility to adjust to diverse severity levels of DED.

V.IV Eyedrops of human origin

According to the TFOS DEWS II Management and Therapy Report, autologous serum drops show a benefit in the treatment of ocular surface damage.

Autologous serum drops are rich of proteins and contain several growth factors and vitamins. They lubricate the ocular surface and have a positive effect on the corneal

epithelium regeneration. Furthermore, they have the ability to reduce the cytokines on the ocular surface. [74]

Albumin is as serum protein with a molecular density of ~66 kDa. Albumin containing eye drops are used in the therapy of severe dry eye such as in SS.

Additionally, their usage also improves the corneal regeneration abilities. As a study from 2003 has shown that rabbits treated with albumin have an increased regeneration rate of corneal epithelial defects. Due to the fact, that albumin is also an integral part of the human serum, albumin drops can be seen as an alternative to autologous serum drops. [74,75].

V.V Eye lid therapy

Massage of the eye lids are frequently used by patients suffering from MGD. Warm and wet compresses are applied on the closed eyes. The increased temperature and moisture have a positive effect on the viscosity of the meibum. It can reduce its stasis and opens blocked meibomian orifices. The subsequent massage then enables the expression of the meibum to the ocular surface. This increases the tear film stability and can reduce further damage progression of the ocular surface.

Some patients might also benefit from the application of lid scrubs and intensified lid hygiene. Crusts and debris are getting removed. However, the TFOS DEWS II Management and Therapy Report points out that there are no official guidelines for these therapeutic options yet. [74]

Methods

The study was conducted with SS patients from the dry eye unit of the Department of Ophthalmology, Medical University of Graz. The searching window for valid patient data was from January 1st, 2010, until December 31st, 2019. Patients had to be diagnosed with pSS or sSS. Patients who received their SS diagnosis after 2016, were classified with the ACR/EULAR classification, patients with an earlier diagnosis were classified with the older AECG criteria. A continuously CsA intake for minimum one year was required. The minimum inclusion age was set at 18 years, since SS is a rare disease among younger patient groups [25]. Women and men were included equally. Furthermore, it was required that a meibography was recorded prior the first CsA application and at the time of the last required examination. Patients with adverse reactions after CsA administrations, insufficient tear film and ocular surface parameters and a discontinuously usage of CsA administrations were excluded.

The data were classified into pre- and post-examination dates. Pre-examination dates had to be before the first application of CsA. The post examination dates had to take place at minimum one year after the first CsA administration. Following data sets were evaluated:

Patient related data: sex, date of birth, age, date of examination (pre/post), follow up in months, diagnosis of SS, other diagnosed diseases, allergies, CsA treatment (pre/post), additional treatment for SS (pre/post).

Eye related data (pre/post): OSDI-score, VAS score, break-up time, corneal grading with NEI-score, Schirmer test, lissamine-green staining (van-Bjiesterveld score and Marx line status), eye lid vascularization, meibomian gland secretion, meibomian gland expression, meibomian gland dropout and distortion of meibomian glands.

Patient data with insufficient records were excluded and not listed in the file.

In this period 760 Patient were treated with CsA, women, and men similarly. However, not all of those patients were diagnosed with pSS/sSS or meet the required inclusion data. According to the inclusion and exclusion criteria, 14 of these patients were identified with their examinations providing sufficient data that they

were using CsA at minimum one year continuously without the onset of adverse reactions.

Only authorized personal (diploma student and thesis supervisors had access to the patient data file. Patient records were analyzed retrospectively via EyeMed, the patient record data system of the Department of Ophthalmology, Medical University of Graz. The data were obtained after positive approval of the ethics committee of the Medical University of Graz. Therefore, the whole study underlies ethical guidelines. All data were gathered in an excel sheet and the statistical evaluation was then performed in SSPS by IBM.

The main statistical elements of our study are median, mean, minimum and maximum values, as well as the standard deviation. We compared pre and post examination data with the Wilcoxon Test for combined samples, due to our related study cohort (pre/post examinations of the same study group).

The gathered data was acquired in two parts. Part one was image processing, the other part contains various data samples which were gathered from slit-lamp examination. The core element of the image processing is the meibography, an infrared examination of the eyelids. The used scoring systems for the meibography, as well as the slit lamp data and tests will be explained in the following pages.

Meibography

As mentioned above, the meibography is an infrared imaging tool for ophthalmologists. It is used for detailed presentation of the eyelids and more precisely the meibomian glands. The glands are visualized in a high quality, this allows their grading for atrophy, destruction, tortuosity and therefore enables the grading of the meibomian gland morphological status. The examination itself is fast, non-invasive, and painless.

It can also be used for the differentiation between EDE and ADE, due to the fact that a pure ADDE consists rather of tear loss, instead of the hyper-evaporation and the meibomian gland destruction in EDE. It enables a more specific diagnosis and therefore a more specific therapy. However, it should also be stated that the

connection between glandular dropout area and the severity of MGD is not adequately researched yet [76].

Technical aspects: The meibography is a contactless infrared imaging of the eyelids. The eyelids must be everted. Topical anesthetics, fluorescein staining or mydriatic/miotic eyedrops were not required in the conducted examinations. An OCULUS Keratograph 5M (Oculus GmbH, Wetzlar, Germany) was used in this study. The working distance is between 78 and 100 mm, with an achieved accuracy of +/-1 diopter. The light source is infrared (IR) light with a wavelength of 840 nm. The image itself is taken with a Digital CCD camera.

