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RESEARCH ARTICLE

A NEW APPROACH FOR DIAGNOSIS OF THE PRIMARY SJOGREN' SYNDROME

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ABSTRACT

Introduction Aims: Primary Sjögren's syndrome (pSS), is considered a rare disease, but also present in countries with high prevalence not exactly high economic development and technology. The correct diagnosis is made with the criteria established by the American-European Consensus Group (AECG): presence of both ocular and salivary sign, the appearance of specific auto antibodies in serum and / or positive histopathological test of the salivary glands. This diagnosis is most expensive, because it requires the collaboration by several specialists and a laboratory service for histological exams. Our aim is to propose a diagnostic method performed by "physician of general medicine" after a short training perform the Shimmer's test , the salivary flow test, and the detection of antibodies anti-Ro (SSA), anti-La (SSA) with a quick test in the saliva of the patients.

Materials and Methods: Twenty ,of about 8,000 patients, visiting five primary care clinics showed suspected history of (pSS), with dry mouth and dryness conjunctival .On these, was made of salivary flow testing, the detection of auto antibodies ANA / ENA in saliva with Immuno Blot assay, an eye medical history and conduct of the Schirmer' stest. The diagnostic criterion is indicated by (AECG). For the positive patients were then carried all the classics specialist examinations as provided for by the protocol to confirm the diagnosis

Results and Discussion: Seven patients were positive for the Schirmer's test and salivary flow were present in the saliva of four anti-Ro (SSA), and according to the protocol (AECG), suffering (pSS). One patient tested positive for suspected Histones SLE, while for the other two, the immuno blot are very slight bands SMD1 dificili valutare. I by subsequent checks have confirmed the presence of four p SS, a case of SLE and two secondary SS for LES.

Conclusions: This research proposes a new reliable diagnostic procedure, for the diagnosis of pSS accordance with the criteria (AECG)., and may be carried out as basic screening, at low cost, even in simple medical structures by primary care physicians .There may also be used with sufficient diagnostic reliability for the secondary Sjogren's syndrome and other autoimmune diseases, since in most cases the concentration of antibodies in saliva is very low.

INTRODUCTION

Primary Sjögren's syndrome (pSS), or Sicca syndrome, is a chronic inflammatory disease characterized by dry eyes (xerophthalmia) and mouth (xerostomia), an autoimmune pathogenesis, due to a reduction in the secretion of the tear glands and salivary... the SS may also be secondary, in association with other autoimmune diseases such as rheumatoid arthritis, lupus erythematosus and progressive systemic sclerosis pSS may be accompanied by lymphocytic infiltration of different organs and tissues and can lead to nephritis and / or interstitial pneumonia and is associated with an increased risk of developing lymph proliferative disorders. The pathogenesis of pSS is yet to be clarified and that is probably multifactorial, genetic and environmental.

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The importance of the genetic factors according to the most recent studies is confirmed by the association between pSS and some of the HLA phenotypes (Bolstad, 2002), in particular for the genes present: D in the sub-region DR region, encoding HLA class 2 antigens, namely:

- DRW52
- DR3: form associated with anti-SSA antibodies and -SSB
- DR4: form associated with rheumatoid arthritis

The prevalence of the female sex, (9: 1), can be probably attributed to the influence of estrogens (4), which increase the polyclonal activation of B lymphocytes and the formation of auto antibodies, activating the immune system. There are many studies about the correlation between SS and infections; in particular the cytomegalovirus (CMV) and Epstein Barr virus (EBV) (Sjogren Syndrome, 2010), are considered possible inducers of the disease. These viruses have, in fact,

