New Perspectives of Ocular Surface Evaluation in Primary Sjögren's Syndrome

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ABSTRACT

Introduction: The diagnosis of Primary Sjögren's Syndrome (pSS) is sometimes challenging, taking into account the clinical heterogeneity and the variability of diagnostic criteria fulfillment. This study aims to evaluate the tear meniscus (TM) and corneal sub-basal nerve plexus (SBNP) in a population with pSS.

Material and Methods: Cross-sectional study of 50 patients with pSS and 20 healthy controls. Conventional dry-eye disease evaluation was performed, using Schirmer test I without anaesthesia, tear break-up time and corneal fluorescein staining. TM height and area were analyzed using anterior segment optical coherence tomography (AS-OCT) and corneal SBNP was evaluated using *in vivo* Confocal Microscopy (IVCM), with study of nerve density, length and tortuosity. Differences in pSS ophthalmic features were analyzed according to phenotype profile. Data analysis was conducted by IBM-SPSS Statistics 25.0.

Results: TM height was significantly lower in pSS in contrast to the controls ($p < 0.001$). Corneal SBNP density and length were significantly lower and tortuosity significantly higher in pSS patients comparing to controls ($p < 0.001$, $p = 0.004$ and $p = 0.001$, respectively).

Conclusion: AS-OCT and IVCM may be useful in the evaluation of lachrymal unit dysfunction in pSS, complementing the conventional methods used in the evaluation of dry-eye disease.

RESUMO

Intrudução: O diagnóstico de Síndrome de Sjögren's Primário (SSp) é, por vezes, desafiador, atendendo à variabilidade da sintomatologia e do cumprimento dos critérios diagnósticos. Este estudo tem como objectivo a avaliação do menisco do lacrimal (ML) e plexo do nervoso sub-basal da córnea (PNSB) na SSp.

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Materais e Métodos: Estudo transversal de 50 doentes com SSp e 20 controlos saudáveis. Foram realizados o teste de Schirmer I, BUT e coloração corneana com fluoresceína como avaliação convencional de síndrome de olho seco. O ML foi analisado quanto à altura e área, através da tomografia de coerência óptica de segmento anterior (OCT-SA); e o PNSB da córnea foi avaliado por Microscopia Confocal *in vivo* (MCIV), com estudo da densidade, comprimento e tortuosidade nervosa. A análise estatísticas foi conduzida IBM-SPSS Statistics 25.0.

Resultados: A altura do ML revelou-se significativamente menor nos doentes com SSp comparativamente ao grupo controle. O PNSB da córnea revelou uma densidade e comprimento significativamente menores e tortuosidade significativamente maior nos doente com SSp comparativamente ao grupo controle (p <0,001).

Conclusão: O OCT-SA e a MCIV podem ser úteis na avaliação da disfunção da unidade funcional lacrimal na SSp, complementando a metodologia convencional utilizada na avaliação da síndrome de olho seco.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a chronic progressive autoimmune disease characterized by lymphocytic infiltration, damage and consequent loss of function of the exocrine glands.[1] The combination of xerostomia, xerophthalmia, fatigue and joint pain is the characteristic presentation form of this exocrinopathy, which affects mainly caucasian women, beginning around the fourth to fifth decade of life.[2]

Ocular symptoms and signs are preponderant points of the American–European Consensus Group (AECG) criteria for pSS classification,[3] the most widely used since 2002. However, the two ocular objective signs considered in these criteria, namely Schirmer's test I and ocular staining score, are not perfect for diagnosis, since they have low to moderate reproducibility in assessing dry-eye disease and might be insufficient in differentiating pSS from other dry-eye conditions, as well as to assess disease severity and activity.[4–10]

In this sense, the interest in developing novel objective and reproducible tools for pSS assessment is growing.[9,11,12] Regarding the aqueous tear deficiency and the resulting ocular surface damage associated with pSS dry-eye, the objective evaluation of tear meniscus (TM), using anterior segment optical coherence tomography (AS-OCT) and corneal sub-basal nerve plexus (SBNP) by *in vivo* Confocal Microscopy (IVCM), may reveal important information about the status of the ocular surface of these patients.[11,13–16]

Therefore, in this study, we aimed to analyse the usefulness of TM AS-OCT and SBNP-IVCM in detecting pSS, as well as in distinguishing pSS patients according to disease activity, immunological and histological profiles.