Grading score: The morphology is evaluated and classified. This score refers to the loss of meibomian glands [77].

- 0 = no loss of glands
- 1 = loss of glands below 33% of the total glandular area
- 2 = loss of glands 33-67%
- 3 = loss of glands above 67%

Another aspect, which is objectifiable via meibography is the glandular distortion. This score refers to the tortuosity of the meibomian glands:

- 0 = no glandular distortion
- 1 = up to 4 glands show a distortion (a distortion above 45° has to be rated as positive)
- 2 = 5 or more glands show a distortion

Slit lamp

The slit lamp is the standard examination tool for the ophthalmologist. The dry eye unit of the Ophthalmological Clinic, Medical University Graz uses regular slit lamps. All patients were examined with an BQ-900® LED powered slit lamp by Haag-Streit. The attached optical unit is a Galilean microscope, which offers an operating magnification ranging from 6,3x, 10x, 16x, 25x and 40x.

The attached light source is a LED, this leads to a homogeneous illuminated area. Filters for the BQ-900 slit lamp reach from blue, green, grey (10%) to yellow.

Various eye related diagnostical tests can be conducted with the slit lamp. Following tests and scores are involved in this study.

Break-up Time (BUT)

The *break-up time* indicates the time between the last complete or incomplete blink and the start of the break-up of the tear film. This diagnostical method can either be viewed manually with a slit lamp or automated with an Oculus Keratograph-5M.

For the manual method, 1 µl of a preservative-free fluorescein sodium 1% solution (Minims, Bausch&Lomb Pharmaceuticals, Brussels) is added to the ocular surface. The patient is now encouraged to blink, this spreads the fluorescent agent over the ocular surface area. The next step consists of counting the time between the last blink and the first sighting of tear film break-up, therefore the examiner must use a cobalt blue filter. The occurring dry spots of the tear film can now be viewed as growing dark areas/spots in slit light examination. The standard magnification is set to 10x for a better overall view over the corneal area. The time is measured with a stopwatch. The time measurement should be repeated three times. The average of these measurements is the representing value.

Scoring:

- An average score <5 seconds indicates a pathological condition of the tear film.

Corneal-grading score:

Different versions of this score exist, e.g., the "*Oxford" score* and the "*NEI"-score*. Since its higher reliability and repeatability, the NEI score is more often used than the Oxford score [78].

In this investigation 1 µl of a preservative-free fluorescein sodium 1% solution is applied to the ocular surface.

The applied fluorescein detects corneal epithelial defects. These adhesions, also called spots or stains. When illuminated with a blue light source, the emitted color spectrum changes to green. This process can be viewed and graded with a slit lamp.

NEI grading score

The corneal surface is divided into 5 parts. A nasal, central and temporal part, as well as a part superior and inferior to the central area. Each compartment can be graded with a score ranging from 0-3 [79]

1 = mild staining

2 = moderate staining

3 = dense or confluent staining

The scores of all five parts are added together. The total NEI score ranging from 0-15 and represents the overall corneal damage.

Schirmer test

Schirmer test is named after Otto Schirmer. It is used for the diagnosis of insufficient tear production. Produced secretions of the lacrimal glands are measured and can be graded. The procedure consists of the application of an paper strip (sterile Clement Clarke Schirmer Tear Test) between the lower eyelid and the conjunctiva. Those strips are standardized to 35x5 mm. After the insertion, the patient has to close the eyes for 5 minutes. During this time, the tear fluid will spread over the paper strip. The length of the wetted area will then be measured.

- ≤10 mm = pathological low secretion rate
- >10 mm = physiological secretion rate

The lower the score (in mm), the more severe is the decrease of the secretion rate. In specific cases patients need a certain stimulus to produce a sufficient amount of tear film. This stimulus can be a mechanical manipulation of the nasal mucosa with a cotton-wool tip. The application of this extra stimulus can trigger a physiological secretion rate. This test is called Schirmer test with nasal stimulation.

van Bijsterveld score (vBS)

The *van Bijsterveld score* [80] is a scoring system developed to grade different states of ocular surface damage.

For the grading of the ocular surface lissamine green, an organic dye, which can be used as an indicator for damage of the ocular surface, is applicated via wet strips (1 drop of NaCl had to be added) to the ocular surface. Lissamine green ophthalmic strips /Green Glo (HUB Pharmaceuticals, LLC, Rancho Cucamonga, CA) were used in this study, each strip is impregnated with 1,5mg of lissamine green. The light source should be set to white light, an additional use of a red-light filter is optional. Magnification should be set to a low magnifying factor.

The classified areas are a nasal and temporal conjunctival division and a central corneal division, each rewarded with a maximum score of 3 points:

0 = no spots

1 = sparsely scattered surface

2 = densely scattered surface

3 = confluent spots

A total score of 9 points can be reached. Values over 4 or more have to be interpreted as positive and promote the diagnosis of ocular surface damage. The points awarded by the vBS are semiquantitative.

Marx line

The *Marx line* marks the border of the mucocutaneous junction. The Marx line is evaluated after the lissamine green staining of the ocular surface. The course of the Marx line, as well as its anterior or posterior displacement are main subjects of the Marx line score [81].

