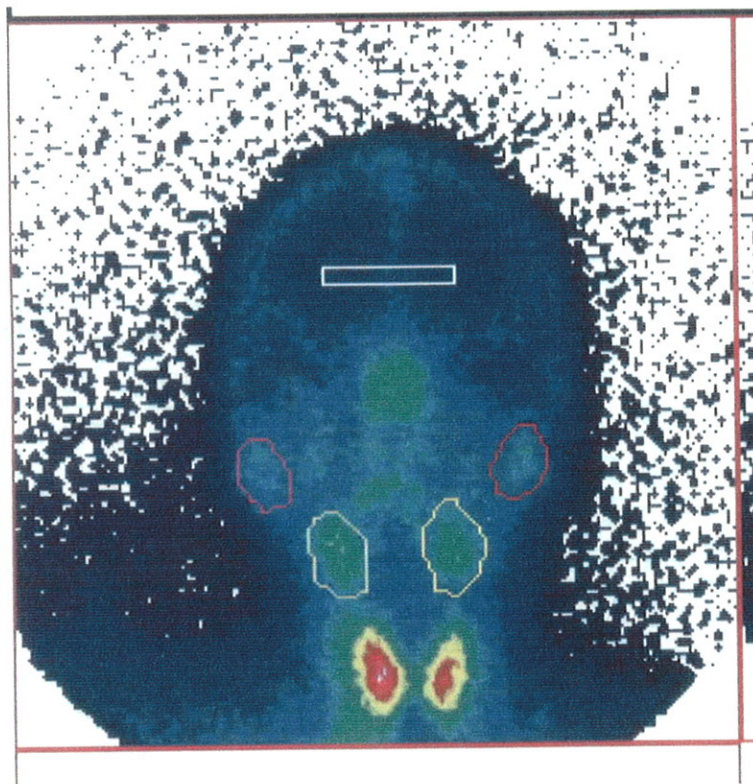# QUANTITATIVE SALIVARY GLAND SCINTIGRAPHY

# IN THE EVALUATION OF PATIENTS WITH SICCA

# SYMPTOMS.

5.årsoppgave I Stadium IV – medisinstudiet ved Universitetet i Tromsø.

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# CONTENTS

# ABSTRACT

**Background:** Abnormal salivary gland scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer is a criterion of most existing classification schemes for Sjøgren's syndrome (SS). However, this description is subjective as it is reader-dependent.

**Objectives:** To determine and compare quantitative data obtained with computerised scintigraphy in patients with SS, keratoconjunctivitis sicca (KCS) and controls.

**Methods:** We studied 24 patients (92% female, median age 50 years, range 27 – 63) with SS (n=8, all fulfilling the preliminary European classification criteria) or KCS (n=16) and 8 controls. Clinical data were extracted from patient files. Salivary Gland Scintigraphy (SGS) was performed with a gamma camera equipped with a high sensitive collimator. The radioactive isotope Pertecnetat (eluat) was injected intravenously in a dose of 200 MBq and a dynamic acquiring protocol was started simultaneously. The data were digitised and computer stored while plotted on time-activity curves. Images were acquired every 30-60 seconds during a period of 15 minutes that describes uptake, concentration phase and spontaneous secretion. Patients were then given lemon juice to study the secretion during the subsequent 5 minutes. Data were analysed with non-parametric tests and regression techniques.

**Results:** Uptake in and excretion by parotic glands was lower with increased time to maximal uptake in SS patients. Significantly more SS patients had abnormal SGS findings than KCS patients. No correlation was found between SGS data and age, focusscore, ESR, serumcreatinin or Immunoglobulin levels.

**Conclusion:** Quantitative SGS is a feasible tool in the classification of SS patients, although it correlates poorly with clinical features in SS and KCS patients.

# INTRODUCTION

Sjøgren's syndrome, named after the Swedish ophthalmologist Henrik Sjøgren (1899-1986)[2], is a chronic inflammatory autoimmune disease of the exocrine glands. Immune-mediated destruction of the salivary and lachrymal glands in particular results in dryness of the eyes (xerophtalmia) and mouth (xerostomia)[3-4]. The role of the frequently present autoantibodies against nuclear antigens and immunoglobulin G in this (often localised inflammatory) process is not well defined. Other exocrine glands in the rest of the body may be affected as well and result in dryness of the skin, nose, throat, airways, vagina and urethra. While reduced exocrine function in the GI-tractus is less frequently symptomatic, it can for instance result in chronic atrophic gastritis and exocrine pancreatic failure leading to malabsorption. The disease is usually multisymptomatic as patients are also prone to systemic manifestations such as extreme tiredness, muscle and joint tenderness, frank arthritis, vasculitis as well as various types of renal, lung, haematological and nervous system involvement[5]. In addition to the risk for infectious complications that follows the reduced epithelial cell function, SS is also associated with a relative risk for heart block in the newborn of a mother with SS (1: 500-600) and malignant B cell lymphoma (relative risk of 40)[5,6]. The disease may be seen alone (primary SS) or in association with other autoimmune diseases (secondary SS), most commonly Rheumatoid Arthritis (RA) and systemic lupus erythematosus (SLE), but also polymyositis, systemic sclerosis, vasculitis[7]. Primary SS is said to be the most common of the systemic connective tissue diseases, with a prevalence of 2-3 %, affecting most often adults with a female: male ratio of 10:1[8]. However, figures on the prevalence of SS differ considerably due to controversies regarding its classification criteria. The prevalence in population studies is reported higher than studies in a clinical setting, because the subjects identified as having SS in population studies often have mild complaints and most of them have not been aware of having a disease[5].

As there is a long delay from onset of initial symptoms to evolution of the full-blown clinical picture, patients may live with vague symptoms for years before the diagnosis of SS is made. No single test alone is specific (diagnostic) for SS and the diagnosis is therefore based on several criteria, a classification set. There currently exist nine different classification systems[6] for SS (see addendum for details). Some criteria sets are based on objective tests alone, while others rely on subjective symptoms as well. Most criteria-sets focus on salivary and lacrymal gland function, and do not reflect the other many problems patients may have. However, none of the classification criteria have been validated and universally accepted. While such classification criteria often serve as diagnostic criteria in practice, this is only correct when sensitivity and specificity of the criteria are close to 100%.