METHODS

Study design and Population

Data were collected from a cross-sectional study of fifty patients with pSS and twenty healthy volunteer women.

pSS was confirmed following the AECG criteria,[3] and patients were considered to have clinical active disease if they scored in any domain of the clinical European League Against Rheumatism-(EULAR) Sjögren's Syndrome Disease Activity Index (ClinESSDAI). The ClinESSDAI was developed by removing the Biological domain from ESSDAI (C3 and C4 complement factors, gammaglobulin and cryoglobulins) and intends to be independent from B-cell derived biomarkers.[17] However, there seems to be a high concordance between both indexes.[18]

The exclusion criteria proposed by the AECG were applied to all subjects in study, as well as other systemic or organ-specific autoimmune disease; history of IgG4-related

disease; neurodegenerative diseases; treatment with drugs of known corneal toxicity or xerogenic effect; eyelid malposition or movement disorders; lacrimal drainage pathway obstruction; ocular surface diseases other than DED; history of previous corneal LASER procedures, refractive, glaucoma or retinal surgeries; contact lens wear within 2 weeks from the ophthalmic evaluation. Additionally, patients were instructed not to use artificial tears within eight hours before their ophthalmic evaluation.

Healthy volunteers, without any sicca symptoms or signs, fulfilling the eligibility criteria, constituted the control group.

A complete ophthalmologic (described in the next section) was performed. Demographic, clinical and laboratorial data were collected from the clinical files, upon which the ClinESSDAI was assessed. Written informed consent has been obtained from all subjects prior to their participation in the study. Approval by the Institutional Ethics' committees was obtained and the tenets of the Declaration of Helsinki were accomplished.

Dry Eye Assessment

All the subjects were evaluated by the same examiner, who was unaware of subject condition, in the same examination room, under standardized conditions of low illumination and temperature (20 - 25°C).[19] Both eyes were examined, however, only the eye recording the highest corneal staining score (CSS) or, in case of equal CSS, the lower score on Schirmer's test I, was selected for statistical analysis.

Firstly, TM was assessed using Visante® OCT (Carl Zeiss Meditec, Dublin, CA), as described by Ibrahim et al.[15] TM scans were captured with a vertical beam, set at a 90° angle, in the protocol "AC Biometry PreOp" and posteriorly lower tear meniscus images were analysed concerning to height and area. Lower TM height (TMH) was manually measured using the software callipers of the device, corresponding to the vertical line formed by the union of the touch points between TM and the corneal surface and the lid margin. TM area (TMA) was measured using Image J software (Java software program developed by the National Institutes of Health, Bethesda, MD). Three images were analysed and the mean value was obtained.

Then, Schirmer's test I without anaesthesia and tear break up time (BUT) were evaluated has previously

defined[8] and CSS was assessed using only fluorescein and graded in six groups according to severity, based on corneal surface staining of the Oxford grading scale: 0 - absent, I - minimal, II - mild, III - moderate, IV - marked and V – severe.[20]

Finally, corneal SBNP - IVCM (Heidelberg® Retina Tomograph III, Rostock Cornea Module) was performed, according to the protocol established by Tavakoli et al.[21] SBNP was characterized in terms of nerve density, length and tortuosity. Nerve fibre density was defined as the total number of major nerves per square millimetre of corneal tissue; nerve fibre length, was defined as the total length of all nerve fibres and branches, in mm per square millimetre of corneal tissue;[22] and nerve fibre tortuosity was classified as grade 0 –almost straight; grade 1 - slightly tortuous; grade 2 –moderately tortuous; grade 3- quite tortuous; Grade 4 - very tortuous.[23]

Data analysis

A descriptive analysis of patients' demographic and immunological features was performed. Frequency analyses were also conducted to assess the distribution of the variables and the non-responses. The comparison between the group of patients and the control group was tested by the Independent Samples t-Test *t* and the Median test. The Independent t-test was also used to examine differences on ophthalmic features according to the disease activity, histological and immunological dichotomous profiles. A categorical multiple regression (CATREG) was done because it was necessary to accommodate categorical and quantitative independent and dependent variables in small samples.[24,25] Data analysis was conducted by IBM-SPSS Statistics 25.0.