0 = the Marx line is located posterior to the meibomian orifices

1 = parts of the Marx line reach some of the meibomian orifices

2 = the Marx line tangents two or more of the meibomian orifices

3 = the complete Marx line is located anterior to the meibomian orifices

The Marx line is graded in the temporal, central and nasal part for the upper and lower eyelid. The three parts are summed which creates a total score reaching from 0-9. The average score of the lower and upper eyelid is listed for the left and right eye.

Vascularization score

The *vascularization score* (*VASC score*) of the lid margin can be increased in symptomatic KCS or MGD patients. Due to the chronical inflammation the blood circulation in the supplying blood vessels is increased and a progredient vascular wall damage proceed on the lid margins.

These changes are visible as increased tortuosity of blood vessels and the presence of telangiectasia. The following grading score was used:

0 = no increased vascular injection of the lid margin

1 = mild vascular injections

2 = moderate vascular injections

3 = highly increased vascular injection

The VASC score displays the average vascular injection of the upper and lower lid margin of each eye.

Quantity and quality of the meibum

The secretion of the meibomian glands can be expressed during slit light examinations. The orifices get stretched using a cotton-wool tip. This promotes the expression of meibum. Magnification should be set to 10x to enable an enhanced evaluation.

The characteristics of the meibum can be graded in two categories: Quality and quantity. Each category has a maximum score of 3 points.

Quantity grading [82]:

0 = normal level of meibum expression

1 = reduced expression of meibum

2 = marginal expression of meibum

3 = no expression of meibum

Quality grading [83]:

0 = clear meibomian secrete

1 = milky meibomian secrete

2 = granulating meibomian secrete

3 = tooth paste like meibomian secrete

(In the case that the expression of meibum is not possible the quality section is graded with 4 points to enable a facilitate statistical analysis).

OSDI

The *Ocular Surface Index* (OSDI) [84] is a scoring system, originally developed by Schiffmann et. al to access the subjective grade of DED. In total 12 questions have to be answered. The answers are valued with points from 0-4. The addressed themes are environmental issues, problems with everyday activities, an impaired vision and ocular problems. The achieved score indexes with the number of answered questions.

The total score can reach from 0-100. A higher score correlates with an increased severity of DED [85].

Normal = 0 to 12

Mild = 13 to 22

Moderate = 23 to 32

Severe = 33 to 100

Visual analogue scale

The *Visual analogue scale (VAS)* is a scoring system which grades the subjective medical conditions and the thereupon associated subjective impairment of the patient. The patients have to use a linear scale ranging from 0-100 to grade their general symptoms and their major symptom.

The marked value represents the applying score in points.

0 = no symptoms and impairment at all

1 to 99 = a linear increase of severity

100 = subjective most severe condition

The average score of both general and main symptoms is calculated and provides more detailed information about the overall grade of subjective restriction of the patient. The minimum score is 0 points, the maximum score can reach up to 100 points.

Cyclosporine A administration

CsA was used in different forms in this study.

A magistral administration which was either CsA mixed in oil (primary peanut-oil) or CsA as an emulsion.

Restasis, an anionic CsA medication approved by the FDA in 2003 and Ikervis, a cationic formulation, which was officially approved by the EMA in 2015 for the treatment of adult patients suffering from DED with severe keratitis.

All CsA applications had to be used at least once a day (usually in the evening) and continuedly applied for minimum one year.

Results

The study was conducted in 14 patients suffering from SS. 12 (85.7%) patients were female, and 2 (14.3%) were male. At the time of the first CsA application, the median age was 61, with a range of 42 years to 88 years.

The follow-up between the first and the last examination had a median duration of 4.0 years. The minimum follow-up duration was 1.6 years, whereas the maximum was 7.5 years.

Out of the 14 patients, 12 were diagnosed with pSS and 2 with sSS (*Figure 5*). All 2 sSS cases were female, with CREST.