The European Classification set[9] is used to classify the patients into SS groups. The European Study Group on Diagnostic Criteria for SS had a preliminary workshop in Italy in 1988, and came up with the European I criteria set. The set were approved during a second meeting in 1992, and published in 1993[10]. Between 1993 and 1995 this criteria set was validated in different European centres, by testing the preliminary disease criteria set on a (new) population of clinically defined patients and controls. This resulted in the modified European-II criteria set, published in 1996[9]. The European-II criteria include 6 items. The 6 items are: I: ocular symptoms, II: oral symptoms, III: ocular signs (Schirmer I, ocular dye), IV: Histopathology, V: Instrumental evidence of salivary gland involvement (whole sialometry, parotid sialography, salivary gland scintigraphy), VI: autoantibodies (SSA/SSB). The classification requires the presence of any four of the six items in patients without any potentially associated disease, and there are also exclusion criteria.

Because of their concomitant musculoskeletal complaints, SS patients are mainly referred to rheumatologists for evaluation. At the department of Rheumatology at the University Hospital North Norway, the diagnosis of SS is made when sicca symptoms are confirmed by objective tests (Schirmer < 5mm and/or sialometry (salivary flow rate) < 1.5 ml after 15 minutes) and also require either the presence of autoantibodies against the intracellular antigens SSA / SSB or a focus score over 1 at salivary gland biopsy.

Different investigation methods are available for the detection of the oral component of the syndrome. These include measurements of salivary flow rate, radiographic contrast sialography, salivary gland scintigraphy and salivary gland biopsy from lower lip. In the European criteria, positive histopathology (more than 50 lymphocytes per 4 mm2 salivary gland) is considered as one separate criterion, while the other investigational methods for the oral component are considered as one criterion, when at least one is abnormal. Lower lip biopsy are invasive, involves some degree of discomfort to the patient and carry a 10-20 % risk of postbiopsy numbness, as small nerve fibres may be damaged during the procedure. As the inflammatory process seen on salivary biopsy results in destruction of acini and ducts, the excretion of saliva will decrease. Salivary gland scintigraphy (SGS) uses technetium pertechnetate to observe and measure the uptake and excretion by the major salivary glands and thus provides a detailed functional evaluation of all four major salivary glands. This study was performed to evaluate the diagnostic value of quantitative SGS in patients evaluated at the rheumatologic department for the presence of SS, in the hope to ultimately find a reliable alternative for salivary gland biopsy.

## MATERIAL AND METHODS

### Patients

The raw data consisted of a cohort of 185 patients with an ICD-9 CR code of 710.2 (sicca syndrome) or 710.9 (diffuse connective tissue disease), who were seen at the department of Rheumatology between 01.01.92 and 31.12.95. This time period was chosen as SGS was performed as a pilot project between May 20 and October 21, 1994. The hospital charts of all 185 patients were reviewed, and patients in whom SGS was performed were selected for further study. None of the 24 patients thus selected fulfilled the exclusion criteria for SS, as defined by the European Classification criteria[9]. The patient files of the 24 selected patients were then extracted for clinical data by one investigator with the use of a predefined data collection form. Data about duration of symptoms/sickness, presence of dry eye and mouth (as defined by the Vitali criteria[9]), results of Schirmer's test, Rose Bengal dye uptake labial biopsy, parotid sialography and unstimulated salivary flow, laboratory tests for anti-Ro/SS-A and anti-La/SS-B were recorded. The files were also extracted for medication use and blood test results (ESR, WBC, Lymphocytes, CRP, Hb, creatinin and liver status (ASAT, ALAT), as well as immunological blood test (ANA, RF Latex and Waaler method, anti-ds DNA (Immunofluorescence and ELISA.)

### Study design

This was a cross sectional study in which scintigraphy was performed as an extended part of the clinical evaluation for SS after informed consent was given. The control group for scintigraphy findings consisted of eight patients with suspected sickness of the thyroid. The hospital records of these patients of these patients were not available, so the only information about the control group comes from the scintigraphy chart (See figures 1 and 2). The chart gives information about initials, date of birth, examination date and scintigraphic data. Information about dry eye and mouth symptoms and antibodies has not been taken into account, although it must be mentioned that no one of these patients were under suspicion of having SS.

### Clinical classification of patients

All patients were given a diagnosis based on the available clinical data, where the result of the scintigraphy was not taken into account (Note: the maximal number of SS criteria a patient could fulfill was thus five instead of six). Patients were classified ass SS when they fulfilled at least four of the European Criteria set, and as having isolated keratoconjunctivitis (KCS), when they fulfilled less than four of the European Criteria set.

## Scintigraphic method

After informed consent, scintigraphy was performed with a gamma camera (Sophia D57) equipped with a high sensitive collimator (General Purpose). The head was immobilized in an anterior position relative to the camera. The projection was from anterior, and the camera was adjusted above the parotid and submandibular glands, and the mouth.

The radioactive isotope Pertecnetat (eluat) was injected intravenously in a dose of 200 MBq. in both the control group and the patient group. A dynamic acquiring protocol was started simultaneously. The data was digitized and stored on a computer (the software was not documented) that plotted data on separate time-activity curves for all four major salivary glands after manual selection of the glands (See figures 1 and 2). Initially the protocol was designed to acquire images every 30 seconds during a period of 20 minutes, where the first 15

 minutes represent the result of uptake, concentration phase and spontaneous secretion. After 15 minutes the patient was given lemon juice to stimulate secretion; then the mouth was then flushed three times with water to empty the oral cavity for radioactivity and during the subsequent 5 minutes imaging continued. After the first 20 studies (7 controls, 4 SS patients, 9 KCS) a change in protocol was made and in subsequent patients (1 control, 2 SS and 9 KCS patients) images were acquired every 60 seconds over a period of 40 minutes, with secretion stimulation by lemon juice after 30 minutes, followed by a secretion phase of last 10 minutes.