RESULTS

Fifty patients with a mean age of 58.2 years (SD = 11.9 years) were included in the pSS group. Twenty subjects with a mean age of 50.9 years (SD = 6.5 years) were included in the control group. All the subjects were female. The characteristics of pSS patients are reported in Table 1.

Table 1 - Patients' Demographic and Immunological Features

	pSS (N=50)
Age (years) [a]	58.2 (11.9)
Gender (Female) (%)	100.0%
Symptoms Onset (years) [a]	46.0 (11.4)
Diagnosis (years) [a]	51.7 (11.8)
Xerostomia, n (%)	48 (96.0%)
Xerophthalmia, n (%)	47 (94.0%)
Minor salivary gland biopsy (FS≥ 1), n (%) [c]	37 (77.1%)
Unstimulated salivary flow (<0.1 ml/min) [c]	20 (71.4%)
Abnormal Submaxillary gland scintigraphy [b e]	2.0
Abnormal Parotid scintigraphy [b e]	2.0
Extraglandular disease ever	21 (42.0%)
Clinical active disease	23 (46.0%)
Anti-Ro/SSA, n (%)	33 (66.0%)
Anti-La/SSB, n (%) [f]	16 (35.6%)
ANA, n (%)	46 (92.0%)
Rheumatoid factor, n (%) [g]	22 (51.2%)
Gammaglobulin ≥ 1.6 g/dl, n (%)	11 (22.0%)

pSS –primary Sjögren's syndrome. [a] Mean and standard deviation were reported. [b] Median was reported (ordered variable, ranged from 0 to 4). [c] N valid = 48. [d] N valid = 20. [e] N valid = 25. [f] N valid = 45. [g] N valid = 43

The results obtain from dry-eye assessment are reported in are reported in Table 2. Schirmer's test I reported means were significantly lower in pSS group comparing with controls ($p < 0.001$). The proportion of low BUT was significantly higher in pSS patients in contrast to normal controls ($p < 0.001$). CSS was significantly higher in pSS group in contrast to the control group ($p < 0.001$).

Concerning to TM evaluation, TMH reported means were significantly lower in pSS group in contrast to the controls ($p < 0.001$). No significant differences were found between the two groups regarding TMA.

In turn, corneal sub-basal nerve plexus density and length were significantly lower and tortuosity was significantly higher in pSS group in contrast with the controls ($p < 0.001$, $p = 0.004$ and $p = 0.001$, respectively).

Table 2 - Patients' Ophthalmic Characteristics - Dry-eye assessment profile

	pSS N=50	Control N=20	*P*
Schirmer's test I (mm)[a]	5.9 (5.9)	22.3 (8.3)	< 0.001[c]
TBUT (proportion of Low)	0.70 (0.46)	0.0 (0.0)	< 0.001[c]
CSS [b]	20 | 30	20 | 0	< 0.001[d]
Tear meniscus height (mm) [a]	0.27 (0.21)	0.43 (0.11)	< 0.001[c]
Tear meniscus area (mm²)[a]	0.05 (0.06)	0.06 (0.03)	n.s.
SBNP density (n/mm²) [a]	28.1 (12.4)	43.9 (12.9)	< 0.001[c]
SBNP length (mm/mm²) [a]	10.3 (6.8)	15.4 (5.1)	0.004[c]
SBNP tortuosity [b]	29 | 21	20 | 0	0.001[d]

pSS – Primary Sjögren's syndrome TBUT – Tear break up time; CSS – Corneal staining score; SBNP – Sub-basal nerve plexus; n.s. – non-significant. [a] Mean and standard deviation were reported. [b] Number of cases <= Median | > Median were reported. CSS is an ordered variable ranged from 0 to 3. [c] t-Test (two independent samples). [d] Median test with Monte Carlo estimation

