This chapter discusses a number of tests used to aid diagnosis of Sjögren’s syndrome, subdivided into eye tests, salivary gland tests and blood tests.

Eye tests

**Schirmer test**
This test measures the tear production. The folded edge of a strip of filter paper 5x30 mm is placed over the rim of the lower eyelid (figure 15.1) and the eyes are then lightly closed. After 5 minutes, the amount of wetting on the filter paper is measured in mm. This is normally more than 5 mm. The Schirmer test is normal in about 20% of patients with Sjögren’s syndrome so a normal test does not exclude the disease. There are two variants: the Schirmer I test and the Schirmer II test. In the Schirmer II test, the measurement is carried out after tear production has been stimulated inside the nose. The Schirmer I test is used in the European criteria (see chapter 4).

**Rose bengal dye test**
Rose bengal stains cells on the surface of the eye red if they are not fully coated by the mucin layer of tear fluid and/or are damaged (figure 15.2). The result of this test is expressed as the Van Bijsterveld score. A score of 4 or higher (maximum is 9) is abnormal according to the American-European criteria. In patients with Sjögren’s syndrome, rose bengal can cause the eyes to sting. The rose bengal test may be painful. Lissamine green is less painful, but is more difficult to evaluate.

**Break-up time**
The purpose of the break-up time test (BUT) is to measure the quality of the tear fluid. Method: a drop of fluorescein is applied to the eye. After blinking once,
the patient must keep his eyes open for 10 seconds without blinking. It is then examined how long the tear film remains evenly distributed over the surface of the eye (cornea). After a while, the tear film starts to break up due to surface tension. This can be seen in the form of dark spots in the layer of fluorescein (see figure 15.3). The tear film normally remains intact for 10 seconds or longer. This test is not only abnormal in Sjögren’s syndrome but also in blepharitis, a condition causing inflammation of the eyelids due to blockage of the Meibomian gland ducts in the eyelid. Blepharitis can also cause a gritty sensation in the eyes, usually at its worst in the morning (in the case of eye problems caused by decreased tear production or too much evaporation, the symptoms are at their least in the morning and increase during the course of the day).

Salivary gland tests

Lip biopsy
In a lip biopsy, a few tiny salivary glands are removed from the inside of the lower lip under local anaesthetic for examination under the microscope. Since it is important for complete glands to be removed, punch or wedge biopsies are not recommended.

Focus score
The biopsy contributes towards the diagnosis of Sjögren’s syndrome if the focus score is ≥ 1 (see chapter 4 on diagnostic criteria). The focus score is the number of clusters (foci) of ≥ 50 lymphocytes per 4 mm² of tissue surface (see figure 15.4). Other diseases can also be detected with a lip biopsy, such as sarcoidosis or non-Hodgkin lymphoma.

The lip biopsy is considered to be an important test for the diagnosis of Sjögren’s syndrome but abnormal biopsies have been found in 15% of healthy volunteers with focus scores ranging from 2 to 6.

Focus scores did not correlate with age, smoking, serologic findings or salivary flow in these persons. Moreover, 18-40% of Sjögren’s patients have a normal lip biopsy. When evaluating the results of the lip biopsy, many sections need to be examined since considerable variation is found. In a recent examination of inter-rater reliability for a group of 5 board-certified pathologists interpreting the same series of labial salivary gland biopsies, the agreement was found to be uniformly poor for judgments of diagnostic status, focus scores, and histological characteristics of biopsy specimens. This lack of reliability is troubling.

Chisholm and Mason grading
In older publications on the evaluation of lip biopsies,
you sometimes come across grade 4 according to Chisholm and Mason. A score of 3 or 4 is equivalent to a focus score of ≥ 1. The meaning of the grades is:

0: absence of infiltrate
1: slight infiltrate
2: moderate infiltrate or < 1 focus
3: 1 focus
4: > 1 focus.

**Sialogram**
A sialogram is an x-ray of a salivary gland, usually of the parotid gland. A contrast medium is injected into the gland via the duct that opens into the mouth. This medium spreads throughout the duct system, making it visible on an x-ray. In the case of Sjögren’s syndrome, there may be dilation or twisting of the ducts and uneven distribution of the contrast medium. A sialogram is therefore only abnormal if the ducts are damaged. Digital subtraction techniques applied to the computer images allow the visual removal of structures other than the duct system. See figure 15.5.

**Salivary flow and sialometry**
Sialometry is a test to measure salivary flow, the speed at which saliva is produced. This done by asking the patient to chew on a paraffin block for 15 minutes and measurement of the amount of saliva produced in that time. There are several variations on this method.

**Sialochemistry**
Sialochemistry is the chemical analysis of saliva. It can measure, for example, how much potassium, sodium, protein or amylase is present in the saliva.

**Scintigram**
A scintigram visualises the uptake and secretion by the salivary glands of a radioactive labelled substance (10 mCl sodium pertechnate $^{99m}$Tc) after this substance has been injected into a vein. Rapid uptake and increased concentration in the salivary glands can normally be seen within 10 minutes. After 20-30 minutes, the substance is rapidly secreted into the mouth. In Sjögren’s syndrome, lower concentration and less secretion into the mouth are seen.