The computer program calculated four different variables (C%, E%, T max and Max counts) from the time-activity curve for each gland. The C% represented the percent distribution of total uptake in each different gland. The E% is the stimulated secretion (i.e. total secretion including spontaneous secretion in that period). E% was calculated as the % reduction in concentration from lemon juice was given until the lowest value achieved. (E%= (1-Min/Max) * 100%.) The Tmax was the time needed to achieve maximum concentration. Due to technical reasons as well as the change in protocol, this required visual reanalysis of the curve in addition to the data plotted by the computer, as in some patients there was a clear discrepancy between the visual findings and the time to maximum uptake registered by the computer. The visual reanalysis ensured that the correct time points were used for this analysis, but also provided a chance to ensure that uptake and distribution for each gland for each minute and their relation to each other were correctly registered, which also was applicable for the information about the excretion. Max(imum) counts were the last variable registered, but was however not available for all patients (6 SS and 14 KCS, but no controls). As the max count variable depends on various individually determined characteristics (i.e.

circulatory integrity, pharmacological distribution surface, blood pressure) and provides no additional information, it was not analysed.

## Statistics

Figures represent mean values (SD) unless indicated otherwise. Data on the various clinical findings were analysed by the appropriate tests with the use of SPSS 11.0 for Windows. Dichotomous data were analysed with cross tables using Fisher's exact test, due to small numbers. Analysis of variance (ANOVA) was performed for continuous variables (laboratory findings and scores of C%, Ex% and Tmax for each gland and group). Correlations between continuous variables were tested by Spearman's rank test. All resulting p-values below 0.05 were considered to indicate statistical significance.

# RESULTS

## Initial classification

Eight patients fulfilled at least 4 of the preliminary European criteria and were classified as SS patients, while the remaining 16 patients were classified as KCS patients for the purpose of this analysis and before SGS results were known. Patient characteristics are summarised in table 1 according to the clinical diagnosis. The majority (92%) of all patients were female and mean age was 46 years (11,5 ; range 27 – 63). The control group also consisted mainly of female (84%) with a mean age of 38 (14 : range 20-67). There was no significant difference in age between the two patient groups, although the control group was younger than the patient groups. In 3/24 (13%) of patients the presumed SS was secondary to RA (one patient) or a connective tissue disease (2 patients).

## Clinical data

The distribution of demographic and clinical data with regard to the sicca symptoms and additional findings is given in table 1. 22 (92%) of all patients had ocular symptoms and 23 (96%) had oral symptoms. The 2 of the 24 patients without ocular symptoms were denying symptoms, while data about oral symptoms were missing in the file of the one patient without oral symptoms. There were no patients that did not suffer from either oral or ocular symptoms. Twelve out of 21 examined patients (57%) had abnormal ocular findings, which consisted of an abnormal Schirmer's test in 12 (o 5 mm) and an abnormal Rose Bengal in 3 patients. SS patients had a significantly higher frequency of abnormal ocular findings (66% versus 40%). Six patients had a focus score over 1 on histopathology, which was significantly more frequent in SS than KCS patients. Possibly because all patients underwent SGS, other tests for salivary glands were not performed (except for one patient who performed sialometry.) 5 of the patients had positive autoantibodies to Ro/SSA and/or La/SSB, and all of them were classified into the confirmed SS group. SSA/SSB had the most significant difference of all the criteria in the criteria set, with a p-value of 0,004. SSA/SSB was not measured in 6 patients. All patients were tested for ANA and all but 2 patients for RF-latex. Four of the five patients with positive SSA/SSB also had a positive RF-latex. Out of all of the blood tests that were registered, ANA was the one with the most significant difference between the groups (p-value 0,000). RF-latex was also significant different (p-value 0,015) in the two patient groups, although 2 KCS patients had a positive value. The patients were also tested for RF-Waaler and anti-DNA (CLIFT and ELISA). RF-Waaler was positive in 4 of the 8 patients, all of them in the confirmed SS group. One of them had not measured SSA/SSB or ANA, but the three other had

positive values. No one had a positive anti-DNA (CLIFT was measured in 6 SS patients and ELISA was measured in 5 SS patients.) Six patients were being treated with hydroxychloroquin, three with corticosteroids, while one patient in the KCS group also used a cytotoxic drug.

**Laboratory findings**

Results for routine laboratory findings for SS and KCS patients are given in table 2. There was a significantly higher mean value of SR, lymphocytes and IgG in the SS group. Although 10 had elevated values for ASAT (range 7-82) and/or ALAT (range 18-105), the mean for the groups was normal.

**Tc-99m scintgraphy**

Quantitative data on scintigraphic findings for the three groups are summarised in table 3.

**Slope:** This was a visual analysis of the shape and duration of the curves.The uptake in the control group was in 4 persons greater in parotic glands and in 3 persons all of the glands showed similar curves. Only one control patient had a greater uptake in the submandibular glands. In the SS and KCS groups the uptake tended to be greater in the (paired) submandibular glands (3 SS + 10 KCS), while only 2 KCS patients had a greater uptake in (paired) parotid glands. In five of the SS patients and four KCS patients there were a discrepancy between the different glands. That is, the paired glands were not following each other and instead the slopes went in different directions. This can not be compared to the control group. Everyone in the control and KCS groups had a defined excretion from the time lemon juice was given and the paired glands were mostly following each other.Four patients in the SS group (7, 9, 10 and 11) had either a negative excretion, i.e. no excretion at all or the excretion lasted only for some minutes and then the slope started to rise again so that the Tmax was reached in the end of the session.

**Uptake/ C%:** There was a significantly reduced uptake in the two parotic glands, with the difference for the left parotic nearly significant on its own. This happened in both the SS and the KCS groups. In the submandibular glands we saw increased uptake in KCS patients, while the SS and control groups had nearly the same mean values. Here the difference was significant if we look at the paired glands as a whole, while right submandibular gland was only nearly significant.