Demographic data of pSS patients according to clinical activity disease, immunological and histological profiles are reported in Table 3, whereas the results obtain from of the comparison between ophthalmic characteristics according to clinical activity disease, immunological and histological profiles are reported in Table 4.

pSS patients with positivity to anti-SSA antibodies presented earlier onset of Sicca complains and were diagnosed at an younger age than SSA-negative patients ($p= 0.058$ and $p= 0.021$, respectively). pSS patients with positive minor salivary gland biopsy, defined as focus score ≥1, were older ($p= 0.029$), presented disease complains later and were diagnosed at an older age, with significant differences compared to pSS patients with negative minor salivary gland biopsy ($p= 0.029$, $p=0.023$ and $p= 0.016$, respectively).

The effect of disease activity on SBNP tortuosity was marginally significant (Beta = 0.24, $p = 0.066$). Patients with clinical active disease showed higher SBNP tortuosity scores (M_{rank} for disease activity += 26.2 and M_{rank} for disease activity – = 24.9).

The effect of SSA on SBNP length was marginally significant (Beta = 0.23, $p = 0.073$). Patients with positive for SSA antibodies showed higher results of SBNP length (M_{SSA+} = 9.25 and M_{SSA-} = 12.3). Finally, the effect of minor salivary gland biopsy on BUT was marginally significant (Beta = 0.28, $p = 0.057$). Patients with negative minor salivary gland biopsy had higher frequency of low BUT (90.9% of patients with FS < 1 had low BUT and that percentage decreased to 62.2% for patients with FS ≥ 1).

Table 3 – Ophthalmic characteristics according to disease activity, immunological and histological profiles

	Disease activity +	Disease activity -	*P*	*SSA+*	*SSA-*	*P*	FS ≥ 1	FS < 1	*P*
	M	**M**		**M**	**M**		**M**	**M**	
Age (years)	56.7	59.5	n.s.	57.1	60.5	n.s.	61.6	49.4	0.029
Symptomsonset (years)	42.5	49.0	0.040	43.8	50.2	0.058	48.5	39.8	0.023
Diagnosis (years)	48.5	54.3	n.s.	48.9	56.9	0.021	54.4	44.8	0.016

Legend: M – Mean; n.s. – non-significant. *Disease activity +* - clinical active disease. *FS* – Focus score.
The independent samples t test was performed to compare means.

Table 4 – Regression results on ophthalmic features according to disease activity, histological and immunological profiles

		Schirmer's test I (mm)		TBUT		CSS		TMH (mm)		TMA (mm²)		SBNP density (n/mm²)		SBNP length (mm/mm²)		SBNP tortuosity	
		Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*	Beta	*P*
Model 1	Age a	-0.01	n.s.	-0.08	n.s.	0.14	n.s.	-0.04	n.s.	-0.01	n.s.	-0.20	n.s.	-0.07	n.s.	-0.01	n.s.
	Disease duration (years) a	-0.13	n.s.	-0.03	n.s.	0.19	n.s.	-0.09	n.s.	-0.04	n.s.	-0.04	n.s.	-0.01	n.s.	0.17	n.s.
	Disease activity	0.15	n.s.	0.16	n.s.	0.04	n.s.	0.21	n.s.	0.02	n.s.	0.07	n.s.	0.13	n.s.	0.24	0.066
Model 2	Age [a]	0.02	n.s.	-0.13	n.s.	0.15	n.s.	-0.04	n.s.	-0.02	n.s.	-0.23	n.s.	-0.09	n.s.	0.10	n.s.
	Disease duration (years) [a]	-0.17	n.s.	0.04	n.s.	0.18	n.s.	-0.11	n.s.	0.05	n.s.	0.00	n.s.	0.01	n.s.	0.20	n.s.
	Anti-Ro/SSA	0.03	n.s.	0.01	n.s.	0.01	n.s.	0.20	n.s.	0.01	n.s.	0.06	n.s.	0.23	0.057	0.05	n.s.
Model 3	Age [a]	-0.04	n.s.	0.03	n.s.	0.15	n.s.	0.01	n.s.	0.11	n.s.	-0.13	n.s.	-0.06	n.s.	0.02	n.s.
	Disease duration (years) [a]	-0.17	n.s.	0.02	n.s.	0.15	n.s.	-0.14	n.s.	0.06	n.s.	0.02	n.s.	-0.04	n.s.	0.26	n.s.
	Minor salivary gland biopsy (FS)	0.08	n.s.	0.28	0.073	0.03	n.s.	0.03	n.s.	0.30	n.s.	0.15	n.s.	0.04	n.s.	0.11	n.s.