**Blood tests**

**Erythrocyte sedimentation rate**
The erythrocyte sedimentation rate or ESR is used to detect and measure inflammation. The ESR is measured by leaving unclotted blood to stand for an hour in a standard test tube. The cells sink to the bottom,
leaving a column of plasma above them. The ESR is the height of the plasma column in millimetres (figure 15.6). It may be elevated in patients with Sjögren’s syndrome, but this is by no means always the case.

An increased ESR does not prove acute inflammation as the ESR is not only influenced by proteins formed by inflammation, but also by antibodies to red blood cells, drugs, anaemia and the concentrations of albumin, IgG and IgM in the blood for example.

**CRP**

CRP (C-reactive protein) is an acute-phase protein. Acute-phase proteins are found in the blood in increased concentration during episodes of inflammation. The purpose of the test is similar to that of the ESR, but the CRP is more sensitive and not dependent on all kinds of other factors that influence the ESR. An elevated CRP always indicates inflammation.

**Leukocytes**

Leukocytes are white blood cells. These include neutrophilic, eosinophilic and basophilic granulocytes, lymphocytes and monocytes (see figure 15.7). The number of leukocytes is decreased in a quarter of Sjögren’s syndrome patients, usually due to a decreased number of lymphocytes. The decrease is virtually always relatively slight and has no negative effect on resistance to infection. The number of individual white blood cells can also be counted. This is known as leukocyte differentiation.

**Hb**

The Hb test (haemoglobin concentration) is carried out to detect anaemia. Haemoglobin is the red colouring in red blood cells and serves to transport oxygen from the lungs to the body’s tissues.

**Thrombocytes**

The number of thrombocytes (platelets) may be decreased by antibodies to thrombocytes or antiphospholipid antibodies. The decrease is often slight with no consequences for clotting. A decreased thrombocyte count is found in 11% of patients with Sjögren’s syndrome.

The number of thrombocytes may also be increased in Sjögren’s syndrome as a result of inflammation. This is likewise of no consequence and does not cause increased susceptibility to thrombosis.

**Immunoglobulins**

Immunoglobulins is the collective name for antibodies. There are five classes as follows: IgG, IgM, IgA, IgE and IgD. IgG is the most important with regard to resistance to infectious diseases. In Sjögren’s syndrome, the IgG may be elevated as an indication that the disease is active. The IgA or IgM are also sometimes elevated. Furthermore, monoclonal abnormalities may also be found (see protein screening test). A greatly decreased IgG level is rare in Sjögren’s syndrome and causes infection with capsular bacteria such as streptococci or staphylococci.

**Complement proteins**

Complement proteins play a role in resistance to infection in combination with antibodies. The concentrations of C4 and C3 are sometimes decreased in Sjögren’s syndrome. This mainly occurs if they are depleted by the formation of immune complexes (see below) which can lead to inflammation of small blood vessels.**
Elevated counts may be caused by inflammation.

**Immune complexes**

Immune complexes consist of antibodies, antigens and complement proteins (figure 15.8). They play a role in vasculitis where they are formed on the inside wall of the blood vessels. They often lead to binding and cleavage (depletion) of complement proteins (see above). Immune complexes can be determined in the blood, but for clinical purposes the results are not meaningful.

**Cryoglobulins**

Cryoglobulins consist of antibodies, antigens and complement that form a gel at a relatively low temperature, thereby making the blood stickier. Cryoglobulins can cause circulation problems. They occur in malignant blood diseases, auto immune diseases and infections with hepatitis B or C virus. Cryoglobulins may cause symptoms resembling Raynaud’s phenomenon so patients with Raynaud’s phenomenon should be tested for cryoglobulins.

Antibodies in cryoglobulins may be rheumatoid factors (see below) and these can cause symptoms at relatively low concentrations.

**Protein electrophoresis**

Protein electrophoresis shows the concentration of proteins, particularly albumin and globulins, in the blood or other fluids. Antibodies belong to the (gamma-)globulin group (figure 15.9A). In patients with Sjögren’s syndrome, protein screening can show a number of abnormalities such as polyclonal elevation of gammaglobulins (figure 15.9B) or monoclonal and oligoclusal abnormalities (figures 15.9 C and D).

Polyclonal refers to antibodies originating from different plasma cells, while monoclonal refers to identical antibodies (also called M-proteins). Oligoclusal means that there are several monoclonal abnormalities present at the same time. All these abnormalities can occur in Sjögren’s syndrome. Testing is important to be able to evaluate the severity of the disease, such as the risk of non-Hodgkin lymphoma.

**Amylase**

Amylase is an enzyme that plays a role in the digestive process because it breaks down starch. In approximately one third of patients with Sjögren’s syndrome, the level of amylase in the blood is moderately elevated due to inflammation in the salivary glands. If there is a very high level of amylase in the blood, it is possible to determine whether it comes from the salivary glands (S-amylase) or from the pancreas (P-amylase).