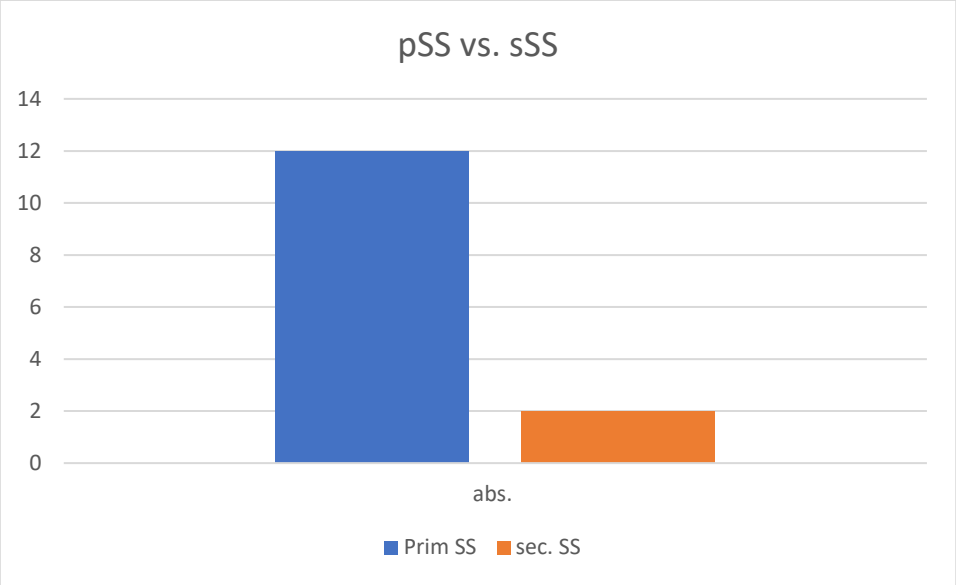

Figure 5 - Distribution of pSS vs. sSS

Eleven patients (78.6%) began their therapy using a CsA emulsion/peanut-oil solution. 2 patients (14.3%) were treated with Restasis and 1 patient (7.1%) with Ikervis. In contrast to this, at the last examination 6 patients (42.9%) had a prescribed CsA emulsion/peanut-oil solution. Restasis users were reduced to 1 (7.1%). The number of patients medicated with Ikervis increased to 7 (50.0%). (*Figure 6*)

All patients used preservative-free dry eye drops as an additional therapy during the whole study period. The number of patients applying lid margin massage changed from 7 (pre) to 11 (post), albumin eye drop users from 1 to 4 patients.

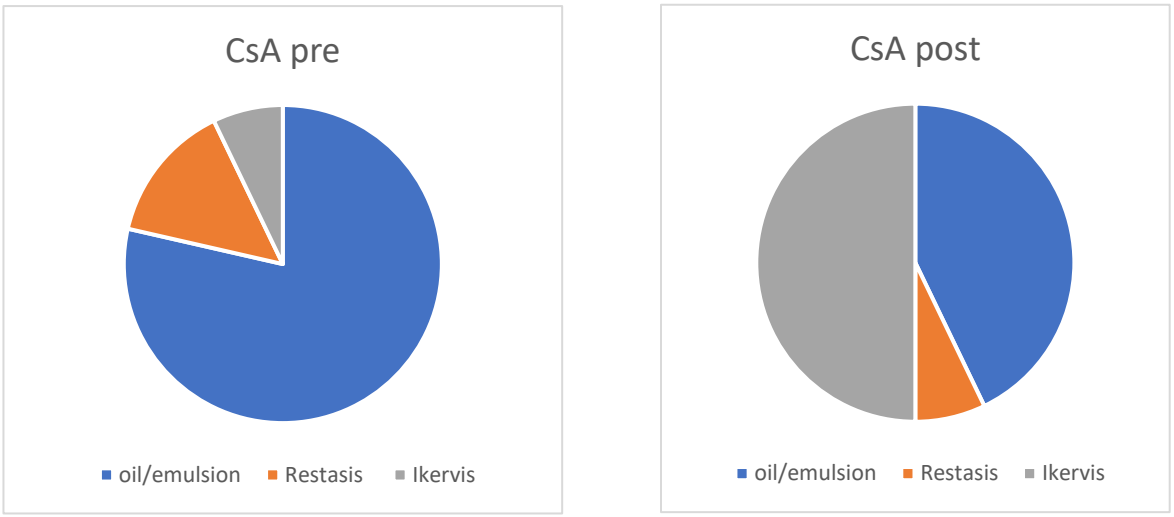

Figure 6 - Distribution of the used CsA medication (Cyclosporine A peanut-oil/emulsion, Restasis or Ikervis) at baseline (pre) and follow-up (post).

Dropout and tortuosity of the meibomian glands, Marx line, VASC score and Meibum expression:

As seen in *Table 3*, under a continuous CsA therapy the *meibomian gland dropout* levels were unchanged (p=0.943). The same applies to the *tortuosity* levels. Although it improved from 0.80 ±0.44 to 0.71 ±0.48, no significance was given (p=0.517), meaning that the CsA application could not change the objective grading parameters of the meibography in our study cohort. (*Figure 7*)

Table 3 - Meibomian gland scoring *- Differences between the baseline and follow-up scores of the Marx line, DO=the meibomian gland Drop Out, TUO=meibomian gland Tortuosity, and EXP=exprimation of meibum, VASC=vascularization of the eye lid.*

	Average	standard deviation	Median	Significance
MARXpre	4.42	2.20	4.13	0.285
MARXpost	5.18	1.92	5.50	
DOpre	1.32	0.68	1.13	0.943
DOpost	1.38	0.70	1.38	
TUOpre	0.80	0.44	0.75	0.517
TUOpost	0.71	0.48	0.75	
EXPpre	2.35	0.50	2.38	0.025
EXPpost	1.98	0.52	2.00	
VASCpre	1.94	0.65	2.00	0.395
VASCpost	1.75	0.77	2.00	