TBUT – Tear break up time; CSS – Corneal staining score; SBNP – Sub-basal nerve plexus; n.s. – non-significant; TMH – Tear Meniscus Height; TMA – Tear Meniscus Area
[a] Age and disease duration (years)– control variables

DISCUSSION

Primary Sjögren's syndrome is a challenging disorder, in terms of diagnosis, severity and activity assessment.[10] The objective ocular signs considered for pSS diagnosis[3] are highly variable with ambient and patient characteristics, which has led to widespread dissatisfaction among the medical community.[4,5,26] Thus, novel objective and reproducible tools to respond to the unmet needs in pSS ocular assessment are needed.

In this study, 50 pSS patients and 20 healthy controls were evaluated in order to demonstrate the value of TMH and corneal SBNP in detecting pSS. We also aimed to demonstrate if ophthalmic features were affected by clinical active disease, immunological and histological profiles of pSS patients. To our best knowledge, and to this date, this is the first study evaluating the differences of conventional dry-eye tests, TM and corneal SBNP in pSS patients, according to disease phenotype.

The results confirmed the value of TMH and corneal SBNP density, length and tortuosity in detecting pSS comparing to healthy individuals. In fact, the diagnostic value of TM for dry-eye disease and pSS has been proven. AS-OCT enables non-invasive, cross sectional high-

resolution images of the TM, thus considered to be a valid tool for the evaluation and diagnosis of aqueous deficient dry-eye disease.[12,14,15,27] Concerning the corneal nerve plexus evaluation, high-resolution and reproducible images of the ocular surface, obtained by IVCM[16] enable the objective assessment of corneal SBNP and the detection of nerve variations associated to dry-eye disease, associated or not with pSS[11,28,29]

However, the value of the referred tools in assessing pSS should not be taken as absolute, but integrated with the clinical picture and with other items in the AECG classification criteria. Indeed, rheumatic diseases are usually identified by the presence of a combination of clinical and laboratory manifestations.[3]

In turn, the effect of disease activity, immunological and histological profiles, corrected for disease duration, had only a marginal effect on patients' ophthalmological features. Patients with clinical active disease showed higher SBNP tortuosity, whereas patients with positive SSA antibodies showed higher results of SBNP length and patients with negative minor salivary gland biopsy had higher frequency of low BUT. This could mean that TM and SBNP are independent parameters, not influenced by phenotype profile, but also that they may have limited value in assessing disease activity, immunological and histological profile, which have been previously demonstrated to be associated with important clinical consequences.[30–32]

TM may change with different blink intervals, with lid position and ambient conditions,[15,33] whereas SBNP variations are indicators of nerve fibre damage, thus, not specific of dry-eye disease.[22,34,35] We also acknowledge that, being the sample limited to pSS, the interpretation of the results in terms of TM and SBNP diagnostic value may be limited. Also, the availability of TM AS-OCT and SBNP IVCM assessment equipment, as well as examination duration, still limit the applicability of these techniques in routine practice. However, their use in a scientific setting has brought valuable information about in differential diagnosis, follow-up and therapeutic approaches of pSS.[11,12,36]

CONCLUSION

Novel objective and reproducible tools for the assessment of the ocular component of pSS are needed. AS-OCT and IVCM may be useful in the evaluation of lachrymal unit dysfunction in pSS, complementing the conventional methodology used in the evaluation of dry-eye disease. More studies are needed to clarify the value of these ophthalmic features in the assessment of disease impact and severity, as well as their possible use as a biomarker for the diagnosis and classification of pSS.

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