Efficacy of N-acetylcysteine for treating dryness symptoms of Sjögren's disease: randomised placebo-controlled double-blind clinical study

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Abstract

Objective

N-acetylcysteine (NAC) is used in Sjögren's disease (SjD) based on limited evidence. The aim of this study was to assess the efficacy of NAC for relieving dryness symptoms in SjD.

Methods

In this placebo-controlled double-blind trial, 60 adult SjD females (with low disease activity) were randomised to receive NAC (1,200 mg/day orally) or placebo. At baseline (D0), 30 days (D30) and 90 days (D90), all participants underwent the following evaluations: EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI), Ocular Surface Disease Index (OSDI), Xerostomia Inventory (XI), Leicester Cough Questionnaire (LCQ), unstimulated/stimulated salivary flow, Schirmer's test, and plasma levels of thiobarbituric acid reactive substances (TBARS), glutathione and NAC.

Results

At inclusion, both groups were balanced for age, ethnicity, disease duration, ESSPRI, OSDI, XI, Schirmer's test, salivary flow, ESSDAI and topical/systemic treatments ($p>0.05$). No significant differences were observed between NAC and placebo groups on D30 and D90 regarding ESSPRI, XI, OSDI, LCQ, Schirmer's test, stimulated salivary flow, ESSDAI and topical/systemic treatments ($p>0.05$). Unstimulated salivary flow was significantly higher in the placebo group on D90 ($p=0.018$). NAC blood concentrations were significantly higher in the NAC group on D30 ($p=0.018$) and D90 ($p<0.001$), however, no differences were found in TBARS and glutathione. Further analysis showed a decrease ≥1 in ESSPRI in the NAC compared with placebo group on D30 ($p=0.045$), a result not found on D90 ($p=0.696$).

Conclusion

NAC is recommended as a rescue therapy for SjD. However, our well-designed study provides novel evidence demonstrating its inefficacy for improving dryness symptoms or reducing oxidative stress. Clinicaltrials.gov-NCT04793646.

Key words

Sjögren's disease, Sjögren's syndrome, N-acetylcysteine, sicca syndrome, dryness, oxidative stress

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Introduction

N-acetylcysteine (NAC), a precursor of cysteine, is a prodrug with antioxidant, anti-inflammatory and mucolytic effects (1, 2). Thus, its potential role as an adjuvant treatment has been investigated in several clinical conditions, and beneficial effects were described in the following chronic illnesses: chronic obstructive pulmonary disease (COPD) (3), chronic kidney disease (4), sepsis (5) and Coronavirus disease 2019 (COVID-19) (6), among others. In this context, NAC has been recommended as rescue therapy for relieving dryness symptoms in Sjögren's disease (SjD), however, with marginal benefits and the evidence supporting its efficacy is inadequate (7).

SjD is a systemic immune-mediated inflammatory illness with prevalence of 0.03% to 4.5% in different countries, affecting predominantly females (9-20:1) aged 40 to 60 years (8). The cardinal characteristic of this disease is the involvement of salivary and lacrimal glands by an intense lymphocytic inflammatory process targeting the acinar and ductal epithelial cells, causing tissue damage, glandular dysfunction and dryness symptoms (9). Additionally, we noted that SjD can manifest with a wide spectrum of organ involvements, including an increased risk for developing B-cell non-Hodgkin lymphoma, which can lead to a more severe disease course and higher mortality (9).

Recent advances have reduced diagnostic delay, including novel classification criteria from the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) (2016) (10). Nevertheless, in real life, clinical management of patients is challenging, since SjD is frequently diagnosed in phases of marked glandular dysfunction, with approximately 90% of patients presenting sicca symptoms at diagnosis (11). Importantly, several studies have shown undesirable impacts of SjD on health-related quality of life, associated with fatigue, joint pain, ocular and oral dryness, itching, lung involvement, sleep disturbances, sexual dysfunction, psychological dysfunction, and reduced physical function (12).

Despite substantial progress in understanding the pathophysiology of SjD, contemporary treatment of glandular manifestations is mainly focused on symptoms, using artificial tears, saliva substitutes and oral muscarinic agonists. According to EULAR recommendations, oral NAC (primarily a mucolytic agent, not a sialagogue) is a rescue therapy for patients who are intolerant or unresponsive to muscarinic agonists (7), with marginal benefits described (13). Additionally, we addressed the limitations of the previous study, including the lack of assessment of the potential effects of NAC on oxidative stress, another possible mechanism of action (1-6), which precludes drawing definitive conclusions about the role of NAC in the treatment of SjD. This previous study in question was a small-randomised controlled trial of NAC (200 mg orally three times a day) including a total of 26 SjD patients who did not meet classification criteria (13). In addition, the sample was heterogeneous, with the majority of the patients [18/26 (69.2%)] having rheumatoid arthritis (RA) associated with SjD (13). The trial showed positive effects of NAC compared with placebo for improving ocular pain and irritability, halitosis and daytime thirst, using a simple question instrument to measure response to NAC therapy (13). However, the study lasted only four weeks and tools, now widely accepted for patient assessment, were not available then (13). Furthermore, the potential impact of NAC on oxidative stress in SjD was not evaluated.

There is evidence of a role of oxidative stress in the pathophysiology of SjD (14-16), with detection of high levels of reactive oxygen species (ROS) in saliva (17, 18), conjunctival epithelium (19) and peripheral blood (14, 16, 20-23) of these patients. Despite this, the literature shows a notable gap relative to potential benefits of oral NAC for symptomatic relief of dryness and elimination of ROS in SjD patients.