easy access (tropism) to the salivary glands and may trigger autoimmune reactions to the same, with both a nonspecific polyclonal B lymphocyte activation mechanism is a mechanism of molecular mimicry, ie, inducing an autoimmune response to antigens viral however capable of also involving self structures, that is, belonging to the body (Sjogren Syndrome, 2010). As for the pathogenesis, the SS is characterized by polyclonal expansion of B lymphocytes (activation of B type lymphocytes) and by a' hypergammaglobulinaemia with the presence of autoantibodies. The median autoantibodies damage to the tear glands causing the destruction of the secretory duct while the salivary levels cause a swelling of the excretory ducts with subsequent atrophy and destruction of the gland itself. Similar alterations may occur at the level of all the body's glands resulting in dry skin, vulva, the bronchial tree, throat and nasal mucosa. The feeling of ocular and salivary dryness is still common among the population and can have causes other than pSS as hepatitis virus infection, the HIV, amyloidosis, sarcoidosis, multiple sclerosis. Moreover metabolic interference caused by certain medications, diuretics, antihypertensives, antidepressants, alcohol abuse, drugs and stress can cause symptom pictures similar to those of the SS, making it difficult differential diagnosis, which in addition to a thorough medical history, must be supported by production tests of tears and saliva, and by immunological examinations in any case, there are however, beyond the beyond the rare disease definition digits that cannot leave indifferent 'also considering the fsatto that in countries with lower technological development it is underestimated The Following table 1 (Bolstad, 2002) attempts to extrapolate the prevalence rate above for Sjogren's Syndrome to the populations of various countries and regions.

These prevalence extrapolations for Sjogren's Syndrome are only estimates, based on applying the prevalence rates from the US (or a similar country) to the population of other countries, and Therefore may have very limited relevance to the actual prevalence of Sjogren's Syndrome in any region the possibility of a certain diagnosis, is therefore based in the respect of widely accepted protocols as that of 'American, (AEGC), (Vitali *et al.,* 2002), shown in table 1, which provides for the recognition of ocular and oral symptoms and signs, as well as positivity to a any histopathological examination (the minor salivary biopsy), and / or to glandular involvement of tests, research with anti-Ro antibodies (SSA), or anti-La (SSB), in serum. The purpose of this study is to show that this long and expensive diagnostic procedure can also be performed in countries with a shortage of laboratory facilities, and / or specialized personnel as the ophthalmologist the rheumatologist, ENT The problem of the lack of Clinical Laboratory Analysis that must perform serological tests, can be overcome by giving the search of autoantibodies to the same basic doctor with the use of a rapid test as that of IMMUNO -Blot, executed, but not on serum on saliva immediately after the examination of the salivary flow. With this study we intend to then test, as is clearly expressed by (AEGC), the ability to diagnose Sjögren's syndrome outpatients in all patients who present anamnesis suspicious symptoms for at least the presence of a "dry symptom" .and in while deriving the data for the prevalence of the sample under esame. In contemporary to this study, the same diagnostic procedure is carried out in facilities where there are ophthalmologists and ENT specialists, and immunoblot test performed by nurses, in order to verify the reliability of the procedure.

MATERIALS AND METHODS

E 'it was carried out a study with collection of anamnestic data on approximately 8000 patients ,,regarding the presence of symptoms and signs, on a possible onset of primary Sjögren's syndrome, namely:

- collecting history data related to ocular symptoms and execution of the Schirmer test: reference value for positivity: ≤ 5mm in 5 minutes:
- examination the salivary flow reference value for positivity: ≤ 1ml /5 min
- examination and research of anti -Ro (SSA), and / or anti -La (SSB), in the sample of saliva resulting from the measure of the salivary flow, using Immuno-Blot technique performed with the kit of the AESKU DIAGNOSTIC, distributed by GRIFOLS ITALY*. the kit contains 24 sticks and has a cost of about 150 €.

Immuno-Blot Assay. Protocol of the test method and modified for use on human saliva. The antigens are fixed in parallel lines on a nitrocellulose membrane. The membrane is stabilized to prevent non-specific reactions, and the strips with specific antigens fixed in well defined positions, are incubated, with agitation for 20 minutes, in the sample of saliva as such in the amount of 1 ml. The antibodies of the subject if present in the sample bind with the antigen e.la unbound fraction is eliminated subsequently, by washing by means of a syringe with 3 ml (x 3) of the buffer solution The anti-human immunoglobulins are then conjugated with 0.8 cc of horseradish peroxidase (conjugate) are incubated and shaken for 20 min and react with the sample antibody antigen complex. The unbound conjugate is subsequently eliminated with only a wash of five cc of buffer solution, which is followed by the addition of 0.5 ml of TMB substrate, incubated for 20 minutes, which causes an enzymatic reaction that converts it into a precipitate color blue

