High levels of rheumatoid factor: association with thyroid antibodies and other analytical thyroid interferences in Sjögren's syndrome

Sirs,

Primary Sjögren's syndrome (pSS) is an autoimmune disease, in which rheumatoid factor (RF) is present in half of the patients (1). In the clinical setting, it is not uncommon for patients with pSS to have both high RF and high thyroid-stimulating hormone (TSH) levels, despite being clinically euthyroid. In the general population, the presence of antibodies to anti-triiodothyronine (anti-T3), anti-thyroxine (anti-T4), thyroid-hormone-binding albumin variants, macro-thyroid-stimulating hormone (macro-TSH), and heterophile antibodies including RF are potential causes of interference with thyroid function tests (TFT) (2-4). These antibodies can cause falsely analytic results by binding to the assay antibodies.

Our aim was to assess a panel of thyroid antibodies (anti-thyroid peroxidase -anti-TPO-, anti-thyroglobulin antibody -anti-Tg-, anti-T3, and anti-T4) in patients with PSS; and to evaluate their association with high RF titres. We also tested the presence of interference of macro-TSH and RF ("macro-RF) with TFTs.

This was a cross-sectional study that included 80 PSS patients according to the ACR/EULAR classification criteria (5), regardless of their thyroid status. We excluded patients with concomitant connective tissue disease. We retrospectively assessed the cumulative ESSDAI score (from diagnosis to last-follow-up). Rheumatoid factor (IgG, IgM, and IgA isotypes) was measured by commercially available ELISA method (Orgentec Diagnostika, Germany). TFT was determined either with commercial systems (CIS-Bio: T4T, FT4, T3T, FT3, TSH, Tg, TGAb; IZOTOP: TPOAb; IMMULITE: TBG) or by home-designed systems (CT3, T4Ab, T3Ab). Interference with macro-TSH and macro-RF was assessed using the polyethylene glycol (PEG) method (3). This study was approved by our IRB. We used descriptive statistics, Pearson correlation coefficient, and logistic regression analysis, and reported OR and 95% CI. We used SPSS 21.

Patients were mainly women (88%), with a mean age of 55.8 years and a median disease duration of 8.7 years. RF was detected in 69 patients (86.2%), being the median titer 220 U/ml (IQR 33.9-1510). The most common anti-thyroid antibody was anti-T4 (n=44, 55%), followed by anti-T3 (n=42, 52.5%), anti-Tg (n=14, 17.5%), and anti-TPO (n=13, 16.2%). There was no correlation between RF and anti-TPO and anti-Tg titers. We found macro-TSH in 5 patients (6.2%) and macro-RF in 24 (30%). When comparing patients in the highest tertile (≥872.36 UI/ml) of RF (n=27) vs the remaining patients (n=53), the former group was younger, had a higher prevalence of antinuclear, anti-Ro/SSA, anti-T3, and anti-T4 antibodies, and a higher cumulative ESSDAI score (Table I). In the logistic regression model, the variables associated with the highest tertile of RF were anti-T4 (OR 1.27, 95% CI 1.05-1.53, p=0.02) and cumulative ESSDAI score (OR 1.02, 95% CI 1.01-1.03, p=0.002).

Herein, we observed a higher prevalence of anti-T4 and anti-T3 antibodies in PSS in comparison to healthy population (0.2-1%), ATD (5-40%), and at two small studies in SS (25%) (6-8). We also evaluated for the first time the presence of macro-TSH (TSH complexed with anti-TSH antibodies) and found it in 6.2% of SS patients. This figure differs markedly from the general population (0.6%) and subclinical hypothyroidism (1.62%) (3, 9). We also observed interference between RF and TFT in 30% of patients. Moreover, patients in the highest tertile of RF had a higher prevalence of anti-T4 (around 50%) and more cumulated disease activity. In this regard, RF has been previously associated with disease activity (10).

In conclusion, pSS patients have a high prevalence of anti-T3, anti-T4, macro-TSH, and macro-RF, all of which are potential sources of TFT interference. Indeed, the highest levels of RF were associated with anti-T4 antibodies and more active disease over time. Our findings should alert clinicians to consider analytical TFT interference in these patients to avoid misdiagnosis of thyroid disease or unnecessary thyroid hormone adjustments.

Table I. Comparison of patients according their RF tertile status.