Therefore, the present randomised placebo-controlled double-blind clinical study aimed to assess NAC efficacy in controlling dryness symptoms in a homogeneous sample of SjD patients, based not only on classificatory crite-

ria (10), but also with a low systemic disease activity index at start of study (24). Furthermore, validated, widely accepted instruments for evaluating oral and ocular dryness symptoms in SjD were applied (7, 25-27). Potential impacts of NAC on oxidative stress were also assessed.

Materials and methods

Study design

This was a randomised placebo-controlled double-blind trial of NAC (600 mg orally twice daily in syrup form) for treatment of dryness symptoms resulting from SjD, lasting 12 weeks. The dosage chosen was based on studies showing benefits of NAC in patients with COPD (3) and kidney transplant recipients (28). Randomisation was carried out by professionals from the institutional Pharmacy Department who were not involved in the study. They used the GraphPad random number generator programme available at https://www.graphpad.com/quickcalcs/randomN1/. The blinding of the vials and the preparation of the placebo were performed by the institutional Pharmacy Department. The placebo was formulated as a syrup identical to the NAC in colour, aroma, flavour and viscosity, indistinguishable from the active drug.

Patients

The convenience sample consisted of sixty consecutive adult female patients (aged 18 to 75 years) with SjD according to the 2016 ACR/EULAR classificatory criteria (10), who were under regular follow-up at the Sjögren's Disease Outpatient Clinic of the Rheumatology Division of the Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil. Patients were randomised to receive NAC (n=30) or placebo (n=30).

- Inclusion criteria

The inclusion criteria were: presence of ocular and/or oral dryness symptoms (according to the American-European Consensus Group criteria) (29); low disease activity [EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI)≤5] (30) for at least 3 months before entering the study; no use of NAC for at least four weeks before entering the study (drug's half-life of <3 hours) (31); and agreement to participate in the study according to signed term of consent.

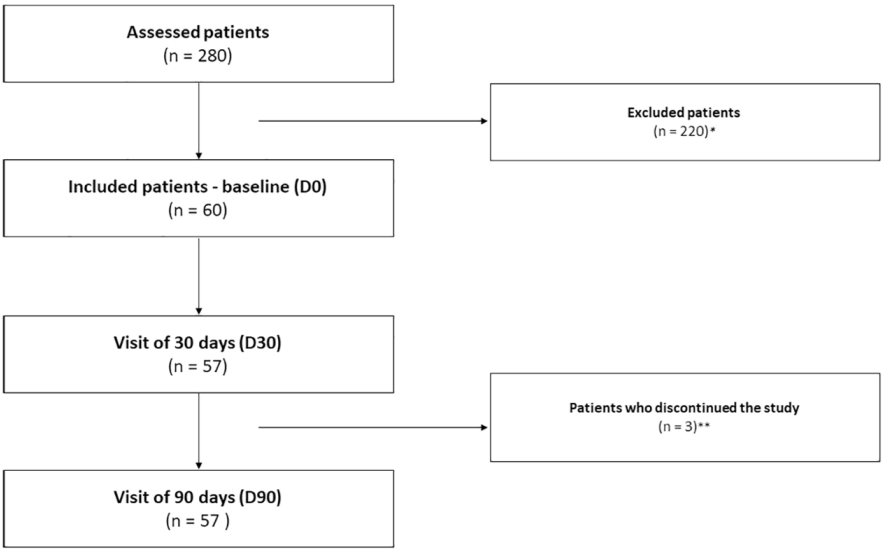

Fig. 1. Flow chart of the study.
*Excluded patients: 28 refused to participate; 3 without dry eye and/or dry mouth symptoms; 6 aged under 18 or over 75 years-old; 9 men; 8 diabetes; 1 sarcoidosis; 1 positive serology for hepatitis B; 15 current use of biologic therapy; 5 current use of tricyclic antidepressants; 1 ESSDAI>5; 3 pregnancy; 1 breastfeeding; 10 other associated autoimmune diseases; 4 current use of pilocarpine; 4 current smoking; 7 chronic kidney disease; 114 use of NAC in the last 30 days.
**Reasons for study discontinuation: unavailability to attend study visits.
ESSDAI: EULAR (European League Against Rheumatism) Sjögren's Syndrome Disease Activity Index; NAC: N-acetylcysteine.

- Exclusion criteria

Exclusion criteria were: associated systemic autoimmune diseases [*e.g.* RA, systemic lupus erythematosus (SLE), systemic sclerosis, dermatomyositis, primary biliary cholangitis and autoimmune hepatitis]; other causes of sicca syndrome (use of tricyclic antidepressants and antihistamines, head/neck radiotherapy, iodine therapy, graft *versus* host disease, positive serologies for HIV, hepatitis B/C, diabetes, sarcoidosis and IgG4-related disease) (10, 29); pregnancy and breastfeeding; current use of prednisone≥20 mg/day, current use of pilocarpine/cevimeline or immunobiological therapy; current smoking (32), alcoholism, cirrhosis and chronic kidney disease.

- Enrolment

Patients were recruited from February 19 to October 22, 2021. In total, 280 patients were systematically evaluated and 220 were excluded, as shown in Figure 1.

Ethics approval, informed consent and trial registration

All procedures carried out in this study were in accordance with the ethical guidelines of the institution's ethical board [Comissão de Ética para Análise de Projetos de Pesquisa (CAPPesq)], which approved the research protocol (24088719.4.0000.0068, report: 3.735.534). All SjD patients signed a term of informed consent before inclusion in the study. The study protocol was registered on Clinicaltrials.gov (NCT04793646).