RESULTS

During a period of about a year he has been made a kind of medical history screening of about 8,000 patients in five local clinics run by general practitioners. With Question wording anamnestic towards the recognition of presence of the main ocular and oral symptoms as expressed by the protocol established by the American-European Consensus Group, twenty patients were identified on which to carry out the Schirmer test, the test of salivary flow and last for the detection of autoantibodies in the saliva by means of the use of the Immuno method –Blot. This screening procedure allows to obtain at the end of the tests confirm the presence or not of Sjogren's syndrome in accordance with the protocol, who see table 1, requires that they be satisfied for a certain positivity pSS of the following conditions:

a) The presence of at least four of the six criteria, provided that in them is positive or the histopathological criterion or one serological; in our case the salivary
b) The presence of at least three of the four objective criteria and that is between III, IV, V, and VI (see Table 2)

Table 1. Who report on sjogren sindrome in 2008 year

Country/Region	Extrapolated Prevalence	Population Estimated Used
Sjogren's Syndrome in North America (Extrapolated Statistics)		
USA	1,079,615	293,655,405[1]
Canada	119,514 WARNING! (Details)	32,507,874[2]
Mexico	385,880 WARNING! (Details)	104,959,594[2]
Sjogren's Syndrome in Central America (Extrapolated Statistics)		
Belize	1,003 WARNING! (Details)	272,945[2]
Guatemala	52,502 WARNING! (Details)	14,280,596[2]
Nicaragua	19,704 WARNING! (Details)	5,359,759[2]
Sjogren's Syndrome in Caribbean (Extrapolated Statistics)		
Puerto Rico	14,330 WARNING! (Details)	3,897,960[2]
Sjogren's Syndrome in South America (Extrapolated Statistics)		
Brazil	676,842 WARNING! (Details)	184,101,109[2]
Chile	58,176 WARNING! (Details)	15,823,957[2]
Colombia	155,554 WARNING! (Details)	42,310,775[2]
Paraguay	22,762 WARNING! (Details)	6,191,368[2]
Peru	101,265 WARNING! (Details)	27,544,305[2]
Venezuela	91,975 WARNING! (Details)	25,017,387[2]
Sjogren's Syndrome in Northern Europe (Extrapolated Statistics)		
Denmark	19,902 WARNING! (Details)	5,413,392[2]
Finland	19,170 WARNING! (Details)	5,214,512[2]
Iceland	1,080 WARNING! (Details)	293,966[2]
Sweden	33,038 WARNING! (Details)	8,986,400[2]
Sjogren's Syndrome in Western Europe (Extrapolated Statistics)		
Britain (United Kingdom)	221,583 WARNING! (Details)	60,270,708 for UK[2]
Belgium	38,045 WARNING! (Details)	10,348,276[2]
France	222,147 WARNING! (Details)	60,424,213[2]
Ireland	14,593 WARNING! (Details)	3,969,558[2]
Luxembourg	1,701 WARNING! (Details)	462,690[2]
Monaco	118 WARNING! (Details)	32,270[2]
Netherlands (Holland)	59,993 WARNING! (Details)	16,318,199[2]
United Kingdom	221,583 WARNING! (Details)	60,270,708[2]
Wales	10,727 WARNING! (Details)	2,918,000[2]
Sjogren's Syndrome in Central Europe (Extrapolated Statistics)		
Austria	30,054 WARNING! (Details)	8,174,762[2]
Czech Republic	4,581 WARNING! (Details)	1,0246,178[2]
Germany	303,031 WARNING! (Details)	82,424,609[2]
Hungary	36,883 WARNING! (Details)	10,032,375[2]
Liechtenstein	122 WARNING! (Details)	33,436[2]
Poland	142,008 WARNING! (Details)	38,626,349[2]
Slovakia	19,939 WARNING! (Details)	5,423,567[2]
Slovenia	7,395 WARNING! (Details)	2,011,473 [2]
Switzerland	27,392 WARNING! (Details)	7,450,867[2]
Sjogren's Syndrome in Eastern Europe (Extrapolated Statistics)		
Belarus	37,906 WARNING! (Details)	10,310,520[2]
Estonia	4,932 WARNING! (Details)	1,341,664[2]
Latvia	8,479 WARNING! (Details)	2,306,306[2]
Lithuania	13,264 WARNING! (Details)	3,607,899[2]
Russia	529,316 WARNING! (Details)	143,974,059[2]
Ukraine	175,485 WARNING! (Details)	47,732,079[2]
Sjogren's Syndrome in the Southwestern Europe (Extrapolated Statistics)		
Azerbaijan	28,927 WARNING! (Details)	7,868,385[2]
Georgia	17,256 WARNING! (Details)	4,693,892[2]
Portugal	38,691 WARNING! (Details)	10,524,145[2]
Spain	148,091 WARNING! (Details)	40,280,780[2]
Sjogren's Syndrome in Southern Europe (Extrapolated Statistics)		
Greece	39,145 WARNING! (Details)	10,647,529[2]
Italy	213,446 WARNING! (Details)	58,057,477[2]
Sjogren's Syndrome in the Southeastern Europe (Extrapolated Statistics)		
Albania	13,032 WARNING! (Details)	3,544,808[2]
Bosnia and Herzegovina	1,498 WARNING! (Details)	407,608[2]
Bulgaria	27,639 WARNING! (Details)	7,517,973[2]
Croatia	16,532 WARNING! (Details)	4,496,869[2]
Macedonia	7,500 WARNING! (Details)	2,040,085[2]
Romania	82,189 WARNING! (Details)	22,355,551[2]
Serbia and Montenegro	39,801 WARNING! (Details)	10,825,900[2]
Sjogren's Syndrome in Northern Asia (Extrapolated Statistics)		
Mongolia	10,115 WARNING! (Details)	2,751,314[2]
Sjogren's Syndrome in Central Asia (Extrapolated Statistics)		
Kazakhstan	55,675 WARNING! (Details)	15,143,704[2]
Tajikistan	25,777 WARNING! (Details)	7,011,556 [2]