**Excretion (E%):** The SS patients had the lowest excretion for all glands, but the differences were only significant in parotic glands. The values for the KCS and control groups did not differ much from each other. For these groups the excretion was lowest in the submandibular glands, while there was no difference between the glands for the SS patients.

**Tmax:** The time to maximal uptake was increased in both SS and KCS patients. The increase was much greater for the SS patients, where the difference was significant, but only for the parotic glands. The difference was not significant for the submandibular glands in any group.

## Correlations between SGS and clinical findings.

To determine if clinical factors might be related to SGS findings, correlations were analysed between parotic uptake or time to peak uptake. No correlation was found with age, focus score, ESR, serumcreatinin or Immunoglobulin levels within the SS or KCS group or within the whole cohort (data not shown).

## Qualitative SGS findings

Seven of the 16 patients initially classified as KCS fulfilled 3 criteria and in these patients abnormal SGS findings would lead to reclassification as SS patients. Nine KCS patients fulfilled less then three criteria and consequently would not be reclassified as SS even with abnormal SGS findings. The European Criteria set requires delayed uptake, reduced concentration and/or delayed excretion of tracer for the scintigraphy to be abnormal. As these descriptions are not further defined, these were arbitrarily defined here as those values outside the mean ± 2SD in the control group. Resulting data on the number of patients with abnormal findings is given for both SS and KCS patients in table 4. Depending on the number of abnormalities one requires 6 (38 %) or 14 (88 %) KCS patients would be reclassificied as SS patients after qualitative SGS.

# DISCUSSION

SGS has been reported to be a sensitive, safe and objective means of evaluating xerostomia and is a recommended investigation by the European SS group[9]. With SGS the functional capacity of all salivary glands can be studied simultaneously and the entire sequence of events can be recorded for later data-analysis. The European classification criteria require the presence of delayed uptake, reduced concentration and/or delayed excretion. However, definitions for these criteria are lacking, indicating that both quantisation of data and the recruitment of controls will have to be done locally in order to define the cut-off levels for normality/abnormality. This study provides such data for the University Hospital of Northern Norway. It shows that sicca patients have a reversed parotis/submandiblar uptake ratio, a prolonged time to maximum tracer uptake and a reduced tracer secretion from especially the parotic glands. Applying cut-off levels that were derived from the control group SGS findings leads to reclassification of a considerable number of KCS patients to SS patients, as defined by the preliminary European criteria.

Already in 1971 Schall and coworkers presented a study on SGS[11], where they concluded that SGS proved to be extremely sensitive in depicting small changes in glandular dysfunction and a good index to follow the natural progress of the sickness or the response to therapy, but concluded that decreased or absent pertechnetate uptake was a non-specific phenomenon. Later Håkansson and coworkers concluded the exact opposite[12], as SGS in their hands was sensitive enough to detect abnormalities between primary SS patients and a control group. They studied time-activity curves for submandibular and parotid glands as pairs, and found the submandibular glands were the most affected in primary SS patients. In contrast, Markusse and co-workers concluded that SGS only has a limited discriminatory value as a diagnostic procedure[3]. They however evaluated uptake and excretion from analogue pictures in a qualitative way, and did not use time-activity curves and quantitative variables.

Tracer uptake is a function that is hard to compare between different persons, as it is a function of salivary gland mass, which varies from person to person. Therefore we could not use maximum number of tracer counts as a variable in this study, while we also did not have information about max counts in the control group. We therefore looked at the uptake as a percent distribution in the different glands (C%). In this patient material, the whole uptake was significantly lower for parotic glands, both for SS and KCS patients. This may reflect a more chronic inflammatory process of the major salivary glands as it was previously reported that reduced uptake occurred only for

submandibular glands[12]. The control group had greater uptake in parotic than submandibular glands, both when we look at the slopes and the variables this may be related to volume of salivary gland, which will likely decrease in sicca patients due to the inflammatory process that leads to scarring. When we look exclusively at the SS patients, the mean uptake was biggest at the left side, both for parotic and submandibular glands, which is likely a chance difference. Visual (qualitative) evaluation of the slopes showed two different patterns, a mixed pattern and greater uptake in submandibular glands. The last one is findings that previously have been reported by others[12].

It took longer time to achieve T max in SS and KCS patients for parotic and submandibular glands for all groups, but the differences were only significant in parotic glands. The Tmax value is an absolute value that does not depend on time, but we have to remember that two different protocols were used and that there was not equal number of patients in the two protocols. Four SS patients reached T max after excretion was stimulated, which resulted in negative excretion values for some patients, which again gave a large standard deviation in the group. To evaluate the Tmax, we can imagine that applying protocol two to these patients, higher values could have achieved if they had been given more time.

Excretion was reduced among SS patients for all glands, but significantly only for parotic glands. While KCS patients also had a lower excretion, the differences with control patients were small, and not statistically significant.

The SGS results for the SS and KCS groups were mostly different, but the difference should really be greater if the KCS group were "clean". We did not take into account the fifth criteria, salivary gland involvement, except for the only patient (patient 7) who had performed whole sialometry. This resulted in a KCS group that consists of many true SS patients, who clinically would be/were classified as true SS patients with a positive SGS. To see whether these seven KCS patients with 3 fulfilled criteria gained a confirmed SS diagnose after the SGS, is not the aim of this study. And to see whether other KCS patients gained a confirmed SS diagnosis at a later stadium, we would need a follow-up study. We must bear in mind that there is median time from start of symptoms to a confirmed diagnosis of SS is reported to be 11 years[5].