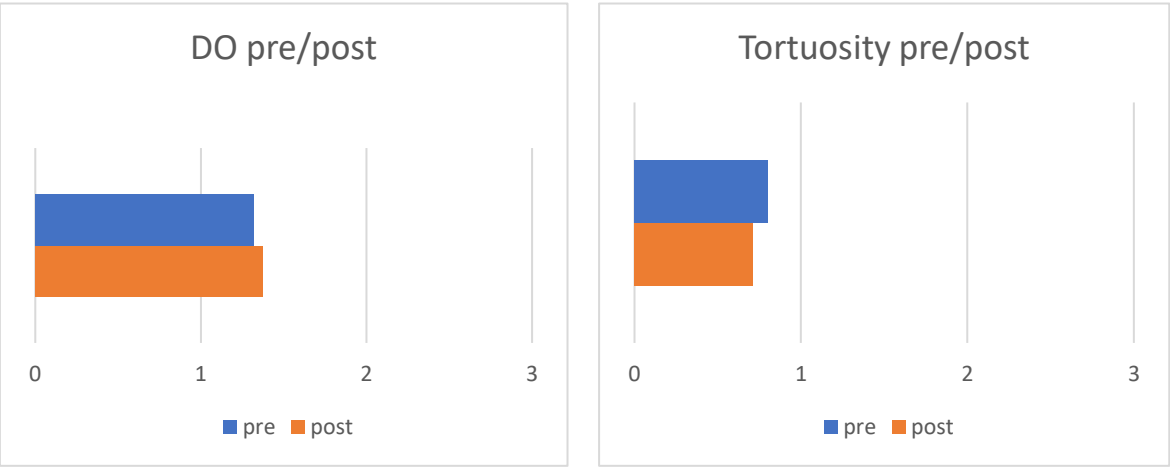

Figure 7 - Changes in the dropout levels (DO) and the tortuosity of the meibomian glands pre and after CsA treatment.

A more detailed breakdown of the meibography scores (*Figure 8*) shows the minimal improvements of the right eye, the stable upper tarsus of the left eye, and a noticeable worse lower tarsus (the dropout score changed from 1.00 to 1.46) on the left eye.

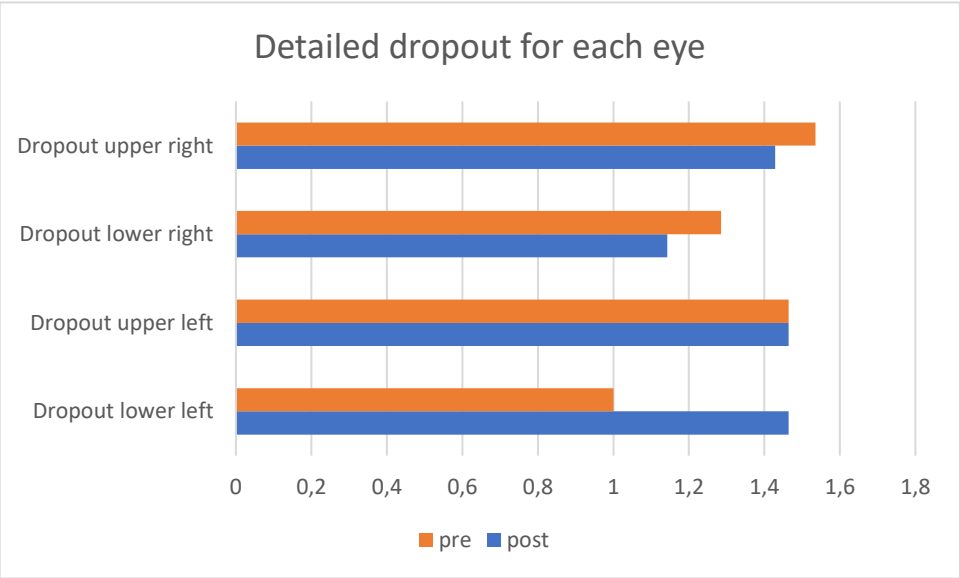

Figure 8 - Detailed distribution of the dropout levels for each eyelid at baseline and at follow-up.

As seen in Table 3 the *Marx line* (p=0.285) and *VASC score* (p=0.395) remained stable prior and after CsA.

A significant change could be seen in the pre/post differences of the quantity level of the expression of the meibomian glands (Table 3). Prior to the first CsA application, an average score of 2.35±0.50 was documented, whereas, after the last CsA application, the average score improved to 1.98±0.52 with p=0.025 (*Figure 9*). This lines along with the enhanced quality level of the expressed meibum. At baseline, 16 out of 32 eyelids showed no expression compared to 6 out of 32 eyelids at the end of the study.

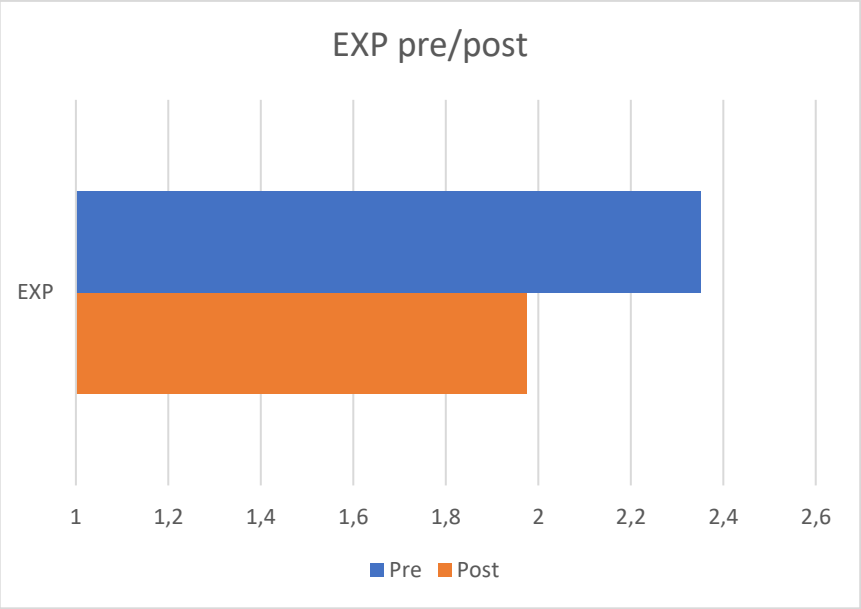

Figure 9 - Changes of the meibum expression levels of the meibomian glands at baseline and follow-up.