……..Continued

Uzbekistan	97,097 WARNING! (Details)	26,410,416[2]
Sjogren's Syndrome in Eastern Asia (Extrapolated Statistics)		
China	4,775,174 WARNING! (Details)	1,298,847,624[2]
Hong Kong s.a.r.	25,202 WARNING! (Details)	6,855,125[2]
Japan	468,136 WARNING! (Details)	127,333,002[2]
Macau s.a.r.	1,637 WARNING! (Details)	445,286[2]
North Korea	83,446 WARNING! (Details)	22,697,553[2]
South Korea	177,329 WARNING! (Details)	48,233,760[2]
Taiwan	83,639 WARNING! (Details)	22,749,838[2]
Sjogren's Syndrome in Southwestern Asia (Extrapolated Statistics)		
Turkey	253,286 WARNING! (Details)	68,893,918[2]
Sjogren's Syndrome in Southern Asia (Extrapolated Statistics)		
Afghanistan	104,829 WARNING! (Details)	28,513,677[2]
Bangladesh	519,634 WARNING! (Details)	141,340,476[2]
Bhutan	8,035 WARNING! (Details)	2,185,569[2]
India	3,915,700 WARNING! (Details)	1,065,070,607[2]
Pakistan	585,280 WARNING! (Details)	159,196,336[2]
Sri Lanka	73,180 WARNING! (Details)	19,905,165[2]
Sjogren's Syndrome in Southeastern Asia (Extrapolated Statistics)		
East Timor	3,747 WARNING! (Details)	1,019,252[2]
Indonesia	876,665 WARNING! (Details)	238,452,952[2]
Laos	22,309 WARNING! (Details)	6,068,117[2]
Malaysia	86,479 WARNING! (Details)	23,522,482[2]
Philippines	317,065 WARNING! (Details)	86,241,697[2]
Singapore	16,006 WARNING! (Details)	4,353,893[2]
Thailand	238,476 WARNING! (Details)	64,865,523[2]
Vietnam	303,907 WARNING! (Details)	82,662,800[2]
Sjogren's Syndrome in the Middle East (Extrapolated Statistics)		
Gaza strip	4,871 WARNING! (Details)	1,324,991[2]
Iran	248,173 WARNING! (Details)	67,503,205[2]
Iraq	93,289 WARNING! (Details)	25,374,691[2]
Israel	22,790 WARNING! (Details)	6,199,008[2]
Jordan	20,629 WARNING! (Details)	5,611,202[2]
Kuwait	8,299 WARNING! (Details)	2,257,549[2]
Lebanon	13,886 WARNING! (Details)	3,777,218[2]
Saudi Arabia	94,838 WARNING! (Details)	25,795,938[2]
Syria	66,238 WARNING! (Details)	18,016,874[2]
United Arab Emirates	9,279 WARNING! (Details)	2,523,915[2]
West Bank	8,497 WARNING! (Details)	2,311,204[2]
Yemen	73,620 WARNING! (Details)	20,024,867[2]
Sjogren's Syndrome in Northern Africa (Extrapolated Statistics)		
Egypt	279,843 WARNING! (Details)	76,117,421[2]
Libya	20,704 WARNING! (Details)	5,631,585[2]
Sudan	143,927 WARNING! (Details)	39,148,162[2]
Sjogren's Syndrome in Western Africa (Extrapolated Statistics)		
Congo Brazzaville	11,022 WARNING! (Details)	2,998,040[2]
Ghana	76,312 WARNING! (Details)	20,757,032[2]
Liberia	12,465 WARNING! (Details)	3,390,635[2]
Niger	41,766 WARNING! (Details)	11,360,538[2]
Nigeria	65,258 WARNING! (Details)	12,5750,356[2]
Senegal	39,897 WARNING! (Details)	10,852,147[2]
Sierra leone	21,631 WARNING! (Details)	5,883,889[2]
Sjogren's Syndrome in Central Africa (Extrapolated Statistics)		
Central African Republic	13,759 WARNING! (Details)	3,742,482[2]
Chad	35,068 WARNING! (Details)	9,538,544[2]
Congo kinshasa	214,400 WARNING! (Details)	58,317,030[2]
Rwanda	30,289 WARNING! (Details)	8,238,673[2]