Within the last three decades there have been introduced nine different classification criteria sets[6]. Manthorpe evaluated these nine criteria set in 2001, and came up with both advantages and disadvantages for all of them. (See addendum for details.) In the light of this evaluation, it would be

interesting is to see whether the patient group would be different by the use of an another criteria set. The fact that there were 7 patients with three fulfilled criteria, and 6 patients with four fulfilled criteria, shows us that there is a gliding scale. Both the clinical and scintigraphic results could be different when we looked at mean values and the groups were so small. The first criteria set that was introduced, was the Copenhagen criteria, invented in 1975-76. It is a simple criterion set, whom requires at least two abnormal objective test results from each organ with no specific test preferred. Since our material includes only two ocular tests (fulfilled by three of four tested patients), this set would reduce the SS group to a minimum. 1984 the first Japanese criteria were described and requires subjective complaints from the mouth and eyes, and at least one fulfilled objective test out of 3 items. That would also seem to be a criteria set useful for our material. All of our 24 patients fulfilled the symptomatic topic, but since we did not take the scintigraphic results into account, and no one had performed sialography, only patients with positive histopathology or two abnormal ocular tests would fulfil this set. We had 5 patients with positive histopathology, and one patient who fulfilled two ocular tests without being in the positive histopathology group. This results in a SS group consisting of 6 patients, where one former KCS patient with positive histopathology was included. In 1997 the Japanese II criteria came. This criteria set requires focal sialadenitis only or two abnormal of three defined items. We would not get a greater SS group with this criteria, since no one of our KCS patients had positive autoantibodies, and we can't use criterion a. The Greek criterion set from 1986 also requires focal sialadenitis only. If not, two of three defined items should then be fulfilled. History of enlargement of at least one parotid gland is one of the items, but our database did not include information about parotid swelling. Another item is modified whole sialometry, which also is a problem for us. The California criteria, published in 1986 and widely accepted require that all out of 5 items are fulfilled. This is a demanding criteria set that is hard to apply to this study because it was not designed for this criteria set. Another point is that they require a focus score over 2 for the histopathology to be positive, present in only 4 of our patients. The Japanese-III criteria were finalised in year 2000, but published in Japan only. For the diagnosis of primary SS at least two of four items should be abnormal. The items are positive focus score, autoantibodies, oral involvement and ocular involvement. Subgroups of the oral and ocular components include total inspection, while the other tests are the common ones. It is reported that Japanese-III criteria are rather similar to the Copenhagen classification criteria[6]. Since many of the items are based on different foundation, it is difficult to put in our data. Item a and b are the same, but since no one of our KCS patients had positive auto antibodies, non of our KCS group would change group. At last we have the EU-US consensus group with criteria from 2000/2001. These criteria are the same as the European-II, discussed in the introduction, but it is an absolute

requirement to have a positive histopathology and/or positive autoantibodies. (The criteria set also have a new exclusion diagnosis list.) Two of our confirmed SS patients would not retain their SS diagnosis with this criteria set, whereas one patient (14) had not been tested for autoantibodies.

Patients with SS have a decreased or abolished function of the exocrine glands and SGS can demonstrate this loss of function, which is the likely result of morphological changes due to chronic inflammation. Anaya and Talal suggested a two-stage model in the pathogenesis of SS[4]. The initial process consists of increased epithelial cell apoptosis, which leads to autoantibody production and subsequent salivary gland lymphocyte infiltration. This is demonstrated in lip biopsy where focal infiltration of lymphoid cells is a progressive process as demonstrated by increase of focus score over time. These lymphocyte infiltrates comprise both B and T cells, and replace the acinar tissue progressively until a final appearance of complete destruction. The lymphocytes are predominantly of CD4+ T helper cells. These cells contribute to B cell hyperactivity and autoantibody production. It has been shown that pertecnetate is actively concentrated in the intralobar ducts, and that 99 mTc⁻ substitutes for Cl- in the Na+/K+/Cl- co-transport system and therefore can serve as a measure of fluid transportation. In SS the ability to trap pertechnetate is reduced. This is suggested to be due to lymphocytic damage to the ductal epithelium and loss of acinar cells. Several studies reports that loss of the acinar component leads to a time-activity curve that gets flatter the more severe and chronic the inflammation gets [12,13].

True autoantibodies against salivary ducts occur rarely, if at all and there are no organ-specific autoantibodies detectable in serum of SS patients. Two precipitating antibodies to nuclear antigens, SSA and SSB are regularly found in SS patient sera. Anti-Ro/SSA and anti-La/SSB are linked non-organ-specific autoantibodies[5]. They are the most clinically important and best characterised autoantibodies in primary SS. Anti-Ro frequently occurs in the absence of anti-La, while anti-La are invariable accompanied by anti-Ro because of a physical association of these molecules in Ro/La ribonucleoprotein particles. There are in fact two Ro proteins that colocalise to surface membrane blebs on apoptotic cells where they may become target of an autoimmune response. Dryness is not solely a result of glandular destruction[4], because normal acinar cells are commonly observed. Autoantibodies directed against muscarinic M3 receptor explain the pathogenesis of both impaired glandular function and features of autonomic dysfunction, because of inhibition of parasympathetic neurotransmission.

A variety of diagnostic modalities can be used when evaluating xerostomia in suspected Sjøgren's syndrome and should be considered when considering SGS in the diagnostic process.

Lower lip biopsy of the salivary glands is considered to have a high specificity for SS, but involves some degree of discomfort to the patient and sometimes gives permanently damages due to excision of nerves, resulting in loss of sensation. The method gives a static picture of the minor salivary glands only, and there is often a problem in getting enough material. To avoid false negatives the material should be 20 mm2 to be representative. There are also reports that suggest that the minor salivary glands become affected at a later stage than the major salivary glands. In the present study, where 18 of the 24 patients had performed lower lip biopsy, only confirmed SS patients got a focus score over 1. However, three of the KCS patients had 2-5 focus of infiltrating lymphocytes and one of them had atrophy. This shows that some of the symptomatic patients also have unspecific changes (sialadenitis), perhaps reflecting a more chronic inflammatory process that probably can develop into more severe abnormalities consistent with SS. Whether SGS should be preferred instead of lower lip biopsy, is another matter. Lower lip biopsy has a traditional place in many diagnostic criteria set, at least in European II. While our data permit no recommendation to replace biopsy with SGS, the given criteria set gives us the possibility to recommend that SGS before lower lip biopsy. If the SGS results are convincing, lower lip biopsy would no longer be necessary.