**Glucose**

Glucose is sugar in the blood. Elevated levels are an indication of diabetes mellitus. The value of this test in Sjögren’s syndrome is to exclude diabetes, particularly if the patient is unable to distinguish properly between dry mouth symptoms and thirst. Whereas Sjögren’s patients have a dry mouth but not thirst, in diabetes mellitus this is the opposite.
Autoantibodies in healthy subjects

Many autoantibodies are found in everyone, including the above-mentioned antinuclear antibodies (ANA) and rheumatoid factor. Usually, but not always, the concentrations are lower in healthy people than in people with autoimmune diseases.

The sensitivity of the laboratory tests used to determine the autoantibodies is adjusted to a certain level so as to ensure that the tests are not positive in more than 5% of healthy people.

TSH

TSH is an abbreviation for thyroid stimulating hormone. This hormone is produced by the pituitary gland (a small gland under the brain) and stimulates the thyroid into producing thyroid hormone. TSH is elevated in people with an underactive thyroid and is lowered in people with an overactive thyroid. Thyroid diseases often occur during the course of Sjögren’s syndrome and are found in approximately 15% of patients.

Lymphocyte subpopulations

Lymphocytes are white blood cells that play a central role in the immune system. With the help of T-lymphocytes, B-lymphocytes can mature into plasma cells that make and secrete antibodies. T-cells are divided into CD4 and CD8 positive T-lymphocytes with different functions.

In Sjögren’s syndrome there may be abnormalities in the numbers of lymphocytes in the blood. This may possibly be connected with the distribution of these cells in tissues and blood. The most common differences with healthy people are that relatively more CD5 positive B-lymphocytes occur in the B-lymphocytes and fewer CD8 positive T-lymphocytes.

Sometimes decreased numbers of all T-lymphocytes and B-lymphocytes are found in people suspected of having Sjögren’s syndrome. This may be an indication of sarcoidosis rather than Sjögren’s syndrome.

Autoantibodies

Antinuclear antibodies

Antinuclear antibodies (ANA, formerly known as ANF or antinuclear factors) is a collective name for antibodies that are directed against structures within cell nuclei. ANA (figure 15.10) are seen in low percentages in numerous diseases and also in approximately 5% of healthy people. The main reason for determining the presence of ANA is to confirm the diagnosis of systemic lupus erythematosus (SLE) because antinuclear antibodies are almost always present in untreated disease. The ANA test is positive in about 50% of patients with Sjögren’s syndrome, but carries no weight in the diagnosis of Sjögren’s syndrome.

ANA contain antibodies to SSA/Ro, SSB/La (see below) and to DNA for example. Since these more specific antibodies are determined using different techniques, discrepancies may occur in the test results. For example, in patients with Sjögren’s syndrome who have antibodies to SSA/Ro, the ANA test is nevertheless sometimes negative in up to 10%.

Antibodies to SSA/Ro and SSB/La

Antibodies to SSA/Ro and SSB/La are the most characteristic antibodies in Sjögren’s syndrome. They can be seen in 60-70% of the patients. SSA/Ro is a complex of three proteins, Ro-52, Ro-60 and calreticulin.

SSA/Ro and SSB/La proteins occur in all body cells of all people and play a role in cell division. It is not clear why patients with Sjögren’s syn drome make antibodies to these proteins. There are several methods of testing to determine antibody to SSA/Ro and SSB/La.

Tests for antibodies to SSA/Ro and SSB/La are important for the diagnosis of Sjögren’s syndrome because they are included in the diagnostic criteria for this disease (see chapter 4). As some patients only have 52 kD SSA/Ro antibodies, tests should be able to detect both 52 and 60 kD antigens. Antibodies to SSA/Ro also occur in patients with subacute cutaneous lupus erythematosus, systemic lupus erythematosus, neonatal lupus and dermatomyositis (anti-SSA/Ro 52 in particular).
Antibodies to SSA/Ro and/or SSB/La can make people more sensitive to sunburn and may cause neonatal lupus in newborn babies (see chapter 12).

**Rheumatoid factor**

Rheumatoid factor is an antibody, usually of the IgM class, that is directed to the Fc component of IgG (figure 15.11). Rheumatoid factor can be seen in 5% of healthy people, in about 70% of patients with rheumatoid arthritis and in about 40% of patients with Sjögren’s syndrome. Determination of rheumatoid factor is only meaningful if rheumatoid arthritis is suspected, but the presence of rheumatoid factors is not sufficient to make this diagnosis.

**Anti-CCP**

Antibodies to CCP (cyclic citrullinated peptide, citrulline is an amino acid, amino acids are the building blocks of proteins) occur in approximately half the patients with rheumatoid arthritis and rarely in people with other diseases. It is a new test, developed in Nijmegen in the Netherlands, which may possibly replace rheumatoid factor tests in the future. This test is slightly less sensitive in rheumatoid arthritis, but far more specific than determination of the rheumatoid factor. The anti-CCP test is virtually always negative in Sjögren’s patients with a positive rheumatoid factor but without rheumatoid arthritis.5

**References**