Sjogren's Syndrome in Eastern Africa (Extrapolated Statistics)		
Ethiopia	262,266 WARNING! (Details)	71,336,571[2]
Kenya	121,257 WARNING! (Details)	32,982,109[2]
Somalia	30,531 WARNING! (Details)	8,304,601[2]
Tanzania	132,613 WARNING! (Details)	36,070,799[2]
Uganda	97,023 WARNING! (Details)	26,390,258[2]
Sjogren's Syndrome in Southern Africa (Extrapolated Statistics)		
Angola	40,362 WARNING! (Details)	10,978,552[2]
Botswana	6,026 WARNING! (Details)	1,639,231[2]
South Africa	163,413 WARNING! (Details)	44,448,470[2]
Swaziland	4,298 WARNING! (Details)	1,169,241[2]
Zambia	40,535 WARNING! (Details)	11,025,690[2]
Zimbabwe	13,499 WARNING! (Details)	1,2671,860[2]
Sjogren's Syndrome in Oceania (Extrapolated Statistics)		
Australia	73,210 WARNING! (Details)	19,913,144[2]
New Zealand	14,683 WARNING! (Details)	3,993,817[2]
Papua New Guinea	19,927 WARNING! (Details)	5,420,280[2]

Table 2. Diagnostic criteria of the American-European Consensus Group in SS

I. Ocular symptoms: a positive response to at least one of the following questions:
1. Dry and ocular discomfort and persistent daily for a period exceeding three months
2. Feeling recurrent sand in the eyes and foreign body
3. Use of artificial tears more than three times a day
II. Oral symptoms: a positive response to at least one of the following questions:
1. Feeling of dry mouth daily for a period exceeding three months
2. parotid swelling recurrent or persistent
3. Use of liquids for ingestion of dry foods
III. Ocular signs: positive to at least one of the following tests:
1. Schirmer test (<5 mm in 5 minutes)
2. Test the Rose Bengal (score> 4 : by Von Bijservedl)
IV. Histopathology: Exhibit of focal lymphocytic sialadenitis in biopsy minor salivary glands obtained from an apparently normal mucosa and, with a focus score ≥ 1, defined as the number of foci of lymphocytes adjacent to the berries of apparently normal mucosa and containing more than 50 lymphocytes per 4mm2 of glandular tissue
V. Salivary glands: salivary gland involvement documented by the positivity of at least one of the following tests:
1. scintigraphy of the salivary glands
2. Sialography parotid
3. Measurement of unstimulated salivary flow (≤1,5 ml in 15 minutes)
VI. Autoantibodies: presence in the serum of the following antibodies: anti-Ro (SSA) and / or anti-La (SSB)
For primary Sjögren's syndrome
In patients with no other pathology potentially associated, the primary SS may be defined as
a) The presence of at least 4 of the 6 criteria is indicative of primary SS, provided they are satisfied theV criteria (histopathology) or VI (serology).
b.) Presence of at least three of the four objective criteria (ie, III, IV, V, VI)
For secondary Sjögren's syndrome
In patients with a potentially associated disease (for example another tissue disease connective), the presence of I or II of the criterion, plus at least two of the criteria III, IV and V, can be indicative of secondary Sjögren's.