Clinical evaluations

At baseline (D0), 30 days (D30) and 90 days (D90) all participants underwent the following evaluations: EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) (25,33), ESSDAI (24, 34), Xerostomia Inventory (XI) (27, 35), Ocular Surface Disease Index (OSDI) (26, 36), Short-Form 36 (SF-36) questionnaire (37, 38), Leicester Cough Questionnaire (LCQ) (39, 40), non-stimulated (NSSF)/stimulated (SSF) salivary flow rates (41) and Schirmer's I test (10). Relative to ESSPRI, the patient's acceptable symptom status was defined as ESSPRI<5, and the minimal

clinically significant improvement was a reduction of at least one point (30).

All medications for the underlying disease prescribed by the patient's physician were recorded at each visit throughout the study. Topical therapies (artificial tears and saliva substitutes) were prescribed for all patients at D0, D30 and D90. Daily air humidity (minimum and maximum) was registered during the study period.

Laboratory tests

At each visit, peripheral blood samples were collected for the laboratory tests included in ESSDAI, and for determining plasma levels of thiobarbituric acid reactive substances (TBARS) (28), glutathione (GSH) and NAC (42). Biological samples were collected after 8-hour fasting on D0, D30 and D90. Samples for determining oxidative stress and NAC were collected in EDTA (edetetic acid) tubes, fractioned and stored at -80°C until use.

Assessment of oxidative stress and NAC levels

Oxidative stress was assessed by measuring TBARS plasma concentration, as previously described for evaluating oral NAC in kidney transplant patients (28). A molar extinction coefficient of 1.56 × 105 mol/cm was used, and serum TBARS concentration was expressed in nmol/mL.

GSH and NAC levels in plasma were quantified by means of liquid chromatography coupled with mass spectrometry (LC-MS/MS). This method is considered the gold standard for quantifying drugs in biological matrices for therapeutic drug monitoring (43). The LC–MS/MS method to measure NAC and GSH plasma levels was developed and validated as previously described (42). The analysis was carried out in the ultra-high-performance liquid chromatography Dionex UltiMate 3000 equipped with DGP-3600 pumps, WPS-3000TRS autosampler and TCC-3000RS oven with two 2-position, and 6-port TitanHT switching valves (Thermo Scientific, San Jose, CA, USA). The coefficients of variation were 4.5% and 5.8% for NAC, at low and high concentrations, respectively, and 6.8% and 17.8% for GSH, at low and high concentrations, respectively. The accuracy at low, medium and high concentration was 88.4 to 114.9% for NAC and 80.1 to 102.0% for GSH. For data analysis, the value of 5 ng/mL was assigned to NAC samples with levels below the limit of quantification (10 ng/mL). For samples with GSH levels lower than the limit of quantification (50 ng/mL), a value of 25 ng/mL was assigned.

Ultrasonography of major salivary glands

Additionally, participants underwent salivary gland ultrasonography (SGUS) on entering the study for evaluating presence and intensity of changes in echotexture according to OMERACT (Outcome Measures in Rheumatology) score (44). SGUS was performed in the equipment MyLab 70 XVG (Esaote SPA, Genova, Italy), utilising a high-frequency linear transducer (6–18 MHz).

Primary outcome

The primary outcome measure was the ESSPRI score (25, 33) with a 3-month time frame compared with baseline. The minimal clinically significant improvement was a reduction of at least one point in the ESSPRI value (30).

Secondary outcomes

Another study aim was to identify improvement in xerostomia on D30 and D90 by decrease in XI values (27, 35) compared with baseline. Other secondary outcomes were: OSDI (26, 36), LCQ (39, 40), SF-36 (37, 38), NSSF, SSF (41), Schirmer's I test (10) and oxidative stress.

Drug adherence

Drug adherence was evaluated at each visit by counting the remaining NAC bottles. Additionally, blood samples were collected at each visit to measure NAC plasma levels.

Statistical analysis

Data were managed using the REDCap web platform (Vanderbilt University, Nashville, TN, USA). For quantitative variables, univariate descriptive statistics were determined by calculating medians, interquartile ranges, means and standard deviations. For qualitative variables, the number of valid observations (n) and respective percentages of occurrence were computed. Subsequently, bivariate analysis was performed to compare means between the two independent groups (NAC *vs.* placebo) at baseline (D0) using the t-test or Mann-Whitney test, as appropriate, based on data distribution verified by the Shapiro-Wilk or Kolmogorov-Smirnov normality tests. For qualitative variables, bivariate analysis was carried out applying the chi-square test, corrected as necessary, or Fisher's exact test, according to the minimum expected frequency. The binomial test was used for comparisons between two groups at each level of qualitative variables with more than two categories.

A comparison was made considering dependent quantitative variables evaluated at three time points (D0, D30 and D90) using repeated measures ANOVA (or the Friedman test for non-parametric parameters), whose adequacy was verified in compliance with the assumptions analysis of variance (residual normality and independence between groups, with visual input from QQ-plot graphs, histograms, box-plots...) and *post-hoc* analysis with parametric Bonferroni or Tukey test or the non-parametric Nemenyi test.

All analyses were conducted at a statistical significance level of 5%, with two-tailed tests, using the open-source software Jamovi, 2022 [Jamovi (v. 2.3) retrieved from https://www.jamovi.org] and R [R Core Team, 2021 (v. 4.1, retrieved from https://cran.r-project.org); Singmann, 2018 (retrieved from https://cran.r-project.org/package=afex); Lenth, 2020 (retrieved from https://cran.r-project.org/package=emmeans)].

A convenience sample of 60 SjD patients was established for the present study, since the only controlled study of oral NAC in SjD included a very small number of SjD patients without other associated systemic autoimmune diseases (n=8) and lasted only four weeks (13). The *post-hoc* power of 99% was calculated using repeated measures ANOVA for three correlated samples for the ESSPRI variable, in

Table I. Baseline data (D0) in NAC and placebo groups.

