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Role of labial salivary gland biopsy in the diagnosis and long-term complications of Sjögren's Syndrome

Dr Michele Bombardieri,
Senior Clinical Lecturer and Consultant Rheumatologist,
Centre for Experimental Medicine and Rheumatology,
William Harvey Research Institute and Barts Health NHS Trust, Queen Mary University of London

Why having a labial salivary gland biopsy is important in Sjögren's Syndrome?

It has long been recognised that the reduction in saliva and tear production, leading to the typical signs and symptoms of dry mouth and dry eyes (sicca) of Sjögren's Syndrome (SS), are dependent on the infiltration of cells of the immune system in the salivary and lachrymal glands. These cells, which are part of the pool of white blood cells and are better known with the name of lymphocytes, normally circulate in the bloodstream and do not populate the glands. However, in the context of SS, a large amount of lymphocytes enter the salivary and lachrymal glands where they remain for long time leading to the persistence and progression of the disease (also described as chronicity).

Because of the easy access compared to the lachrymal glands, the study of labial salivary gland biopsies has been a cornerstone not only for the diagnosis of SS, but also for researchers to understand the causes and the mechanisms driving the disease. A labial salivary gland biopsy is considered a minimally invasive, safe and well-tolerated procedure which consists of a small incision on one side of the lower lip under local anaesthesia; this allows the collection of the small labial salivary glands (normally 3 to 5 glands are harvested) which are then sent to the Pathologist for examination. The labial salivary glands are often referred as "minor", in comparison to the "major" salivary glands such as the parotids and submandibular glands which account to around 80% of the total saliva production.

Historically, the procedure to obtain labial salivary gland biopsies was indicated in order to confirm the diagnosis of SS in patients presenting the typical clinical features of SS but who did not have the signature anti-Ro/SSA and anti-La/SSB antibodies in their blood. In particular, the current criteria that Rheumatologists use for the diagnosis of SS state that a positive labial salivary gland biopsy is required in the presence of a negative blood test

for anti-Ro/SSA and anti-La/SSB antibodies. What does having a "positive" salivary gland biopsy mean? Researchers back in the 1960s noticed that the immune cells which invade the salivary glands in patients with SS were different from other inflammatory diseases of the salivary glands in that these cells did not infiltrate the glands in a random fashion, but formed dense masses or clusters around ducts (the little tubes which transport the saliva from the gland to the mouth cavity). These masses of lymphocytes took the name of foci, with one focus being defined when the number of cells exceed 50. Pathologists who examine the glands under the microscope precisely look for the presence of these lesions to confirm the diagnosis of SS.

Beyond the diagnosis: labial salivary gland biopsies as predictors of severe disease and lymphoma in SS

Although the cause(s) of SS remains unknown, from the initial definition of these focal lesions in the salivary glands as hallmarks

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of SS, 40 years of research of the salivary gland of SS patients have significantly advanced our understanding of the mechanisms driving the disease. Additionally, recent evidences from different investigators have clarified that salivary gland biopsies can not only confirm the diagnosis of SS, but can also inform, at least to a certain extent, on how the disease may progress.

Although some general symptoms of the disease are present in the vast majority of cases (i.e. mouth and eye dryness, fatigue, generalised muscle and joint pain) it is now well recognised that not all patients with SS present with the same disease severity and evolution. Around one third of SS patients experience a more severe disease with significant extraglandular manifestations (i.e. inflammatory arthritis, kidney, lung or skin inflammation etc). In addition, 5% of all patients with SS will develop a form of cancer called lymphoma. This 5% translates to up to a 44-higher risk of lymphoma in SS patients compared to healthy individuals. Most frequently, lymphoma in SS arises within the salivary glands (in particular parotids) and although it is normally not as aggressive compared to other forms of lymphoma, it is the most serious complication of SS and the only factor which influences the observed increased death rate in SS.

Comparative studies between the labial (minor) and the parotid (major) salivary glands have now clarified that the same cells (the lymphocytes described above) that infiltrate the salivary glands at early stages of SS are also responsible for the evolution towards lymphoma. Specifically, one particular type of lymphocytes, called B cells, is the culprit of over 80% of lymphomas in SS (thus referred to as B cell lymphomas). Normally, B cells are not present in the salivary glands of healthy individuals and are only found in biopsies from SS patients. Nevertheless, different patients with SS can display a very different amount of B cells in their salivary glands. In particular, in 30% of SS patients B cells form dense and large rings which are defined as germinal centres. In normal situations, germinal centres are only present in lymphoid organs such as the spleen or the lymph glands where they form the factories in which B cells produce antibodies. Antibodies can be divided in “good antibodies” and “bad antibodies”. Good antibodies are those that protect us from infections by helping the body in the clearance of pathogens such as viruses and bacteria. Conversely, in conditions such as SS, antibodies can react against the body own cells and can cause damage in different organs, thus the definition of autoimmune diseases. In patients with SS, the bad antibodies, such as the anti-Ro/SSA and anti-La/SSB, can be made in the germinal centres in the salivary glands, which thus directly contribute to the glandular dysfunction.