Table 3. Screning evaluation for oral and eyes symptoms

Pz	Oral dryeness	Parotid swelling	use liquid intake for dry foods	dry eye discomfort	Sensation sand in eye	Use of tears artificia
1F	+	-	+	+	-	-
2F	+	-	+	+	+	-
3F	+	+	+	+	+	-
4F	+	-	+	+	-	-
5F	+	-	-	-	-	-
6F	+	-	+	+	+	-
7F	+	-	+	+	+	-
8M	+	-	+	+	+	-
9F	+	-	+	+	+	-
10F	+	+	+	+	-	-
11F	+	-	+	-	-	-
12M	+	-	+-	-	-	-
13F	+-	-	+	-	-	-
14F	+	-	+	-	-	-
15F	+	-	-	+	+	-
16F	-	-	+	-	-	-
17F	+	.-	+	+	-	-
18F	+	-	-	+	+	-
19F	+	-	-	+	-	-
20F	+	-	-	+	+	-

Legend ;(+) = This Symptom (-) Symptom Absent ; (+) = this symptom (-) symptom absent;
Key (+) schirmer test ≤ 5mm / 15 minutes (+) test salivary flow S≤1.5 ml/ 15 minu

Table 4. Test results schirmer; saliva test flow -research antibodies ANA/ENA

Patient	Schirmer test	Flow test	Anti-Ro(SSA)	Anti –La (SSA)	Other ana /ENA
1F	+	+	+	-	-.
2F	+	+	+	-	-
3F	+	+	-	-	-
4F	+	+	-	-	.
5F	+	+	+	-	
6F	+	+	+	-	
7F	+	+	-	-	HISTONES
8 M	+	-	-	-	
9F	-	+	-	-	-
10F	-	+	-	-	-
11F	+	-	-	-	
12M	-	+	-	-	
13F	-	-	-	--	
14F	-	-	-	-	
15F	-	+	-	-	
16F	-	+	-	-	
17F	-	-	-	-	
18F	-	-	-	-	
19F	-	-	-	-	
20F	+	-	-	-	

Table 5. Processing of data on the concentrations (mg / l) of immunoglobulins in plasma and the saliva of the patients with SS, derived from the work of Halse K-A9, and Ben-Chetrit10

Total Ig	Plasma	Saliva
Range	7300 -50300	1 -592
average (SD)	24 500 (12900)	112 (140)
median	26 500	94
p-value	≤ 0.0001	
IgA		
range	430 - 8000	11- 231
average (SD)	2800 (1900)	97 (68)
median	2260	79
p -value	≤ 0.0001	
IgM		
gamma	258 - 1.614	1- 26
average(SD)	783 (352)	6 (7)
mediana	735	4
p.- value	≤ 0.0001	

Table 6. Processing of the data of the literature 9, 10,11 for the values of Ro 52 kD, 60 kD Ro, La and 48 kD IgG, IgA, and IgM isotypes in plasma and saliva of patients with SS The results are expressed as antigen antibody speci®c kU / mg total immunoglobulin isotype. The values refer to comparison p-value according to the Wilcoxon test between antibody levels in plasma and saliva