Variable	Overall cohort n=80	Low/intermediate RF tertiles n= 53	Highest RF tertile n=27	p
Females, n (%)	76 (95)	50 (94.3)	25 (92.6)	1
Age in years, mean ± SD	55.8 ± 13.3	58.9 ± 12.1	49.7 ± 13.5	0.005
Ocular symptoms, n (%)	73 (91.2)	49 (92.5)	24 (88.9)	0.68
Oral symptoms, n (%)	73 (91.2)	48 (90.6)	25 (92.5)	1
NSWSF[+] in ml/15 min, median (IQR)	0.10 (0.05-0.5)	0.2 (0-0.7)	0.1 (0-0.3)	1
Positive Schirmer-I test, n (%)	73 (91.2)	48 (90.6)	26 (96.3)	0.65
Keratoconjunctivitis sicca, n (%)	39/55 (70.9)	26/37 (70.3)	13/18 (72.2)	0.88
Antinuclear antibodies, n (%)	61/76 (80.2)	38 (73.1)	23 (95.5)	0.02
Anti-Ro/SSA antibody, n (%)	68 (85)	41 (77.4)	27 (100)	0.006
Anti-La/SSB antibody, n (%)	40 (50)	24 (45.3)	16 (59.3)	0.34
Cumulative ESSDAI[§] score, median (IQR)	8 (4.25-14)	7 (3-10.5)	12 (6-20)	0.004
Parotid gland swelling, n (%)	38 (47.5)	23 (43.4)	15 (55.5)	0.34
Arthritis, n (%)	34 (42.5)	19 (36.5)	15 (5.6)	0.15
Vasculitis, n (%)	11 (13.8)	6 (11.3)	5 (18.5)	0.49
Lymphadenopathy, n (%)	18 (22.5)	11 (20.8)	7 (25.9)	0.59
Renal, n (%)	5 (6.3)	1 (1.9)	4 (14.8)	0.04
Peripheral neurological, n (%)	15 (18.7)	7 (13.2)	8 (25.9)	0.7
Central neurological, n (%)	2 (2.5)	1 (1.5)	1 (3.7)	0.6
Haematological, n (%)	21 (26.3)	9 (17)	12 (44.4)	0.008
Constitutional, n (%)	10 (12.5)	5 (9.4)	5 (18.5)	0.2
Prednisone ever use, n (%)	46 (57.5)	31 (58.5)	15 (55.6)	0.80
Immunosuppressor ever use, n (%)	47 (57.5)	31 (58.5)	16 (59.3)	1
Antimalarials ever use, n (%)	17 (21.2)	12 (48)	5 (55.6)	1
Low C3, n (%)	7/68 (8.8)	5/44 (11)	2/24 (8.3)	1
Low C4, n (%)	19/68 (23.8)	12/44 (27.3)	7/24 (29.2)	1
Autoimmune thyroid disease, n (%)	31 (38.8)	24 (44.4)	7 (25.9)	0.14
Number of anti-thyroid antibodies, median (IQR)	2 (1-3)	1 (0-2)	2 (1-2)	0.01
Anti-Tg positivity[¥] (≥32 UL/ml), n (%)	14 (17.5)	8 (15.1)	6 (22.2)	0.42
Anti-TPO positivity[¶] (≥38 UL/ml), n (%)	13 (16.2)	10 (18.9)	3 (11.1)	0.37
Anti-T3 antibody[T], n (%)	42 (52.5)	23 (43.4)	19 (70.4)	0.02
Anti-T4 antibody[‡], n (%)	44 (55)	22 (41.5)	22 (81.5)	0.001
Anti-Tg antibody title[¥] (UI/ml), median (IQR)	12 (9-20.7)	10 (8-17)	16 (11-28)	0.10
Anti-TPO antibody title[¶] (UI/ml), median (IQR)	8 (3.2-11)	8 (2.5-11.5)	7 (4-11)	0.83
Macro-TSH[V], n (%)	5 (6.2)	4 (7.5)	1 (3.7)	0.65

[+]NSWSF: Non stimulated whole salivary flow; [§]ESSDAI: EULAR Sjögren's syndrome disease activity index; [¥]Anti-thyroglobulin antibody; [¶]Anti-thyroid peroxidase; [T]Anti-triiodothyronine; [‡]Anti-thyroxine; [V]Macro-thyroid-stimulating hormone.

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References

1. CAFARO G, BURSI R, CHATZIS LG *et al.*: One year in review 2021: Sjögren's syndrome. *Clin Exp Rheumatol* 2021; 39 (Suppl. 133): S3-13. https://doi.org/10.55563/clinexprheumatol/eojaol
2. WAUTHIER L, PLEBAN M, FAVRESSE J: Interferences in immunoassays: review and practical algorithm. *Clin Chem Lab Med* 2022; 60(6): 808-20. https://doi.org/10.1515/cclm-2021-1288
3. FAVRESSE J, BURLACU M, MAITER D, GURSON D: Interferences with thyroid function immunoassays: clinical implications and detection algorithm. *Endocr Rev* 2018; 39(5): 830-50. https://doi.org/10.1210/er.2018-00119
4. NORDEN A, JACKSON R, NORDEN L, GRIFFIN J, BARNES M, LITTLE J: Misleading results from immunoassays of serum free thyroxine in the presence of rheumatoid factor. *Clin Chem* 1997; 43(6 Pt 1): 957-62.
5. SHIBOSKI CH, SHIBOSKI SC, SEROR R *et al.*: 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis* 2017; 76(1): 9-16. https://doi.org/10.1136/annrheumdis-2016-210571
6. SAKATA S, MATSUDA M, OGAWA T, TAKUNO H, MATSUI I, SARUI H: Prevalence of thyroid hormone antibodies in healthy subjects. *Clin Endocrin* 1994; 41(3): 365-70. https://doi.org/10.1111/j.1365-2265.1994.tb02558.x
7. PEREZ-E B, KRAUS A, LÓPEZ G, CIFUENTES M, ALARCÓN-SEGOVIA D: Autoimmune thyroid disease in primary Sjögren's syndrome. *Am J Med* 1995; 99(5): 480-4. https://doi.org/10.1016/s0002-9343(99)80223-x
8. OZGEN A, KESER G, ERDEM N *et al.*: Hypothalamus-hypophysis-thyroid axis, triiodothyronine and thyroid antibodies in patients with primary and secondary Sjögren's syndrome. *Clin Rheumatol* 2001; 20(1): 44-8. https://doi.org/10.1007/s100670170102.9
9. HATTORI N, ISHIHARA T, YAMAGAMI K, SHIMATSU A: Macro TSH in patients with subclinical hypothyroidism. *Clin Endocrinol* 2015; 83(6): 923-30. https://doi.org/10.1111/cen.12643.
10. MAŚLIŃSKA M, MAŃCZAK M, KWIATKOWSKA B: Usefulness of rheumatoid factor as an immunological and prognostic marker in PSS patients. *Clin Rheumatol* 2019; 38(5): 1301-7. https://doi.org/10.1007/s10067-019-04438-z