Unstimulated whole sialometry is another method that determines total salivary secretion during basal conditions. It is a method that is easy to perform and to repeat, and gives a dynamic picture. The sensitivity for SS is high, but the specificity is low. There are great flow variations among healthy individuals, and under varying conditions the same individual can exhibit great variations. There are proposed standardized examination procedures[5]. Since SGS is also a safe, minimally invasive and quantifiable method it should be considered as a replacement for or addition to sialometry.

X-ray sialograpy is reported to be an accurate, but inconvenient tool for the patient and may lead to serious contrast medium reaction[14]. Reports about other radiologic procedures indicate that Magnetic resonance (MR) imaging and MR sialography (both non-invasive methods) give definitive information of morphologic changes in parotid glands[14]. Human polyclonal immunoglobulin (HIG) scintigraphy is another method that recently has been described (as an accidental finding) for diagnosing SS[15] as HIG accumulates in site of inflammation. Doppler-sonography in determining salivary flow is another method that is still under investigation.

In summary, our findings indicate that quantitative SGS can distinguish uptake and excretion for each of the four salivary glands between controls and sicca patients. The most severe abnormalities are seen in SS patients and using standardised data form controls would lead to a considerable number of KCS patients being reclassified as SS patients. As quantitative SGS is easy to perform and the required expertise, instrumentation and radioisotopes are all available at UNN, the use of quantitative SGS can be recommend in the diagnostic process and possibly follow-up of SS-patients.

## ACKNOWLEDGEMENTS

TABLE I

Demographics and classifying criteria for all patients. Figures represent numbers (%) unless otherwise indicated.

| | SS (n=8) | KCS (n=16) | Controls (n=8) | P-value |
|---|---|---|---|---|
| No. females | 7 (88%) | 15 (94%) | 7 | 0,832 |
| Age (years,mean) | 42,6 | 47,3 | 37,6 | 0,206 |
| Ocular symptoms | 8 | 14 | NA | 0,296 |
| Oral symptoms | 8 | 15 | NA | - |
| Abn Schirmer | 3 (38%) | 6 (37%) | NA | - |
| Abn sialometri | 1 (13%) | - | NA | - |
| Anti-SSA | 5 (63%) | 0 | NA | - |
| Anti-SSB | 4(50%) | 0 | NA | - |
| Histopathology | 4 (50%) | 1 (6%) | NA | 0,026 |
| Focus score (mean) | 2,12 | 0,16 | NA | 0,001 |
| Biopsy mm (mean) | 18,1 | 17,3 | NA | 0,897 |
| Positive ANA | 5 (63%) | 0 | NA | 0,000 |
| Positive RF-latex | 5 (63%) | 2 (13%) | NA | 0,015 |
| Positive RF-Waaler | 4 (50%) | 0 | NA | 0,102 |
| Corticosteroid use | 2 | 3 | | |
| Antimalarial use | 1 | 5 | | |

TABLE II

Distribution of laboratory findings for SS and KCS patients number with positive result in the different groups for each criteria.

| | SS | KCS | TOTAL | Reference | P-value |
|---|---|---|---|---|---|
| Hb .......(g/dl) | 12,3 | 13,3 | 12,9 | 11,5-16,0 | 0,131 |
| WBC .........($10^9$/L) | 5,9 | 6,6 | 6,2 | 4,0-11,0 | 0,686 |
| LYMFO........(%) | 48 | 28 | 30 | 20-45 | 0,038 |
| SR........(mm/h) | 36,9 | 17,1 | 23,7 | <21 | 0,034 |
| CRP...........(mg/L) | 4,6 | 4,4 | 4,5 | <5 | 0,718 |
| Kreatinin ....(µmol/L) | 66,6 | 72,9 | 70,6 | 55-100 | 0,349 |
| ASAT..........(U/L) | 25,1 | 25,0 | 25,1 | 10-35 | 0,986 |
| ALAT...........(U/L) | 28,4 | 40,3 | 35,9 | 10-35 | 0,218 |
| IgG........ | 29,9 | 13,7 | 19,1 | | 0,008 |
| IgA ........ | 3,6 | 2,7 | 3,0 | | 0,075 |
| IgM............. | 2,1 | 1,5 | 1,7 | | 0,201 |

Hb: Hemoglobin, WBC: White blood cells, LYMFO: Lymphocytes, SR: Erythrocyte sedimentation rate, CRP: C-reactive protein, ASAT: Aspartate transaminase, ALAT: Alanine transaminase, Ig: Immunoglobulin.

TABLE III

Distribution of salivary scintigraphy findings for SS and KCS patients compared to controls

| Scintigraphy finding | SS | KCS | CONTROL | P-value |
|---|---|---|---|---|
| Peak uptake | | | | |
| L parotic | 23,6 | 21,1 | 26,1 | 0,055 |
| R parotic | 20,4 | 19,0 | 26,9 | 0,046 |
| L submandibular | 24,9 | 29,8 | 23,5 | 0,043 |
| R submandibular | 22,9 | 29,9 | 23,8 | 0,051 |
| Total parotic uptake | 44,0 | 40,0 | 53,0 | **0,018** |
| Total submandibular uptake | 47,8 | 59,7 | 47,3 | **0,029** |
| Tmax | | | | |
| L parotic | 20,9 | 15,6 | 10,6 | 0,043 |
| R parotic | 21,0 | 15,6 | 10,6 | 0,035 |
| L submandibular | 16,3 | 12,6 | 8,5 | 0,096 |
| R submandibular | 14,4 | 11,9 | 10,3 | 0,525 |
| Whole parotic | 18,1 | 10,4 | 9,4 | **0,001** |
| Whole submandibular | 13,7 | 8,8 | 8,0 | **0,014** |
| Excretion | | | | |
| L parotic | 16,3 | 31,6 | 35,88 | **0,010** |
| R parotic | 15,8 | 32,0 | 33,9 | **0,007** |
| L submandibular | 18,1 | 25,3 | 28,1 | 0,117 |
| R submandibular | 16,6 | 25,9 | 26,4 | 0,103 |
| Total Parotic excretion | 16,3 | 31,4 | 34,9 | **0,008** |
| Total submandibulat excretion | 17,4 | 26,7 | 27,3 | 0,073 |