A breakthrough discovery highlighting the importance of germinal centres in SS came from a recent Scandinavian study which enrolled a large population of patients with SS who underwent labial salivary gland biopsies. In this study, the investigators divided the patients as having or not having germinal centres in the salivary glands and asked whether the frequency of lymphoma was different in the two groups up to 20 years after the biopsy. The results of this study are very important because they provided novel observations that can be immediately used in clinical practice. First, they showed that all cases of B cell lymphoma of the salivary glands developed in patients having germinal centres while only 1 case of lymphoma of the lachrymal glands was observed among the germinal centre negative SS patients. The investigators calculated that having germinal centres in the labial salivary glands conferred a 16-fold higher risk of lymphoma. Second, the authors of this work also observed that, independently from lymphoma, having germinal centres was associated with a more active disease and more frequent extraglandular manifestations.

What are the clinical consequences of this work? The first very important advance which is of immediate benefit is that we now have, through the observation of labial salivary gland biopsies, a way of classifying SS patients as having high vs low risk of evolving towards lymphoma many years prior to lymphoma development. It is now possible to ask the Pathologist looking at the salivary gland biopsy to report on whether germinal centres are present. However, a second and potentially most important advance in the long term is that we could be able to prevent severe extraglandular manifestations and lymphoma development by treating high risk patients (i.e. those with ectopic germinal centres in the salivary glands) with appropriate treatment.

Moving the field forward: development of novel drugs targeting B cells and germinal centres for patients with SS

Since the discovery of B cells as central players in SS, a significant interest has been raised in targeting these cells as a new form of treatment for SS patients. Several studies, from initial small reports to larger clinical trials have been performed or are under testing with novel drugs directed against B cells. Most of these studies have been conducted using a drug called Rituximab, which is approved by the NHS for use in other autoimmune disease such as Rheumatoid Arthritis and Systemic Lupus Erythematosus. This drug selectively wipes out B cells from the peripheral blood leaving the other white blood cells largely untouched.

Although not all data from these studies have been in agreement, the general consensus is that Rituximab is of benefit in a subset of patients in alleviating the symptoms of SS. In particular, patients with very active disease and shorter disease duration which were given Rituximab more than once (the drug is administered in intravenous infusions) seems to respond better. A large multicentre UK study, led by Prof Simon Bowman in Birmingham, has completed the recruitment of over 130 patients with SS to test the efficacy of Rituximab. Patients were randomly allocated to either Rituximab or placebo (an inactive compound) with neither the patient nor the doctor aware of the treatment (what we call a double blinded study). The results of this trial will be analysed from early 2015 and hopefully will show a significant improvement in patients who received the active drug. In addition, our group at Queen Mary University of London and Barts Health NHS Trust is looking at labial salivary gland biopsies taken from a smaller subgroup of patients before and after treatment. We aim to understand whether by studying the salivary gland biopsy we can identify before treatment those patients who might respond better (i.e. those with more B cells in the salivary glands may have a better response).

In addition to Rituximab, a very significant number of novel potential treatments directly or indirectly targeting B cells and germinal centres are currently being tested in clinical trials both in SS and other autoimmune disease. This is very important as only some patients with SS respond to Rituximab and some experience side effects which do not allow giving the treatment more than once or twice. The development of novel treatments for SS, a disease which is considered “orphan” due to the lack of clinical studies compared to other rheumatic disease, will require not only interactions between doctors and pharmaceutical industries but also a close and truthful interactions between doctors and patients with SS in order to discuss the potential benefits and the risks of taking part in such clinical trials.

In an ideal scenario, with the development of such new treatments, the challenge will be to identify prior to the initiation of treatment which patients with SS may respond to a particular drug in order to maximise benefit and minimise exposure to potential side effects. In this regard, the use of labial salivary gland biopsies, in parallel with other studies in the peripheral blood, may offer such opportunity once we better clarify the different mechanisms driving different forms of disease.