	Ro 52kD		Ro 60 kD		La 48 kD	
	Plasma	Saliva	Plasma	Saliva	Plasma	Saliva
IgG						
Range	2 – 6973	0 – 6468	0 – 540	0 – 309	1 – 2634	0 - 4878
Average (SD)	2258 (2517)	1689 (2087)	76 (148)	54 (91)	414 (705)	781 (1187)
median	1061	913	5	5	105	518
p value	0.01		0.39		0.57	
IgA						
Range	1-351	0 -439	2 – 72	0 – 330	1 – 2921	0-7578
Average(SD)	73 (91)	134 (156)	18 (26)	70 (113)	323 (705)	898
median	57	63	5	22	77	208
p value	0.30		0.20		0.001	
IgM						
Range	10 -824	0 – 1162	6 – 104	0 – 451	2 – 1588	0 - 2797
Average(SD)	165 (218)	316 (389)	29 (25)	63 (117)	356 (484)	529 (839)
median	60	156	20	0	117	232
p value	0.047		0.96		0.51	

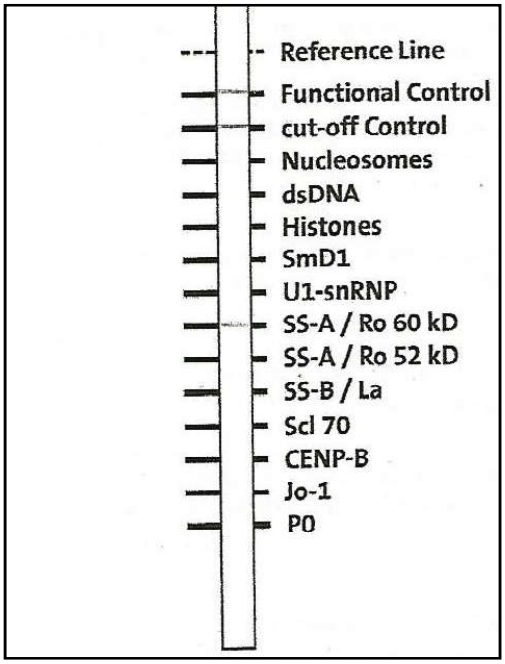

Image 2. Positive histones control

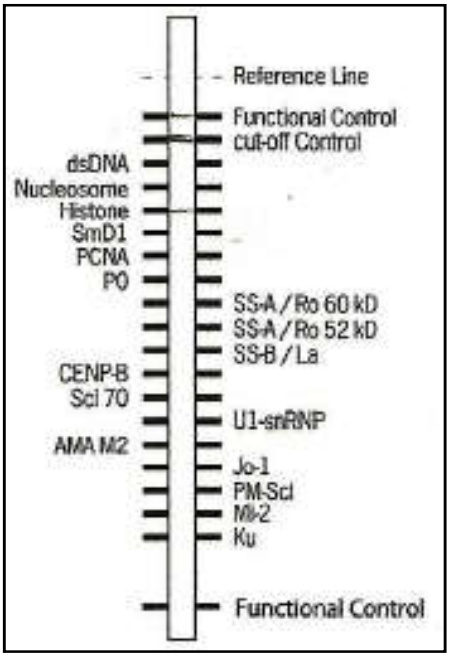

Image 1. SS-A/Ro60 KD Positive Control

From a first reading of the admission medical history of patients see Table 2, you can see for them the following information

a) Prevalence of female = 90%
b) The prevailing oral symptom, dry mouth = 80%
c) The prevailing oral symptoms: dry and / or ocular discomfort 70%

Table 3 also deduced the prevalences of objective signs

a) oral sign, positive flow test in 35% of cases
b) Eye Schirmer test positive sign in 35 % of cases

The results in Table 3 indicate that in four patients is positive research SSA autoantibodies, (see Image 1). For these you can establish a firm diagnosis of primary SS according AECG criteria in Table 4 it is noted that in a case presents saliva, positivity Histones, (Image 2), at the hands of other autoimmune diseases, probably LES. For two others positive patients to eye and ENT signs, the immuno-blot test show the appearance of a slight band relative to SMD1. The diagnosis of pSS in the test-positive patients was confirmed later with the execution of the serological tests that confirmed the presence of autoantibodies, in addition to their title the results indicates that the sensitivity of the Protocol is 100%.