TABLE IV

Qualitative salivary gland scintigraphy findings for SS and KCS patients ; numbers reflect the number of patients (% of group) with values outside the mean ± 2SD range of controls.

| Scintigraphy finding | SS (n=8) | KCS (n=16) | P-value |
|---|---|---|---|
| **Reduced uptake** | | | |
| L parotic | 0 | 0 | ND |
| R parotic | 1 (12,5) | 5 (30) | 0,3 |
| L submandibular | 0 | 0 | ND |
| R submandibular | 0 | 0 | ND |
| Total parotic uptake | 3 (37,5) | 7 (42) | 0,7 |
| Total submandibular uptake | 1 | 0 | 0,5 |
| **Prolonged T max** | | | |
| L parotic | 6 (75) | 0 | 0,0006 |
| R parotic | 6 (75) | 2 (12) | 0,004 |
| L submandibular | 6 (75) | 2 (12) | 0,004 |
| R submandibular | 3 (37,5) | 0 | 0,03 |
| Whole parotic | 6 (75) | 0 | 0,0006 |
| Whole submandibular | 6 (75) | 9 (54) | 0,6 |
| Delayed Excretion | | | |
| L parotic | 5 (62,5) | 2 (12) | 0,02 |
| R parotic | 4 (50) | 1 (6) | 0,03 |
| L submandibular | 4 (50) | 0 | 0,006 |
| R submandibular | 1 (12,5) | 0 | 0,3 |
| Total Parotic excretion | 6 (75) | 3 (19) | 0,03 |
| Total submandibular excretion | 3 (37,5) | 0 | 0,03 |

## TABLE V

Summary of qualitative scintigraphy findings in SS and KCS patients.

| Overall Scintigraphy result | SS (n=8) | KCS (n=16) | P-value |
|---|---|---|---|
| Number with normal findings (%) | 0 | 2 | 0,53 |
| Number with 1 abnormal result (%) | 2 | 8 | 0,38 |
| Number with >1 abnormal result (%) | 6 | 6 | 0,21 |

## FIGURE 1

Salivary Gland Scintigraphy scheme for patient 7, a 50 year old woman.
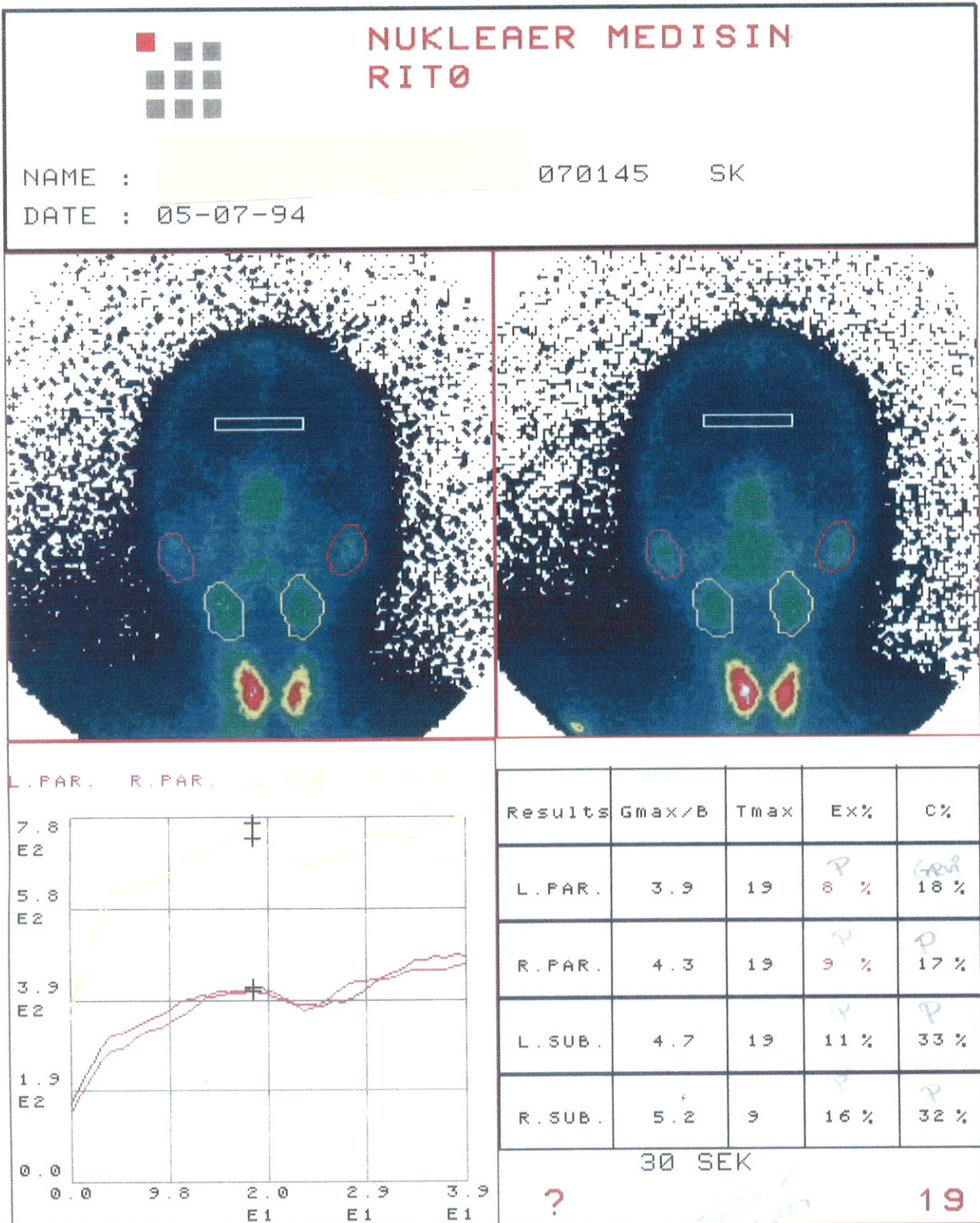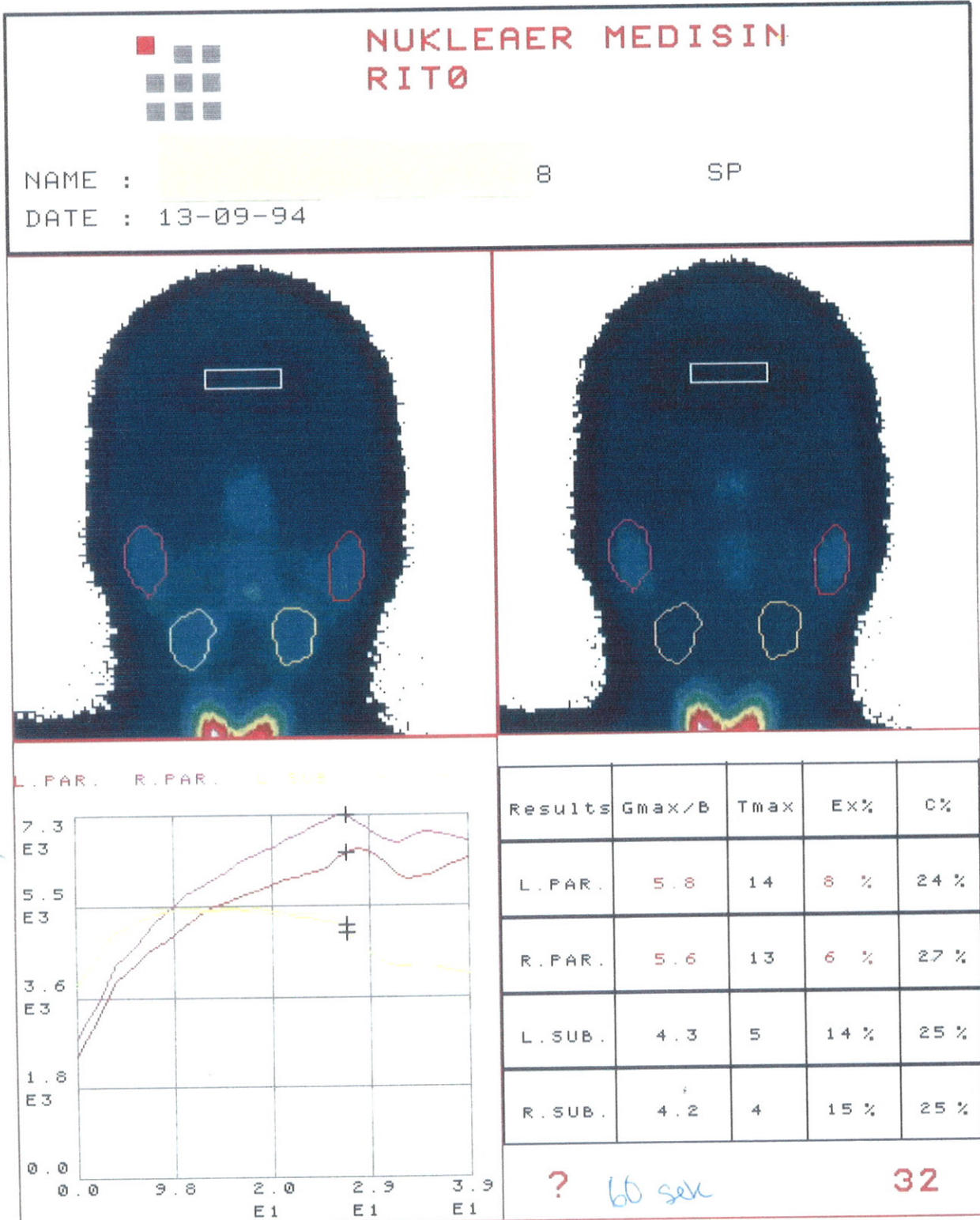
# FIGURE 2