It should be stated, that the meibum quality levels of the patients could not be compared directly, 6 out of 14 patients did not have valid patient data and 16 out of 32 eyelids did not show pre-expression scores and therefore, could not be used for a quantitative pre/post comparison.

Slit-lamp examinations including corneal and tear grading scores (NEI, vBS, BUT and Schirmer):

Pre- and post-data results of the NEI, vBS, BUT and Schirmer test are relatively stable and showed no significant changes; this can also be seen in Table 4 and Figure 10.

Table 4 - Objective parameters *- Difference between the baseline and follow-up scores (NEI, BUT=Break-up Time, Schirmer test, vBS=van Bijsterveld Score).*

	Average	standard deviation	Median	Significance
NEIpre	6.57	5.32	4.88	0.900
NEIpost	6.05	2.71	6.06	
BUTpre	2.17	0.98	2.00	0.666
BUTpost	2.21	1.21	2.42	
SCHIRMERpre	2.46	2.14	1.50	0.501
SCHIRMERpost	2.10	2.12	1.50	
VBSpre	5.99	2.09	5.75	0.850
VBSpost	5.84	2.14	6.13	

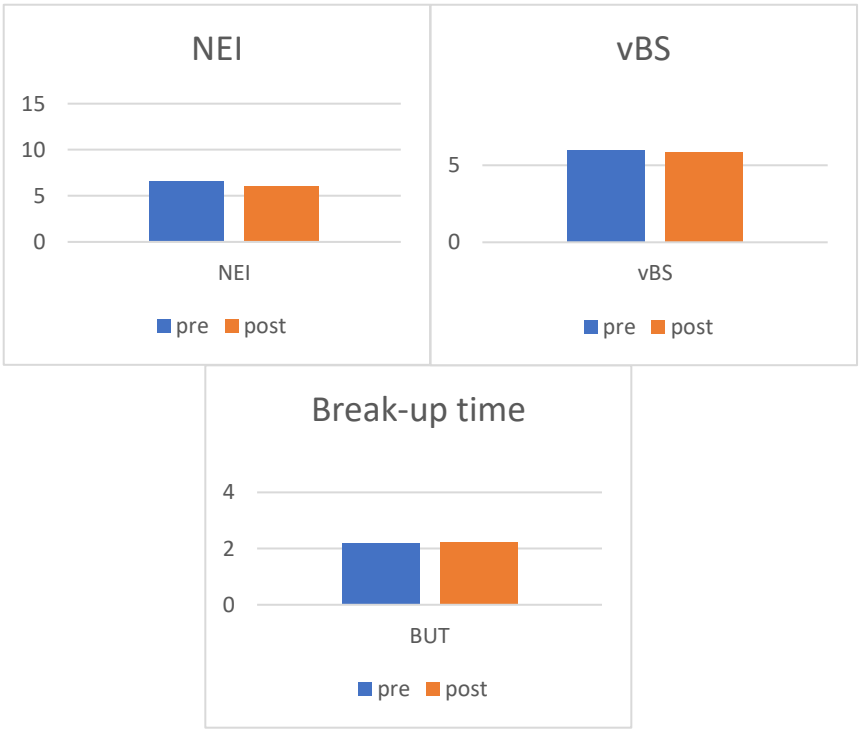

Figure 10 - NEI, van Bijsterveld Score (vBS) and Break-up time differences at baseline and follow-up.

Subjective patient scores (OSDI and VAS):

The ocular surface disease index showed no significant change (52.42±17.56 to 44.84±20.03 points). The visual analog scale changed from 49.15±24.28 to 46.14±16.63 points. (Table 5)

No significance could be reached for the OSDI (OSDI p=0.875) However, the VAS showed a tendency of improvement (VAS p=0.132). (Table 5 and Figure 11)

Table 5 - Subjective patient parameters *- Differences of the VAS=visual analogue score and the OSDI=ocular surface disease index at baseline and the follow-up.*

	Average	standard deviation	Median	Significance
VASpre	49.15	24.28	46.87	0.132
VASpost	46.14	16.60	47.72	
OSDIpre	52.42	17.56	55.00	0.875
OSDIpost	44.84	20.03	50.00	