	NAC n=30	Placebo n=30	*p*-value
Demographic features			
Age (years)	49.8 ± 12.0	49.9 ± 13.2	0.984
Education (years)	10.6 ± 4.3	11.7 ± 3.6	0.323
Age at SjD diagnosis (years)	42.9 ± 12.5	43.3 ± 14.4	0.848
Duration of illness (years)	7.0 ± 6.2	6.6 ± 8.7	0.327
Ethnicity			0.596
White	20 (66.7)	17 (56.7)	
Afro-Brazilian	10 (33.3)	13 (43.3)	
Socioeconomic classification*			0.292
A1, B1 and B2	20 (66.7)	16 (53.3)	
C1, C2, D and E	10 (33.3)	14 (46.7)	
Menopause	17 (56.7)	16 (53.3)	0.795
Age at menopause	45.4 ± 6.1	48.3 ± 8.6	0.374
Previous smoking	4 (13.3)	5 (16.7)	1.000
ESSPRI	6.3 ± 2.3	5.8 ± 2.2	>0.05
ESSDAI	1.8 ± 1.8	1.3 ± 1.6	>0.05
Unstimulated salivary flow rate (mL/min)	0.1 ± 0.1	0.2 ± 0.2	0.054
Stimulated salivary flow rate (mL/min)	0.3 ± 0.3	0.4 ± 0.3	>0.05
Schirmer's I test	9.6 ± 10.2	4.9 ± 7.0	>0.05
OMERACT US**			
≥1 gland with ultrasound score 1, 2, 3	27 (100)	27 (100)	-
≥1 gland with ultrasound score 2 or 3	27 (100)	27 (100)	-
≥1 gland with ultrasound score 3	16 (59.3)	11 (40.7)	0.174
≥2 glands with ultrasound score 1, 2, 3	26 (96.3)	26 (96.3)	1.000
≥2 glands with ultrasound score 2 or 3	25 (92.6)	22 (81.5)	0.420
≥2 glands with ultrasound score 3	10 (37.0)	10 (37.0)	1.000
Topical therapies			
Artificial tears	25 (83.3)	25 (83.3)	1.000
Saliva substitutes	6 (20.0)	10 (33.3)	0.243
Current treatments			
Hydroxychloroquine	21 (70.0)	23 (76.7)	0.559
Prednisone	9 (30.0)	8 (26.7)	0.774
Dose (mg/day)	7.8 ± 4.4	7.2 ± 4.1	0.398
Methylprednisolone pulses	0 (0)	0 (0)	-
Methotrexate	3 (10.0)	6 (20.0)	0.488
Leflunomide	1 (3.3)	1 (3.3)	1.000
Azathioprine	5 (16.7)	2 (6.7)	0.288
Mycophenolate mofetil	2 (6.7)	2 (6.7)	0.554
Cyclophosphamide	0 (0)	0 (0)	-
Rituximab	0 (0)	0 (0)	-
Belimumab	0 (0)	0 (0)	-
Abatacept	0 (0)	0 (0)	-
SjD phenotype			
Xerostomia	29 (96.7)	30 (100)	1.000
Xerophthalmia	29 (96.7)	29 (96.7)	1.000
Parotitis	15 (50.0)	14 (46.7)	0.796
Articular involvement	18 (60)	21 (70)	0.417
Cutaneous involvement	6 (20)	6 (20)	1.000
Raynaud's phenomenon	2 (6.7)	4 (13.3)	0.671
Respiratory involvement (pneumonitis and/or bronchiolitis)	5 (16.7)	8 (26.7)	0.347
Renal involvement	2 (6.7)	0 (0)	0.492
Muscular involvement	0 (0)	0 (0)	-
Central nervous system involvement	0 (0)	1 (3.3)	1.000
Peripheral nervous system involvement	2 (6.7)	3 (10)	1.000
Haematological involvement	1 (3.3)	4 (13.3)	0.353
Gastrointestinal involvement	0 (0)	1 (3.3)	1.000
Immunological profile			
Antinuclear antibodies	29 (96.7)	30 (100)	1.000
Anti-Ro (SS-A)	27 (90.0)	28 (93.3)	1.000
Anti-La (SS-B)	18 (60.0)	14 (48.3)	0.366
Rheumatoid factor	18 (60.0)	12 (42.9)	0.192
Cryoglobulins	0 (0)	0 (0)	-
Low complement C3	4 (13.3)	2 (6.7)	0.670
Low complement C4	7 (23.3)	6 (20.0)	0.754
Comorbidities			
Dyslipidaemia	5 (16.7)	5 (16.7)	1.000
Diabetes	0 (0)	0 (0)	-
Hypertension	5 (16.7)	11 (36.7)	0.080
Hypothyroidism	9 (30.0)	9 (30.0)	1.000
Hyperthyroidism	0 (0)	0 (0)	-
Previous smoking	4 (13.3)	5 (16.7)	1.000

Data presented as number (percentage), or mean ± standard deviation (SD).
ESSDAI: European League Against Rheumatism (EULAR) Sjögren's Syndrome Disease Activity Index; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; NAC: N-acetylcysteine, SjD: Sjögren's disease.
*A/B1/B2/C1/C2/D/E: socioeconomic classes according to the Socioeconomic Classification of the Brazilian Association of Research Companies (45).
**OMERACT US: Outcome Measures in Rheumatology Grey-scale Ultrasound Scoring System for salivary glands (44).

two groups of 30 individuals each, with a maximum alpha error of 5% and an effect size of 0.25 within the groups. It should be noted that an intention-to-treat analysis was conducted for randomised patients, so those who withdrew from the study remained in the randomised groups.