DISCUSSION

The diagnosis of SS is typically obtained after a diagnostic procedure complex enough, the patient complaint some disorders, sometimes only defined as sensations not well specified especially in the time and therefore not properly classifiable as required by AECG protocol. In addition, the physician will often tend to overlook some of these disorders misled during anamnesis by other confounding factors such as taking medication, stress, consumption and / or abuse of cigarettes and alcohol The proposal, which is the basis of the following study instead provide a clear and strict protocol for the following diagnostic steps to be performed in the outpatient setting:

a) Medical history, to search for any oral and ocular symptoms, according to the protocol AECG
b) In a positive response to at least two of the parameters identified in the protocol, such as dry mouth and / or eye, are made of the Schirmer test routine and the flow test
c.) based on these results, and being in the outpatient setting, and then not being able to perform histological tests, to have a confirmation of the presence of a pSS, must be made the search for anti-Ro antibodies (SSA), anti-La (SSA), the patient's saliva with Immuno Blot method. This test is sold by manufacturers to be used on serum samples and is not expected to suo'utilizzo investigations on human saliva. This limit is rightly place, because saliva by its nature has many diagnostic advantages, such as non-invasive, easy collection, and the relative stability of the sample differently from that serum, obtained by venipuncture, and plasma, after centrifugation requires a conservation to ± 20³Cfino analyzed. The saliva limit is generally due to the anabolites concentration to search that is much lower than in serum (Pink *et al.,* 2007). In particular, this research Immuno Blot assay in serum ANA and ENA the concentration of which is in saliva even a thousand times lower, (Haga *et al.,* 1999), see Table. 5) In this study, it has been postulated to be able to use this method, according to the recent studies on salivary composition of immunoglobulins, and in particular those on the concentration of anti-Ro (SSA), and anti-La (SSA), (Halse *et al.,* 2000) as you can see from the table (Vitali *et al.,* 2002), concentrations in saliva and serum Ig are very similar and the study results also reveal a significant statistical correlation, this work also demonstrates that the pSS increases levels of immunoglobulin in the plasma but, proportionally much more in saliva. The hypotheses that can justify such concentrations are essentially two, and that is that there is a transfer of proteins from blood vessels spraying the tissues of the salivary glands, but more likely as recent studies have shown, (Ben-Chetrit *et al.,* 1993; Busamia *et al.,* 2010), for a' hyperproduction of Ig response to the inflammatory process. This research has highlighted the results correlated to the literature cited: they are bands were detected in seven samples of complex / conjugate autoantibody-Ro (SSA), very evident and the same intensity of the control line, (see Images 1). In additio we have for a single patient the presence, of his tone (see Image 2), and traces of SmD1 for two others men, without the possibilty to confirm a diagnosis of secondary sjogren 'sindrome. In this study we were evaluated, the intensity of the bands if not visually, but it is possible to quantify the concentration of autoantibodies with the use of a scanner and dose the exact title within the stated ranges. Outside the territorial structures serological analyzes were performed to confirm the results of the tests on the saliva. With the benchmarks of standard laboratory values accepted for ANA and ENA titles, the results of the analysis confirmed the diagnosis detected in this study. In this context it can be said that the determination of the qualifications is unnecessary for the purposes of research and the proposal of this work being the purpose of the proposal is to make a diagnosis of pSS, regardless of the stage of disease activity that under no circumstances, can be correlated with the title of the various autoantibodies. In fact, the value that is given to the result of ENA is indicative of a more or less marked positivity by convention, are an exception, but this case does not fall within the scope of this study, the values of anti1-RNP, which if detected at high titer, are specific markers for mixed connective tissue. In the case of a detection of autoantibodies SSA, these fluctuate over time, not disappearing in the disease remission phases. In any case, taking into account that according to this study, which has as its ultimate goal only a proper diagnosis of pSS, and not the state of activity, just remember that the repetition of the research test the saliva of ENA, can be reasonably be taken into account only in the case of a secondary Sjogren's syndrome to verify a modification of the clinical picture, since at present antibodies, one can add other positivity of ENA.

Conclusions

The problem of diagnosis of pSS has been addressed in this preliminary search not only for the purely biomedical aspect, in agreement with the AECG protocol, but also for the logistics, time and cost Certainly with this type of integrated approach, should be reassessed and skills of general practitioners, which in low economic and technological developing countries, are faced with solving a problematic diagnostic and complex, but did not seek medical pole

structures. As mentioned in the introduction of this work, they are examining the first results obtained with this system with the same methods performed by a specialist in ophthalmology and otolaryngology what. The first results are in perfect alignment and validate the results of this study.

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