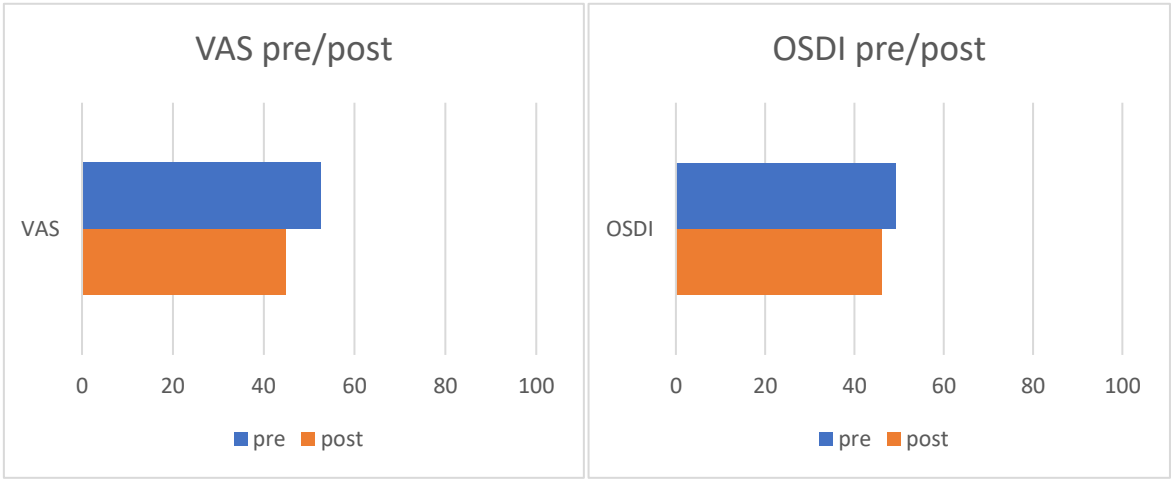

Figure 11 - The pre and post comparison of both the VAS=visual analogue scale and the OSDI=ocular surface disease index.

Discussion

This retrospective study shows that a continuous topical CsA therapy does not lead to significant changes in the morphology of the meibomian glands. Patients receiving CsA eye drops do not show significantly improved or worsened conditions. Even though there was no significant improvement in the meibography, there was no deterioration as well.

As already described in Table 3, the *dropout level* remained stable due to an insignificant p-value. The therapy with CsA did not influence the meibomian gland dropout level induced by both primary and secondary SS.

The minimal change from 1.32 to 1.37 may be explained by age-related changes in this small study population. A larger study group and further DE parameters like the osmolarity of the tear film, impression cytology etc. would have given further information.

The fact that the *tortuosity* showed a minimal improvement from 0.80+- 0.44 to 0.71+- 0.48 can be explained due to the minimal worsening of the glandular dropout. Shorter glandular ducts mean that there is less room for tortuosity, resulting in a decreased tortuosity score. In addition to the stable meibography scores (p=0.943), the insignificant changes in the tortuosity (p=0.517) should also be interpreted as a sign for a stable condition.

Interestingly, patients in our study group showed fewer signs of subjective discomfort. This can be seen in *Figure* 11. Although the *VAS* (p=0.132) showed no statistical significance, there was a tendency of improvement. Compared to this, the OSDI was unchanged (p=0.875). A larger study group with a prospective study design could provide clarification in both cases. Moreover, even if there are no significant improvements, our results can assist the fact that CsA may stabilize the subjective symptoms of patients and the progression of the ocular surface damage due to the underlying pathological condition in SS.

However, the bias of missing patient data should also be considered, due to the strict exclusion criteria not all patients could be included. This is particularly critical for patients, who did not apply their prescribed CsA therapy continuously for over

one year. Therefore, only patients who are reliant to a continuous CsA medication were part of this study.

Sjögren Syndrome induced DED is a condition that cannot be cured at the moment. The main goal is to achieve reduced or at least stabilized symptoms and enhanced subjective comfort. Reduced VAS scores could indicate that the situation on the ocular surface has improved or at least stabilized. The insignificantly altered objective markers of the ocular surface (NEI score, vBS, BUT) confirmed that our study did not show a deterioration under a continuous CsA therapy. However, at this point it should be restated that there was no control group without the usage of CsA.

A finding of this study which should be highlighted is the significant change in the expression levels of the meibomian glands ($p=0,025$). Increased expression levels can mean that the applied CsA in combination with the additional DED therapy, had potentially reduced the hyper-keratinization of the ducts and reopened the pathologically obstructed meibomian gland orifices. This could assist in stopping the progression of the glandular dropout and the relatively stable ocular surface.

A point that should also be mentioned is the possible positive effects of the additional applied therapies (like unpreserved eye drops and the regularly use of lid margin massage). Our findings show that a topical CsA therapy, combined with those additional therapies can improve meibum expression. This could be due to the applied warm compresses. Warmth can dissolve the stasis of the meibum and enhance the quantity and quality of the expressed secretion.

Most patients had received the recommendation to perform lid margin massage (11 out of 14) and all patients used preservative free dry eye drops throughout the study. The unpreserved eyedrops could also aid in reducing the inflammation on the ocular surface and the connected irritation of the meibomian glands. This could also lead to a reopening of the orifices of the meibomian glands and an improved secretion.

However, the addition of both lid massage and lubricants could enhance the positive effect of CsA on the ocular surface. Even if eye drops and lid margin therapies themselves do not inhibit the auto-immune genesis, inflammatory reactions can be alleviated, and corneal healing can be induced.

Due to the retrospective design the exact extents of these additional therapies could not be determined. A control group with a sole CsA therapy compared to a CsA therapy combined with lid margin massage, and unpreserved artificial tear applications would show more detailed results.