Results

Comparative analysis between NAC vs. placebo groups at baseline (D0)

NAC and placebo groups had a comparable mean age [49.8±12.0 *vs*. 49.9±13.2 years, respectively (p=0.984)] and ethnicity (p=0.596) (Table I). At inclusion, the two groups were also similar relative to several disease parameters, such as: disease duration, symptom and disease activity scores, glandular function tests, salivary gland echotexture on US, systemic phenotype, autoantibody profile, current topical and systemic medications and comorbidities (p>0.05) (Table I). Previous pilocarpine use was observed in 2 (6.7%) patients in the NAC group and 4 (13.3%) patients in the placebo group (p=0.389).

Comparative analysis of disease parameters between NAC vs. placebo groups at baseline (D0) and post- intervention (D30 and D90)

At baseline (D0), both groups presented comparable values of the following tools and disease parameters: XI, OSDI, ESSPRI, ESSDAI, LCQ, NSSF, SSF, Schirmer's I test and SF-36 (p>0.05) (Table II). All these variables, except NSSF, remained similar between the NAC and placebo groups post-intervention on D30 and D90 (p>0.05) (Table II). NSSF was higher in the placebo group on D90 (p=0.018) (Table II).

Notably, in the NAC group 17 patients (58.6%) had a reduction of at least 1 point in ESSPRI (Δ ESSPRI≤1) from D0 to D30, while only 9 patients (32.1%) in the placebo group achieved this improvement (p=0.045). From D30 to D90 and from D0 to D90, no significant differences were observed in frequencies of patients with Δ ESSPRI≤ 1 in NAC and placebo groups (p=0.509 and p=0.696), respectively. Frequen-

Table II. Comparative analysis of disease parameters between NAC and placebo groups at D0, D30 and D90.

	NAC n=30 Median ± IQR	Placebo n=30 Median ± IQR	*p*-value	*p*-value
XI				
D0	39.0 ± 12.0	42.0 ± 13.0	>0.05	0.999
D30	33.0 ± 14.0	36.0 ± 13.5	>0.05	
D90	37.0 ± 15.0	38.5 ± 14.0	>0.05	
OSDI				
D0	11.0 ± 1.0	11.0 ± 1.0	>0.05	0.879
D30	23.0 ± 21.0	18.0 ± 35.5	>0.05	
D90	29.0 ± 33.0	18.5 ± 36.0	>0.05	
ESSPRI				
D0	6.3 ± 3.3	5.7 ± 2.3	>0.05	0.723
D30	5.0 ± 1.7	5.3 ± 3.7	>0.05	
D90	5.0 ± 3.3	5.3 ± 4.3	>0.05	
ESSPRI (dryness)				
D0	8.0 ± 4.0	7.0 ± 3.0	>0.05	0.066
D30	7.0 ± 3.0	6.0 ± 3.0	>0.05	
D90	7.0 ± 2.0	6.0 ± 2.0	>0.05	
ESSPRI (fatigue)				
D0	7.0 ± 5.0	5.0 ± 5.0	>0.05	0.682
D30	5.0 ± 5.0	5.5 ± 6.0	>0.05	
D90	4.0 ± 5.0	6.0 ± 4.5	>0.05	
ESSPRI (pain)				
D0	6.0 ± 4.0	5.5 ± 7.0	>0.05	0.910
D30	4.0 ± 5.0	5.0 ± 5.0	>0.05	
D90	5.0 ± 5.0	5.5 ± 8.0	>0.05	
ESSDAI				
D0	1.0 ± 3.0	1.0 ± 2.0	>0.05	0.613
D30	4.5 ± 5.8	3.5 ± 4.4	>0.05	
D90	5.8 ± 5.8	4.5 ± 4.6	>0.05	
LCQ				
D0	20.2 ± 4.2	20.2 ± 4.9	>0.05	0.075
D30	21.0 ± 1.4	20.9 ± 3.3	>0.05	
D90	21.0 ±2 .4	20.7 ± 4.7	>0.05	
NSSF (mL/min)				
D0	0 ± 0.1	0.1 ± 0.3	0.054	0.024
D30	0.1 ± 0.2	0.2 ± 0.3	0.091	
D90	0.1 ± 0.2	0.2 ± 0.4	0.018	
SSF (mL/min)				
D0	0.2 ± 0.5	0.4 ± 0.4	>0.05	
D30	0.3 ± 0.6	0.4 ± 0.6	>0.05	0.303
D90	0.3 ± 0.5	0.6 ± 0.7	>0.05	
Schirmer's I test				
D0	5.5 ± 18.5	2.0 ± 4.5	>0.05	
D30	4.5 ± 13.5	3.5 ± 6.5	>0.05	0.300
D90	2.0 ± 9.8	2.0 ± 10.0	>0.05	
SF-36				
Physical functioning				
D0	65.0 ± 35.0	65.0 ± 40.0	>0.05	
D30	66.0 ± 25.0	67.5 ± 35.0	>0.05	0.324
D90	75.0 ± 20.0	75.0 ± 47.5	>0.05	
Role physical				
D0	37.5 ± 100.0	75.0 ± 100.0	>0.05	
D30	75.0 ± 50.0	100.0 ± 62.5	>0.05	0.429
D90	100.0 ± 50.0	100.0 ± 50.0	>0.05	
Bodily pain				
D0	52.0 ± 23.0	51.5 ± 31.0	>0.05	
D30	62.0 ± 21.0	61.0 ± 32.0	>0.05	0.789
D90	52.0 ± 21.0	56.0 ± 27.0	>0.05	
G eneral health				
D0	52.0 ± 25.0	63.5 ± 20.0	>0.05	
D30	57.0 ±4 0.0	57.0 ± 23.5	>0.05	0.680
D90	62.0 ± 40.0	67.0 ± 30.0	>0.05	
Vitality				
D0	52.5 ± 20.0	60.0 ± 25.0	>0.05	
D30	60.0 ± 15.0	65.0 ± 30.0	>0.05	0.783
D90	65.0 ± 15.0	65.0 ± 37.5	>0.05	
Social functioning				
D0	62.5 ± 62.5	68.5 ± 50.0	>0.05	
D30	87.5 ± 37.5	75.0 ± 50.0	>0.05	0.576
D90	87.5 ± 37.5	87.5 ± 37.5	>0.05	
Role emotional				
D0	66.7 ± 100.0	67.0 ± 100.0	>0.05	
D30	100.0 ± 33.3	100.0 ± 66.7	>0.05	0.898
D90	100.0 ± 66.7	100.0 ± 66.7	>0.05	
Mental health				
D0	70.0 ± 24.0	60.0 ± 28.0	>0.05	
D30	72.0 ± 28.0	64.0 ± 32.0	>0.05	0.390
D90	72.0 ± 20.0	68.0 ± 22.5	>0.05	
Air humidity				
Minimum (g/m^3)				
D0	0.5 ± 0.1	0.5 ± 0.3	>0.05	
D30	0.5 ± 0.3	0.5 ± 0.3	>0.05	0.553
D90	0.5 ± 0.3	0.6 ± 0.2	>0.05	
Maximum (g/m^3)				
D0	0.9 ± 0.1	0.9 ± 0.1	>0.05	
D30	0.9 ± 0.1	0.9 ± 0.1	>0.05	0.715
D90	0.9 ± 0.0	0.9 ± 0.0	>0.05	