Salivary Gland Scintigraphy scheme for patient 21, a 26 year old woman.



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**Addendum** - Overview of the various classification criteria for Sjøgren's dyndrome.

| Criteria set | Diagnostic demands for SS | Items |
|---|---|---|
| Copenhagen | At least 2 abnormal oral and ocular tests | |
| Japanese I | Symptoms from eye and mouth and 1 abnormal item | a: two abnormal ocular tests<br>b: focal sialadenitis<br>c: abnormal sialography/scintigraphy. |
| Japanese II | Focal sialadenitis only or 2 of 3 abnormal items | a: abnormal sialography only, or abnormal scintigrap stimulated whole sialometry<br>b: two of either schirmer-I, rose Bengal or fluorscein<br>c: positive anti-SS-A and or –SS-B |
| Japanese III | 2 of 4 abnormal items | A: positive focus score<br>B: autoantibodies (either SSA or-B) _<br>C: oral involvement: a: total inspection, b: scintigrap sialography, d: stimulated whole sialometry for 10 m than 10 ml, e: Saxon test for 2 minutes with less thar<br>D: ocular involvement: a: total inspection, b: Schirm Bengal using score 0-9 and d: fluorscein test |
| Greek | Focal sialadenitis only or 2 of 3 abnormal items | a: subjective keratoconjunctivitis sicca and abnormal abnormal rose Bengal<br>b: history of enlargement of at least one parotid glan<br>c: subjective xerostomia and stimulated modified wh |
| Californian | All 4 items abnormal | a: focal sialadenitis with more than two foci per 4 m<br>b: subjective xerostomia<br>c: abnormal unstimulated whole and stimulated sialo<br>d: abnormal Schirmer-I and rose Bengal tests<br>e: positive RF or ANA titer, or pos anti-SSA or –B a |
| European | Preliminary criteria. ANA and RF was included in f. | a: ocular symptoms<br>b: oral symptoms |
| European II | 4 of 6 abnormal criteria | c: ocular signs |
| EU-US consensus group | 4 of 6 abnormal criteria, but d and/or f must be abnormal | d: Histopathology<br>e: Instrumental evidence of salivary gland involvem<br>f: autoantibodies (SSA/SSB) |

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