After all, the positive effect of a topical CsA therapy should not be negated. Ho-Yun Kim et. al showed in their retrospective analysis that the usage of CsA could potentially increase subjective, as well as objective DE parameters [86]. This lines along with Pinnita Prabhasawat et al. randomized double-masked study from 2012 with significantly enhanced follow-up scores after the topical usage of CsA [87]. However, this is in direct contrast with our results, which were unchanged with insignificant p-values for most objective and subjective patient parameters. At this point it should be mentioned that our study cohort was relatively small and could therefore lead to a higher insignificance. Furthermore, the retrospective design in combination with the bias of missing or excluded patient data, could decrease our significance levels. Nevertheless, the biggest limitation of the mentioned follow-up studies was the relative short follow up period of 3 month and the missing meibography data.

CsA could therefore significantly enhance subjective and objective patient parameters during a shorter follow up period and according to our study data, it could be possible that CsA provides a stabilization of these parameters and the disease in the long run.

A limitation of our study is the already described small study group and the retrospective study design. A larger study cohort and a control group would have been needed for more detailed results for the usage of CsA. Although SS is a widespread disease, only 14 patients of the archive of the dry eye unit were left to meet the study´s inclusion criteria.

Both, a time coherent meibography with a slit lamp examination and a continuous CsA application are needed to be included. The meibography often does not have timing proximity with the induction of the CsA therapy. Therefore, patients with a meibography 3 months prior or after the first application of CsA had to be marked as falsified data sets and could not be part of the study group. Another major point

are missing meibographies. A meibography in combination with a time-related slit-lamp examination was not always existent for all patients. Nowadays, a meibography is part of the standard procedure of the dry eye unit. In 2012 and before, not all patients were examined with the keratograph 5M, meaning that CsA was initiated without an infrared picture of the meibomian glands. Leading to the fact that there is no documented baseline condition of the meibomian glands. Therefore, changes could not be validated or graded.

Patients with an adverse reaction towards the use of CsA or a non-tolerated CsA therapy (ocular burning or itching) were also excluded. This also applied to patients, with mild cases of SS, which received a CsA therapy for less than a year. Resulting in the fact, that our study mainly included moderate or severe cases of pSS or sSS. Potential significant changes in the meibography could therefore still be possible for patients with mild cases of SS.

Another weakness is the examiner bias. Although there are official grading scores, the subjective reception of qualitative graduation could lead to altered scores between examiners. One way to prevent this could be the usage of quantitative scores or the utilization of automated examination processes.

One way to receive more detailed results could be the usage of artificial intelligence (AI) for enhanced grading. Recent developments in deep learning enable the possibility of a computer-aided/AI-aided classification. Wang et al. developed a digital grading program that graded atrophic regions with a meiboscore accuracy of 95.6%; however, the identification and specific scoring of individual morphological characteristics were insufficient [89].

A benefit of using AI-aided grading would be the related scoring method. As already described, the Arita classification is ranging from 0-3, with no intermediary steps. In the case of borderline scores, this can lead to an increased inaccuracy. AI can grade the percentage of the dropout area and, therefore, enables more detailed and comparable results. Using an AI-aided scoring method could help in the question of whether a CsA therapy can enhance changes of the meibomian glands or not.

A problem could occur since the conducted images must be assessed in a specific way. The upper and lower eyelids have to be everted in total to receive an optimal

image quality (complete infrared representation of the inner tarsus). This quality is needed for a reliable and comparable grading. Patient records in the archive reach back up to 20 years; therefore, not all images reach the guidelines for AI-aided grading.

Another score that could be improved is the measurement of the break-up time. The break-up time was measured with stopwatches and the application of fluorescein. The subjective reaction time of each examiner can vary. This could potentially lead to falsified break-up times. Furthermore, no further details about the area of increased break-up are documented in the slit-lamp examination. The automated method with the Oculus Keratograph 5M is done without fluorescein instillation. The Keratograph creates a reflection of multiple round spheres on the corneal surface. During the process of evaporation, the reflection alters. The time between the last blink and the start of the reflecting alterations is measured.

The keratography 5M shows a detailed break-up map of the ocular tear film and a corresponding timeline. The integration of frequent uses of the Keratograph 5M for BUT could lead to more standardized values.

Another advantage in the usage of the Oculus Keratograph 5M comprises additional conducted examinations, for example the measurement of the tear meniscus height or conjunctival redness. Therefore, standardizing the usage of the Keratograph 5M also contributes to an easier access for additional examinations and more information about the ocular health of the patients.

To conclude, a scheduled CsA therapy for patients diagnosed with KCS and SS is still the therapy of choice in severe cases. Our findings show that the meibography results, like glandular dropout and tortuosity, remain stable. This lines up with the subjective and objective ocular parameters, which were also unchanged, except that the expression of meibum was significantly increased with the applied therapies. CsA stabilize the damage of the ocular surface and could decelerate the progression of MGD. Additional therapies like eyelid massage and artificial tears also contribute to the positive effects of CsA. This leads to the recommendation that a CsA therapy should be complemented with the mentioned additional therapeutic options. Regular examinations are also required to ensure an optimal treatment. Regarding to this

and due to its comparability, the meibography should be a part of the follow up for DED patients, including MGD and SS.

Glossary

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