ESSDAI: European League Against Rheumatism (EULAR) Sjögren's Syndrome Disease Activity Index; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; IQR: interquartile range; LCQ: Leicester Cough Questionnaire; NAC: N-acetylcysteine; NSSF: non-stimulated salivary flow rate; OSDI: Ocular Surface Disease Index; SF-36: 36-Item Short Form Health Survey questionnaire; SSF: stimulated salivary flow rate; XI: xerostomia inventory.

cies of patients with ESSPRI<5 on D0, D30 and D90 were comparable between the NAC and placebo groups: D0 [6 (20.0%) *vs*. 8 (26.7%), *p*=0.542], D30 [13 (44.8%) *vs*. 12 (42.9%), *p*=0.881] and D90 [13 (44.8%) *vs*. 12 (42.9%), *p*=0.881], respectively.

The percentages of patients who reached an ESSPRI ≤3 at D30 (17.2% *vs*. 21.4%; *p*=0.689) and at D90 (13.8% *vs*. 25%; *p*=0.284) were comparable between the NAC and placebo groups, respectively. Likewise, the percentages of patients with a reduction ≤1.5 in ESSPRI (Δ ESSPRI≤1.5) from D0 to D30 (55.2% *vs*. 71.4%, *p*=0.203), from D30 to D90 (86.2% *vs*. 71.4%; *p*=0.171) and from D0 to D90 (62.1% *vs*. 78.6%; *p*=0.173) were comparable between the NAC and placebo groups, respectively. During the study, air humidity conditions were comparable between the NAC and placebo groups (*p*>0.05) (Table II).

Regarding ESSDAI, a mean reduction from D0 to D90 (Δ ESSDAI) of 0.00±3.50 was observed in the NAC group, compared with -1.26±2.82 in the placebo group (*p*=0.013). There were no differences between the NAC and placebo groups relative to Δ ESSDAI from D0 to D30 (-0.21±3.10 *vs*. -0.82±2.37 *p*=0.142) and from D30 to D90 (0.21±1.10 *vs*. -0.26±2.35, *p*=0.156), respectively.

Comparative analysis between NAC vs. placebo groups at D0 and post-intervention (D30 and D90) relative to adherence, plasma NAC levels and oxidative stress

Adherence, assessed by counting the remaining NAC bottles, was similar in NAC and placebo groups (*p*=0.383) (Table III). NAC plasma concentrations were significantly higher in NAC group on D30 (*p*=0.018) and D90 (*p*<0.001), but no differences in TBARS and glutathione concentrations were found between the two groups throughout the study (Table III). Figure 2 shows the plasma NAC curve levels in the treatment and placebo groups. In the former, an upward curve in NAC plasma levels occurred from D0 to D30, which reached a plateau from D30 to D90 (Fig. 2).

Table III. Comparative analysis of plasma NAC levels and oxidative stress between NAC and placebo groups at D0, D30 and D90 regarding adherence.

	NAC n=30 Median ± IQR	Placebo n=30 Median ± IQR	*p*-value	*p*-value
Remaining NAC bottles (n)				
D30	12.0 ± 4.0	11.0±3.0	>0.05	0.383
D90	4.0 ± 3.5	2.0 ± 3.0	>0.05	
Plasma NAC levels (ng/mL)				
D0	16.3 ± 15.1	5.0 ± 13.2	0.227	**0.000**
D30	**64.2 ± 134.0**	**5.0 ± 13.4**	**0.018**	
D90	**88.6 ± 216.8**	**5.0 ± 14.3**	**0.000**	
Oxidative stress				
Serum TBARS (nmol/mL)				
D0	4.6 ± 5.2	4.7 ± 5.3	>0.05	0.498
D30	4.5 ± 5.8	3.5 ± 4.4	>0.05	
D90	5.8 ± 5.8	4.5 ± 4.6	>0.05	
Serum GSH (ng/mL)				
D0	298.3 ± 623.2	258.2 ± 393.6	>0.05	0.565
D30	270.4 ± 550.1	321.9 ± 434.0	>0.05	
D90	327.7 ± 460.0	220.6 ± 441.4	>0.05	

GSH: glutathione; IQR: interquartile range; NAC: N-acetylcysteine; TBARS: thiobarbituric acid reactive substances.

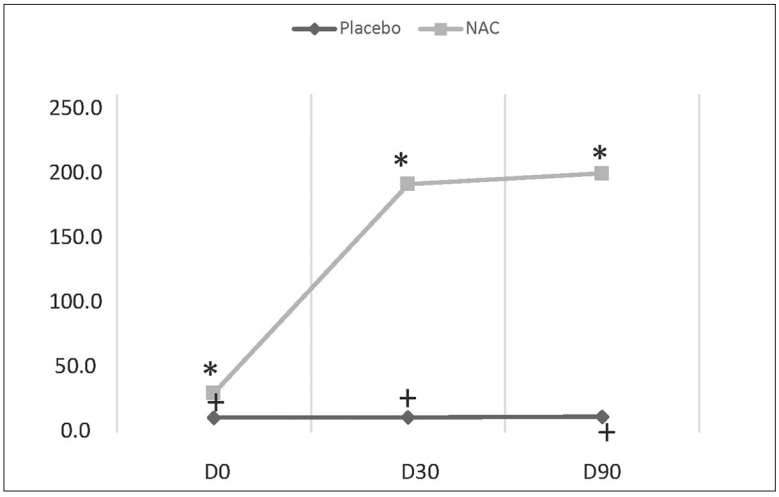

Fig. 2. NAC plasma levels (ng/mL) at baseline (D0), 30 days (D30) and 90 days (D90) in NAC and placebo groups.
**p*-value in the NAC group: D0-D30=0.013; D30-D90=1.000; D0-D90=0.000; +*p*-value in the placebo group: D0-D30>0.05; D30-D90>0.05; D0-D90>0.05. NAC: N-acetylcysteine.

Comparative analysis of adverse events between NAC and placebo groups

Adverse events were comparable between NAC and placebo groups on D30 and D90 ($p>0.05$) (Table IV). Side events were mild and more associated with gastrointestinal symptoms: vomiting (3.4% *vs*. 0%), diarrhoea (6.9% *vs*. 3.7%) and abdominal pain (17.2% *vs*. 11.1%) in NAC and placebo groups on D30, respectively ($p>0.05$) (Table IV). Furthermore, only 13.8% *vs*. 3.7% and 3.6% *vs*. 3.6% patients needed to reduce the dose by half at D0-D30 ($p=0.353$) and at D30-D90 ($p=1.000$) in NAC and placebo groups, respectively. No patient withdrew from the study due to adverse events.

Comparative analysis of glucocorticoids, immunosuppressive drugs and topical medications between NAC and placebo groups post-intervention (D30 and D90)

Throughout the study, glucocorticoid use was comparable between the NAC and placebo groups. There was no difference between NAC and placebo groups relative to frequency of prednisone use on D30 [10 (33.3%) *vs*. 9 (30%); $p=0.781$] and D90 [12 (40%) *vs*. 9 (30%); $p=0.417$], respectively. Prednisone dose was also comparable between NAC and placebo groups at these two time points (D30: 10.50±6.32 *vs*. 6.39±5.17 mg/day; $p=0.121$) and (D90: 9.79±5.48 *vs*. 8.06±5.56 mg/day; $p=0.472$), respectively. Similarly, no difference was found relative to frequency of immunosuppressive drug use between NAC and placebo groups on D30 and D90 [15 (50%) *vs*. 10 (33.3%); $p=0.190$] and [15 (50%) *vs*. 10 (33.3%); $p=0.190$], respectively. Frequencies of artificial tear use on D30 [29 (96.7%) *vs*. 28 (93.3%); $p=1.000$] and D90 [29 (96.7%) *vs*. 28 (93.3%); $p=1.000$] and saliva substitutes on D30 [29 (96.7%) *vs*. 29 (96.7%); $p=1.000$] and D90 [29 (96.7%) *vs*. 29 (96.7%); $p=1.000$] were also comparable between NAC and placebo groups, respectively.

Discussion

The present study was the first randomised placebo-controlled double-blind trial of oral NAC specifically targeting dryness symptoms in patients with well-defined SjD without other associated systemic autoimmune diseases. Oral NAC did not lead to improvement in dryness symptoms according to currently validated tools in SjD, nor any reduction in plasma oxidative stress biomarkers.

The study had several strengths, including its randomised placebo-controlled double-blind design. It evaluated a homogeneous population with well-defined SjD according to the 2016 ACR/EULAR classificatory criteria (10), without other associated systemic autoimmune diseases (29). Another advantage of this trial was its strict exclusion criteria that ruled out other causes of xerophthalmia and xerostomia, such as use of tricyclic antidepressants and antihistamines, smoking, head/neck radiotherapy or iodine therapy, graft versus host disease, hepatitis B and C, HIV, diabetes, sarcoidosis and IgG4-related disease (10, 29, 32).

Moreover, the inclusion of only female SjD patients is relevant since this disease affects predominantly women (8,9), and there are phenotypical differences in comparison with male patients, particularly in dryness symptoms (46). Furthermore, the inclusion/exclusion criteria optimised homogeneity of the population evaluated and minimised the possible influence of other factors, such as moderate to high disease activity (ESSDAI>5) (30), high glucocor-

Table IV. Comparative analysis of adverse events between NAC and placebo groups at D0, D30 and D90.

	NAC n=30	Placebo n=30	*p*-value
Number of patients with adverse reactions			
D30	8 (26.7)	4 (13.3)	1.667
D90	1 (3.3)	1 (3.3)	1.000
Hypersensitivity/allergy			
D30	0 (0)	0 (0)	-
D90	0 (0)	0 (0)	-
Headache			
D30	2 (6.9)	0 (0)	0.492
D90	0 (0)	0 (0)	-
Tinnitus			
D30	0 (0)	0 (0)	-
D90	0 (0)	0 (0)	-
Tachycardia			
D30	0 (0)	1 (3.6)	0.491
D90	0 (0)	0 (0)	-
Vomiting			
D30	1 (3.4)	0 (0)	1.000
D90	0 (0)	0 (0)	-
Diarrhoea			
D30	2 (6.9)	1 (3.7)	1.00
D90	0 (0)	0 (0)	-
Stomatitis			
D30	0 (0)	0 (0)	-
D90	0 (0)	0 (0)	-
Abdominal pain			
D30	5 (17.2)	3 (11.1)	0.707
D90	0 (0)	1 (3.6)	0.491
Fever			
D30	0 (0)	0 (0)	-
D90	0 (0)	0 (0)	-
Low blood pressure			
D30	0 (0)	0 (0)	-
D90	0 (0)	0 (0)	-
Bronchospasm			
D30	0 (0)	0 (0)	-
D90	0 (0)	0 (0)	-
Dyspnoea			
D30	0 (0)	0 (0)	-
D90	0 (0)	0 (0)	-
Dose reduction			
D0-D30	4 (13.8)	1 (3.7)	0.353
D30-D90	1 (3.6)	1 (3.6)	1.000

Data presented as number (percentage).
NAC: N-acetylcysteine.

ticoid doses (prednisone ≥20 mg/day) (47), immunobiological treatment (48), use of pilocarpine (49) and tricyclic antidepressants (10, 29).

Although there was a decrease in ESSPRI (ΔESSPRI≤1) in NAC compared with placebo group in the first month of intervention, this result was not sustained throughout the study. Thus, the present clinical trial did not reach its primary endpoint of improvement in ESSPRI values.

We reinforced these findings by extending analysis of NAC efficacy to other dryness symptom assessment tools used in SjD, such as OSDI (7, 26, 36) and XI (27, 35, 50). These tools showed no improvement in dryness symptoms either, nor were there any improvement in Schirmer's I test and unstimulated/stimulated salivary flow rates. Furthermore, we evaluated dryness symptoms using a cough questionnaire since xerotrachea is common in SjD, and it is associated with impaired health-related quality of life (51). Consistent with all the above findings, we observed no improvement in the cough questionnaire or SF-36.

At baseline (D0), there was a trend towards a lower NSSF in the NAC group compared to the placebo group (p=0.054) (Table I). This trend may have influenced the better NSSF result observed in the placebo group on D90 (p=0.018) (Table II). Regarding SSF, although baseline values were lower in the NAC group compared to placebo group, this difference was not statistically significant (p>0.05). In contrast, baseline values for the Schirmer's I test were higher in the NAC group than in the placebo group, but again without reaching statistical significance (p>0.05) (Table I). These last two parameters remained comparable between both groups throughout the study (p>0.05) (Table II), therefore there was no evidence of NAC benefits.

In the present study, another relevant aspect was that adherence was not the underlying cause of NAC inefficacy, since plasma levels of NAC were adequate throughout the study, according to its quantification by the LC-MS/MS method (42). Moreover, randomisation ensured adequate comparability between NAC and placebo groups relative to several factors that could possibly have influenced the dryness symptoms results, such as: age (52), duration of the disease (53), ethnicity (54), menopause (55), topical and systemic treatments (49), socioeconomic features, comorbidities (10, 29, 32), and daily air humidity conditions during the study (56). On entering the study, we used SGUS to assess the degree of parotid and submandibular gland involvement, which was also comparable between the two groups. This assessment was an important point since high SGUS scores are associated with more severe glandular and systemic disease (57).

Relative to response of oxidative stress products in blood, GSH and TBARS, there were no differences between the NAC and placebo groups. This finding contrasts with studies involving other clinical conditions (1, 2, 58). However, only SjD patients with low disease activity (ESSDAI≤5) were included in the present study, which could have influenced these results. Indeed, a recent study showed that patients with ESSDAI<5 had lower oxidative stress compared with those with higher disease activity values (16).

Additionally, although the NAC dose in the present study (1,200 mg/day) was higher than that previously used in SjD patients (600 mg/day) (13), a dose-dependent effect should be considered. In this regard, in a randomised double-blind placebo-controlled trial including SLE patients, only NAC doses ≥2,400 mg/day improved disease activ-

ity scores, but with worsening in drug tolerance (59).

Another possible factor of non-response to NAC in the present study could be the severity of glandular involvement, as evidenced by the SGUS score. Indeed, the majority of our patients had ≥2 glands with SGUS score 2 or 3 according to OMERACT (44). However, further sub-analysis including only patients with SGUS score <3 in the four glands in NAC (n=11) and placebo (n=16) groups showed no significant differences between these groups relative to ESSPRI, XI, OSDI and LCQ on D30 (p>0.05) and D90 (p>0.05), respectively.

On the other hand, a limitation of the present study was that it did not assess oxidative stress in saliva, due to the difficulties inherent to SjD, such as the small volume of saliva available, and influence of impaired oral health of these patients (50) on ROS (60). Furthermore, studying GSH levels in this matrix was complex due to interference from intense enzymatic activity (61). Another limitation of our study is that Ocular Staining Score was not obtained due to logistic difficulties during the COVID-19 pandemic.

In conclusion, oral NAC (1,200 mg/day) did not lead to improvement in dryness symptoms according to contemporary validated metrics or reduction in oxidative stress biomarkers in SjD patients with low disease activity. NAC is currently recommended as a rescue therapy for SjD (7, 62, 63), and our rigorous and well-designed study provides novel evidence demonstrating its inefficacy for this indication. In fact, the randomised double-blind placebo-controlled design, coupled with the inclusion of a homogeneous patient population and strict exclusion criteria, ensures the reliability of our results. Future studies with higher doses of NAC could be considered.

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